



The Pennsylvania State University

Surgery in Research Animals

Animal Resource Program
University Park, PA

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This document contains information on performing surgical procedures in research animals at PSU. Although surgery in rodents is emphasized, the basic principles discussed here apply to surgery in all animal species. Successful surgical outcomes require understanding of fundamental concepts in anesthesia and analgesia, aseptic technique and tissue handling. Surgery is a complicated undertaking that requires planning and practice to do well. The new surgeon should be aware that there is much to learn. Performing a surgical technique while also maintaining sterility and monitoring patient well-being and anesthetic depth is a challenge for even the most experienced surgeons. Poor surgical training and technique not only endangers animal welfare but may invalidate research results. Investigators are encouraged to use this document and other PSU training resources to help their staff and students develop proficiency in research surgery.

General Principles of Surgery

Prepare in Advance

- Use a pre-surgical checklist (*see appendix B*) to ensure that necessary supplies (instruments, drugs, syringes, clipper, scrub, suture, heating pads, etc.) are available and preparatory steps completed before beginning surgery.
- *Arrange to have help available during the surgery.* While it is not impossible to perform surgical procedures alone, it is extremely difficult to do them well. A ‘non-sterile’ assistant should be used whenever possible.
- The surgeon should have a thorough understanding of the surgical procedure and practice on cadaver animals before using live animals when possible.

Use Aseptic Technique

- Aseptic technique encompasses all procedures designed to prevent the introduction of microbial contamination into the surgical wound. Failure to use correct aseptic technique may result in post-operative *wound infections, animal suffering and invalid research data.*

Handle Tissues Gently

- Handle living tissue as gently as possible during surgery. Rough handling or crushing of tissues will lead to *swelling, inflammation and post-operative pain and discomfort* for the animal.
- Develop your surgical technique by practicing on representative models or cadaver animals before proceeding to live animals.

Use Appropriate Anesthetic and Monitoring

- General anesthesia is *required* during surgery. Anesthesia must be deep enough that the animal cannot feel pain yet not so deep that breathing and heart function are compromised. Respiration, cardiac function and anesthetic depth must be regularly monitored while the animal is anesthetized. Exactly how this is done will depend on the species of animal and type of surgical procedure.

Post-Operative Care

- Post-operatively, animals must be kept warm and *monitored closely* to ensure they recover from general anesthesia.
- Appropriate *analgesic medications* must be administered as needed.
- For 7 to 14 days after surgery, *daily observation and evaluation* is necessary to ensure there are no surgical complications (such as infection, bleeding, or poor wound healing) or unnecessary pain and distress.
- Complete surgical and post-operative *records are required.*

Location for Surgery

The surgical area should be uncluttered and easily disinfected. For rodents, the area should be dedicated to surgery during the procedure but may be used for other purposes when not being used for surgery. *Patient preparation and recovery should take place in a location separated from the surgical area although it may be in the same room.*

Surgical Attire

Rodent survival surgery must be performed using *sterile gloves, a clean lab coat or gown and a facemask*. A head covering is recommended to reduce the risk of stray hair falling into the operative field.

Record Keeping

Investigators are responsible for maintaining accurate records of anesthesia, surgery and postoperative care (including analgesic administration). Records also serve as documentation that the procedures were conducted humanely and by appropriately trained individuals. Information on groups of rodents treated similarly may be entered on a single medical record. Non-rodent species require an individual record for each animal. *Records must be kept in the animal room or an accessible area nearby for easy access by veterinary and IACUC personnel.*

Information to Include on Surgical Records

- Date of surgery
- Name of surgeon
- A description of the surgical procedure**
- Animal identification i.e., number or group name
- Name(s), dosage(s) and route(s) of administration of anesthetic and analgesic drugs
- Name(s) and dosage(s) of experimental agents administered
- Post-operative monitoring that clearly documents daily observation and health status
- Medical treatments administered during or after surgery

Surgical records should be kept in a notebook. It is strongly recommended that a detailed description of each surgical procedure be kept in the front of the notebook.

** Rather than rewriting the surgical description on each record, provide the name of the procedure that corresponds to the description provided in the front of the notebook. Record any deviation from the detailed description on the surgical record.

Anesthesia

General anesthesia, a condition in which the animal is unconscious and completely insensible to pain, is required for surgery. There are two types of general anesthetics used. **Inhalation (gas) anesthesia** requires special equipment to deliver a controlled dose and anesthesia lasts only for as long as the animal is breathing the gas. Animals usually recover within minutes from the inhalant anesthetic (isoflurane) that is most commonly used at Penn State. **Injectable anesthetics** are administered using a needle and syringe. The duration of a surgical plane of anesthesia with injectable anesthetic drugs varies but is generally ~ 20 minutes. However, recovery from injectable anesthesia is prolonged (1 hour or more). Drugs are available that can shorten ('reverse') anesthesia time for certain injectable anesthetics, but these are not generally given until at least 30-60 minutes after anesthetic administration. Please consult with an ARP veterinarian regarding anesthetic choice.

Inhalant Anesthetics

- The delivery of inhalant anesthetics, such as isoflurane, requires the use of an *anesthetic vaporizer* and oxygen supply source.
- The duration and depth of anesthesia is *easily controlled and recovery is rapid*.
- Chronic exposure to inhalant anesthetics is a *human health hazard* and must be avoided.
- Appropriate anesthetic *scavenging equipment* must be used for gas anesthesia.
- You must be *trained to use this equipment*.

Injectable Anesthetics

- These drugs must be prepared and stored under *sterile conditions*. Sterile needles and syringes are used to inject the animal with the drug. A new needle and syringe must be used for each animal.
- The amount injected is calculated based on *animal body weight*.
- The duration and depth of anesthesia is *not easily controlled and recovery is slow*.
- Drugs *regulated by the U.S. Drug Enforcement Administration* must be purchased and stored following DEA regulations.

Preparation of Injectable Anesthetic Drugs

While injectable drugs are a convenient way to administer anesthesia to rodents in biomedical research, incorrect administration of these drugs can lead to adverse events such as over- or under-dosing and post-operative infections. The *amount of drug administered to each animal is based on its body weight*. Each animal must be weighed on the day of anesthesia. Mice and rats, especially young, growing animals, can change weight rapidly. A body weight recorded last week may not be accurate today. *These drugs must also be prepared and stored under sterile conditions*. Sterile needles and syringes are used to inject the animal with the drug. A new needle and syringe must be used for each animal.

Many factors influence how a specific anesthetic drug and dosage will affect an individual animal. *Variables such as strain, sex, age, and stress levels can result in variations in anesthetic depth and recovery times*. Try a test administration on non-experimental animals prior to using unfamiliar drugs, drug combinations or groups of animals.

Dilutions of Injectable Anesthetic Mixtures for Use in Rodents

Multiple injectable anesthetic drugs are often combined and diluted for use in small rodents. This makes calculation of doses for individual animals much simpler and reduces the likelihood of dosing errors. Instructions for mixing and dosing [ketamine/xylazine drug mixtures](#) for rodent anesthesia are available on the ARP website. Remember that all supplies must be sterile and aseptic technique used during mixing and administration of injectable anesthetics.

Controlled Drugs

The United States Drug Enforcement Administration (DEA) regulates (controls) certain drugs used for anesthesia, analgesia, and euthanasia because of the potential for human abuse. Ketamine and buprenorphine are examples of commonly used “controlled drugs”. Investigators using controlled drugs must apply to the [DEA](#) for a license and are responsible for complying with all DEA regulations including storage in a substantial locked cabinet or safe and maintenance of written records accounting for quantities received and dispensed.

Monitoring the Depth of Anesthesia

The ideal dose of general anesthesia acts on the central nervous system to eliminate the sensation of pain as well as consciousness. Under-anesthetized ('light') animals can still feel pain even though they may not be able to move. Animals given too much anesthetic ('deep') can die due to drug-induced heart and respiratory depression.

The goals of monitoring are to:

- Verify that the animal is *not able to feel pain*. Firmly pinch the rear paw or tail. If the animal moves it is too lightly anesthetized.
- Make sure the animal is not too deeply anesthetized by monitoring respiration, heart rate and mucous membrane color. The breathing and heart rates will slow as the animal becomes more deeply anesthetized. Although respiratory and heart rates are not commonly counted during anesthesia in rodents, with practice, the speed and general character of these parameters can be recognized. Changes (shallow, infrequent breathing, pale or blue mucous membranes, decreasing heart rate) can be indicators that the animal is too deeply anesthetized. *Note: anesthetized animals that appear to be gasping with an open mouth are often dying.*
 - Instruments such as a pulse oximeter may be used to monitor the heart rate and blood oxygen levels.
 - Capnographs can be used to measure the amount of carbon dioxide expelled during each breath and are another way to monitor respiratory function during anesthesia.

Frequency of monitoring:

- Perform toe or tail pinch about every 5 minutes
- Although it is ideal to monitor the character of breathing while you work, it is easy to concentrate so deeply on the surgical procedure that you are not paying attention to breathing. Therefore, you should make a point of checking respirations about every 5 minutes when you check toe/tail pinch.
- **Important:** It is easy to forget about monitoring the animal when you are concentrating on the surgical procedure. Consider using an alarm set to beep at 5-minute intervals to remind you to check.

Prevention of Fluid Loss

Rodents may experience extensive fluid loss during and after surgery due to evaporation from exposed body tissues and cavities, bleeding and/or lack of fluid intake perioperatively. To reduce intra-operative fluid loss the surgeon should control blood loss by clamping and/or ligating bleeding vessels and may gently irrigate exposed tissues with warmed sterile saline. In addition, warm, sterile fluids (e.g., 0.9% NaCl) may be injected subcutaneously (Mouse: 40 ml/kg total, not > 1.0 ml per site; Rat: 25 ml/kg total, not > 5.0 ml per site), divided between 2-3 sites prior to anesthetic recovery. The animal's water and food intake and body weight should also be monitored as part of post-operative care.

Analgesia

Post-operative analgesic medication must be provided for research animals subjected to procedures expected to induce more than momentary or slight discomfort. Pain adversely affects the welfare of animals and if not controlled, is a *variable that can confound the interpretation of experimental results*. Investigators are responsible for the assessment and management of pain in their research animals and must include a detailed pain management plan in their IACUC protocol.

It is often advisable to give analgesic medication either immediately before or after the surgical procedure. This is termed *preemptive analgesia*. The administration of analgesia before surgery and recovery from anesthesia may reduce the severity and duration of post-operative pain and decrease the amount of analgesic medication needed to provide effective pain relief.

Preemptive analgesia ensures that all *animals receive analgesic medication prior to the onset of post-operative pain*. However, this requires the investigator to make an accurate prediction of how much post-operative pain the surgical procedure will cause the animal. Certain surgical procedures are associated with greater post-operative pain and discomfort than others. There are also different types of pain (e.g., acute versus chronic; neuropathic versus visceral pain) and each must be treated appropriately to provide effective relief for the animal. Post-operative pain may also continue well past the day of surgery. *Investigators must observe, evaluate and treat each animal as long as the potential for post-operative pain and discomfort exists*. More information on recognizing and treating [pain in laboratory animals](#) may be found on the ARP website.

Analgesic drugs are usually administered by injection. However, there are occasions when analgesic drugs may be added to drinking water or food for oral delivery. Please consult with an ARP veterinarian about analgesic use when planning an experimental protocol.

Surgical Instruments and Tissue Handling

The basic goals of any surgical procedure:

- Create a wound and expose a target tissue
- Close the wound so that rapid healing occurs
- Prevent post-operative infection
- Animal survives and returns to 'normal' with minimal pain or distress

Achieving these goals is directly dependent on the surgeon's skill in selecting and using instruments and handling tissues.

Instrument Selection and Use

Instruments used for rodent surgery are delicate and typically designed for a specific function. Incorrect use of these instruments will damage them and make it difficult, if not impossible, to use them correctly. For example, hemostatic forceps are designed to grasp and hold tissue such as blood vessels or skin. While they can be used to hold a needle for suturing in an emergency, routine use of hemostatic forceps instead of a surgical needleholder is not recommended. These forceps are not designed to securely grasp the needle and using them for suturing will damage the tips making them useless for their intended purpose. Instruments should also be of the appropriate size for rodent surgery. Using instruments that are too large will exaggerate hand motions and decrease surgical precision resulting in poor technique and increased tissue trauma.

It has been reported that repeated use of glass bead sterilizers will make instruments brittle and dull over time. Any instrument that is used frequently, handled roughly or cleaned with abrasive materials can be damaged or dulled. Instruments should be assessed on a regular basis and replaced when necessary. Scissors and other sharp instruments can sometimes be re-sharpened to prolong life. Instruments must be gently cleaned after each use to ensure that all blood and tissue is removed. An ultrasonic cleaner can assist in this process. Proper care will extend the life of your instruments and help preserve your investment.

Reference:

How to Select and Procure Surgical Instrumentation for Rodent Surgical Research. 2012. S Baran, E Johnson, and M Perret-Gentil. <https://www.alnmag.com/article/2012/09/how-select-and-procure-surgical-instrumentation-rodent-surgical-research>

Instrument Preparation

Prior to surgery, instruments and other supplies are placed in a 'surgical pack'. The surgical pack must not only hold the instruments and supplies during the sterilization process, but also maintain the sterility of the contents until used in surgery. A variety of packing methods exists, including:

- Instruments, with or without an instrument tray, are packed inside a folded cloth or paper wrap and sealed with tape designed to indicate when sterilization has been achieved. Other types of sterilization indicators may be placed inside the pack.
- Peel packs –self-sealing 'envelopes' used for steam or gas sterilization. A sterilization indicator is included on the envelope.

Keeping the Instruments Sterile During Surgery

Use an extra piece of sterile drape material or the inside of the surgical wrap or envelope as a sterile space to place instruments during surgery. *One of the most common errors for an inexperienced surgeon is setting instruments down on an unsterile surface.*

Sterilization Procedures

Surgical instruments and other material or equipment that will contact the surgical site must be sterile prior to use. The process of sterilization kills all forms of life, including bacterial spores and viruses. *Chemical disinfection is not the same as sterilization and is not acceptable as the primary method of instrument preparation for surgery.* Methods of instrument sterilization include steam (autoclave) and gas (ethylene oxide) sterilization. Dry heat sterilization using a glass bead sterilizer is not acceptable for initial sterilization of instruments prior to surgery but may be used during ['batch' surgeries](#) in rodents.

Steam Sterilization

- Steam or autoclave sterilization is the most common method of instrument sterilization.
- Instruments are placed in a surgical pack and exposed to steam under pressure.
- A *sterilization indicator* (required) such as autoclave tape or an indicator strip is used to identify instruments that have been sterilized.

Ethylene Oxide

- Ethylene oxide gas is used to sterilize items that cannot withstand the high temperature and steam of an autoclave.
- Ethylene oxide is highly toxic. Items sterilized with ethylene oxide must be aerated before use to allow the gas to dissipate.

Glass Bead Sterilizers:

- Glass bead sterilizers may be used to re-sterilize instruments during a surgical procedure but are not acceptable as an initial method of sterilization.
- Glass bead sterilizers have a central well filled with glass beads heated to high temperature (approximately 500°F).
- Blood and tissue must be removed from surgical instruments before placing the tips of the instruments into the hot glass beads for approximately 10-15 seconds.
- *Only the tips of the instruments are re-sterilized* in this process.
- Instrument tips become extremely hot and must be cooled before use.
- Glass bead sterilizers may be used between animals when one set of initially autoclaved instruments is used on a group of up to 5 animals.

Reusing Instruments

When the same surgical procedure is performed on multiple rodents during one surgical session, the same set of instruments may be used on more than one animal under the following conditions.

- The instruments are *initially sterilized by autoclaving or gas sterilization.*

- Instruments are *wiped with alcohol or sterile saline to remove blood and tissue particles* between animals.
- The tips of instruments are then re-sterilized in a *hot bead sterilizer* before use on the next animal.
- *A new set of sterile instruments must be used after every 4-5 animals.*

“Instrument tip” technique for certain rodent surgical procedures

Although challenging, instrument tip technique may be useful for specific rodent surgery situations. Generally, this is limited to surgeries in which incisions involve only the skin and manipulation of internal organs does not occur. Keep the following points in mind when using “tips only” technique:

- Prior IACUC approval is required to use instrument tip technique in research rodents.
- The animal must be maintained in a surgical plane of anesthesia throughout the procedure.
- Begin surgery with sterile instruments and handle them aseptically throughout the procedures.
- The same set of pre-sterilized instruments may be used for up to five similar surgeries provided aseptic technique is followed throughout all procedures.

Sterile surgical gloves do not have to be worn if the following criteria are strictly adhered to:

- Gloves are changed after completing the surgical scrub such that new clean gloves are donned at the beginning of the surgical procedure.
- The gloved hands never touch the working (tip) end of the instruments, the suture, suture needle or any part of the prepped surgical field.
- Only the sterile tips of the instruments are used to handle the animal’s tissues.

Outline of procedures in “tips only” surgery

- The surgeon must wear a mask, and *clean* lab coat or gown. A head covering (cap or bonnet) is recommended.
- Place the anesthetized and surgically prepped animal on a warming device that has been covered with a clean paper bench towel.
- Open your sterile surgical pack and any other sterile equipment needed (e.g., suture material) without touching or handling the sterile contents.
- Wash your hands with soap and water and put on clean examination gloves.
- Drape the surgical site. The surgeon must handle the sterile drape only by its edges so that it does not become contaminated. (The drape should contain a ‘pre-cut’ opening that allows access to the surgical site as it will be difficult for the surgeon to cut an opening without contaminating the drape.)
- Once the instruments are handled by the surgeon, *only the tip of the instrument is considered sterile*. If the surgeon lays the instrument down, the sterile tip must be placed on a sterile gauze sponge or drape. The instrument handle should not come in contact with the sterile surface to avoid contamination of that surface.
- If the tip of a surgical instrument becomes contaminated, the tips may be re-sterilized by wiping away blood or tissue using a moistened gauze or alcohol pad and placing the tips in a hot bead sterilizer.
- *Keep in mind that the surgeon cannot touch the instrument tips, suture material or wound clips, suture needle or any part of the prepped surgical field with his/her hands during the surgery.*
- Gloves should be rinsed with 70% alcohol between surgeries. If you must handle another mouse to anesthetize and prep it, you must change gloves before performing the next surgery.

While performing surgery, be careful not to get paper or cloth instrument drapes wet. Wet material acts as a wick to pull bacteria through from the non-sterile surface below. When this occurs, the instruments should be considered contaminated and re-sterilized before further use.

Animal Preparation

Protect eyes– Anesthetized animals lose the ability to blink and their eyes remain open. *Ophthalmic ointment* must be applied in the eyes of anesthetized animals to prevent corneal drying.

Remove fur from the surgical site - Aseptic surgical preparation includes *removal of the fur* surrounding the incision site by clipping, plucking or depilatories (e.g., Nair). This must take place in an area separate from the location of surgery. Note: Since depilatories can irritate the skin they should be applied for a short period of time (< 1 minute) and then thoroughly rinsed from the skin.

Protect the animal from hypothermia – Rodents lose body heat quickly during anesthesia. For all procedures, insulate the rodent from underlying cold surfaces. Animals must be provided with *supplemental heat when anesthesia (including recovery time) will last more than a few minutes*. Far infrared or warm water circulating pads are recommended as safe, controllable sources of heat. Electric heating pads or heat lamps are not recommended as they can easily burn the skin.

Animal positioning – Animals must be positioned so that *breathing and/or blood flow is not restricted*. Animals can die quickly from lack of oxygen if the head and neck are bent sharply or objects are placed on top of the chest such that they cannot breathe. If it is necessary to secure an animal to keep it in a particular position during surgery, be careful not to restrict breathing or blood flow.

Scrub the surgical site - The incision site should be cleaned with a non-irritating, germicidal soap such as povidone-iodine (Betadine) or chlorhexidine scrub.

- A sterile cotton swab can be used to scrub rodents.
- The scrub should start in the center of the surgical area and gradually work outward in concentric circles around the incision site.
- The site should be rinsed with sterile water or 70% alcohol following the same pattern.
- Repeat the scrub and rinse a second time. *It is critical that rodents are not soaked with water or alcohol, which can lead to hypothermia.*



Work outward in concentric circles when scrubbing the surgical site.

Conducting Surgery

Open the surgical pack – Place the pack in a convenient location near the animal. It must be opened before donning sterile gloves (or have an assistant open the pack). Do not allow sleeves, loose hair or other objects to hang over the opened pack when working.

Drape the surgical site - The skin and hair surrounding the surgical site should be covered with a sterile surgical drape (paper, cloth or other acceptable sterilized material). The drape effectively enlarges the sterile area around the surgical site and helps prevent contamination of sterile instruments, suture material, and the surgeon's gloves.

Maintain Sterility – Surgeons and support staff must work together to maintain sterility during the surgical procedure. Sterile articles must touch only sterile surfaces and all sterile surfaces should be kept as dry as possible. *See Appendix A for an illustration of sterile and non-sterile areas of the surgical field during surgery.* If contamination of surgical gloves or equipment is suspected, new sterile gloves or equipment should be substituted for the contaminated material.

- It is advisable to have an assistant available during the procedure.
- Place sterile instruments on a sterile surface when not in use.

Wound Closure and Suturing

Basic Principles of Wound Closure

1. Minimize bacterial contamination
 - a. Aseptic technique must be maintained during wound closure. Suture material, wound clips, instruments, gloves, etc. must start out sterile and remain sterile during surgery.
2. Remove foreign bodies and damaged tissue
 - a. Foreign material (e.g., gauze, excess suture material, hair) must be removed from the incision if present. If left inside the body or under the skin, an inflammatory reaction and post-operative infection will occur.
3. Control bleeding
 - a. Use appropriate hemostasis techniques prior to closing an incision. Bleeding under the closed wound will lead to poor wound healing, breakdown of the incision and post-operative infection.
4. Handle tissue gently
 - a. Trauma will lead to inflammation, animal pain and discomfort, tissue death and increased chance of post-operative infection
5. Approximate, don't strangulate!
 - a. In general, when closing a wound, you are trying to bring two wound edges in contact long enough for the body to repair the incision. Tying sutures tightly or excessive pressure on wound clips will traumatize the tissue (see #4 above).

*(Taken from Boston University School of Medicine Suturing Basics,
<http://www.bumc.bu.edu/surgery/training/technical-training/suturing-basics/>)*

Incisions through multiple tissue layers must be closed separately. Poor wound closure technique will result in delayed healing and increased incidence of post-operative infection, as well as animal discomfort and pain. Incisions in tissues underneath the skin (e.g., muscle, subcutaneous tissue) are typically closed using absorbable suture material. Proper suture technique requires practice. Surgical knots must be tied using the correct method and sutures not tied too tightly. Tight sutures inflame the tissues and may cause the animal discomfort. Consult with a veterinarian for advice on wound closure and suture material selection.

Rodent skin incisions are typically closed with stainless steel wound clips. These clips must be sterile and applied aseptically. The clips must be observed daily post-operatively and can usually be removed in 7-10 days if the wound is healed. Wound clips must not be left in the skin beyond 14 days post-operatively.

Surgical Needles and Suture Material

Needle types:

- Taper Point – Round needle shaft. Used for soft, pliable tissue (e.g., muscle layers, abdominal organs)
- Cutting – Triangular needle shaft with a sharp edge. Used for tougher tissue (e.g., skin, tendons).

Suture Material:

Suture comes in a variety of sizes on a scale from 11-0 (smallest) to 7 (largest). Sizes are either a whole number or a specific number of zeros (#2>#1>0>2-0 (00)>3-0 (000)). Suture size reflects the diameter of the suture. With sizes less than #1, the smaller the diameter of the suture material, the greater the number of zeros. Most rodent surgeries are done using 4-0 (rat) or 5-0 (mouse) suture material, although certain procedures require smaller sizes (e.g., microsurgery). Packages of sterilized suture material with attached needles are the easiest and most efficient choice for surgery.

Package of suture material:

1. Suture size (in this example, 5-0)
2. Brand name and composition (Webcryl, Polyglactin 910)
3. Type and color of suture (braided, absorbable, undyed = white)
4. Length of suture (30 inches)
5. Size, shape and type of needle (19 mm length, 3/8 circle, FS-2 reverse cutting)



Suture material also has an expiration date; in this photo is it September 2020 (see lower center of photo). As with drugs and other medical materials, surgeons may not use expired suture material for survival surgery.

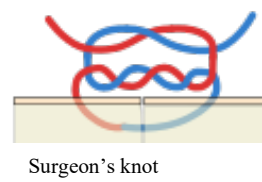
Suture material may be absorbable (broken down and removed by the body within a short period of time) or non-absorbable (remains within the body permanently). In general, absorbable suture is used under the skin or within the body and non-absorbable suture is used in the skin (sutures must be removed after the wound is healed). However, because rodents tend to chew out skin sutures, skin incisions in these species are usually closed using metal wound clips that are removed after the skin incision is healed.

Suture also comes in either monofilament or multifilament (braided) types. Monofilament suture is made of a single strand of material and can be compared to fishing line. Multifilament suture is composed of many strands braided around each other. In general, although multifilament suture material is easier to work with it is not used in skin incisions or in contaminated wounds due to the tendency to allow wicking of bacteria-contaminated liquid along the strands.

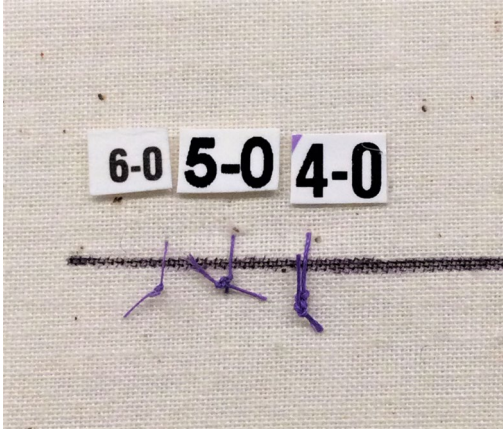
Always use the smallest diameter suture and needle required to hold the wound edges together. The larger the suture and needle size, the more damage caused while passing through tissue and the longer the wound will take to heal.

Suture Knots and Patterns

In rodent surgery most sutures are tied using an 'instrument square knot'. An instrument square knot consists of two simple knots reversed, one on top of the other. The knot is tied using a surgical needleholder. If there is tension on the wound edges or the suture material tends to slip open after tying the first knot, an extra loop can be added to the first simple knot for increased stability. This is called a 'surgeon's knot'. Correct technique in suture tying is important because incorrectly tied sutures often leave too much suture material in the knot which delays healing and increases the chance of post-operative infection. In addition, incorrectly tied sutures tend to slip and allow wound edges to open which may lead to life threatening complications.



Most rodent surgical incisions can be closed using a simple interrupted suture pattern. Each suture is placed and tied separately. This helps prevent failure of the entire suture line should one suture break or slip open.



The photo above shows the *differences in instrument square knot size using 6-0, 5-0 and 4-0 polyglactin 910 (absorbable, multifilament) suture material*. Each knot was tied using four throws. Knot size increases significantly as suture size increases. Suture material is a foreign body and causes an immune response in the animal. The more suture material that is present, the greater the response as the immune system tries to eliminate it.

Standard sutures are tied using four throws. Inexperienced surgeons may try to reduce knot size by using fewer throws, which reduces knot security. A study by van Rijessel EJ, et al. showed that *increasing the number of throws does not significantly increase knot size or tissue reaction*. Instead, the main determinant of knot size and tissue reaction is type of suture material and suture size. Therefore, use the smallest suture size that adequately holds the tissue edges together to reduce the amount of suture material present in the wound. Recommended suture sizes for muscle and skin closure: Mice = 5-0 or 6-0; Rat = 4-0 or 5-0. When suturing internal organs, vessels or performing microsurgery procedures, recommended suture sizes are often smaller than this.

Reference: van Rijssel EJ, Brand R, Admiraal C, Smit I, Trimbos JB. Tissue reaction and surgical knots: the effect of suture size, knot configuration, and knot volume. Obstetrics and Gynecology. 1989 Jul;74(1):64-68.

Ligation

Ligation is the use of suture or other wound closure material to tie off a blood vessel (or other hollow anatomical structure, e.g., vas deferens) during surgery. With blood vessels, a hemostat may be clamped across the vessel to crush the tissue (and start the coagulation cascade) followed by ligation. The vessel is then cut with a scissors distal to the ligation (and hemostat if used). In some cases, additional hemostatic clamps are placed across the vessel prior to ligation to ensure that the severed ends of the vessel remain secured (to check for bleeding after the vessel is cut). Ligation is used in rodents during surgical procedures such as carotid artery and jugular vein catheterization and removal of abdominal organs such as the spleen.



Wound Clips

Most rodent skin incisions are closed using stainless steel wound clips. Wound clips and applicators must be sterilized prior to use and must be applied using aseptic technique. Various sizes and styles of wound clips and applicators are available. Correct technique must be used to ensure that the wound clip does not fall out. Wound clips must be removed after the wound is healed and before the hair regrows around the incision site. Generally, this will be 7-14 days after surgery.

Post-Operative Care

Immediate Post-Operative Care - During the immediate post operative period animals *must* be observed until they are awake and able to maintain sternal recumbency (see photo).

Extended Post operative Care - The animals and the surgical wound should be observed and evaluated at least daily until the wound is healed and wound clips or sutures have been removed. Wound clips are generally removed at 7-14 days post-operatively. You must document your daily observations in the surgical record. Abnormal findings and treatments must be recorded.



Mouse in sternal recumbency

Unexpected Complications

Unexpected complications occasionally occur in association with surgery or post-operative recovery. Veterinary advice is frequently helpful. A veterinarian can be reached 24 hours a day by calling the Animal Resource Program Office at 865-1495 (This phone number is posted in all animal facilities). If calling after hours, a recorded message will provide veterinary contact information.

Common Problems

- **Hypothermia** – Animals that have become cold during the surgical procedure will recover very slowly and will often die. It is extremely important to keep animals warm during and after the procedure.
- **Prolonged recovery** – Hypothermia, hypoglycemia, dehydration, and anesthetic overdose may all contribute to prolonged recovery.
- **Anesthetic overdose** – Repeated injections of anesthetics during lengthy procedures may lead to prolonged recovery and occasionally death. The veterinarian may be able to suggest modifications to the anesthetic regime for prolonged procedures.
- **Swollen inflamed surgical wound** – may be caused by rough tissue handling, poorly placed sutures or infection.
- **Abnormal Behavior** – The animals may be in pain or distress. You should be familiar with the signs a rodent may display if it is in pain.

Recognizing Pain in Rodents

A rodent experiencing mild to moderate pain may display only subtle behavioral signs associated with its discomfort. Moderate to severe pain in rodents leads to more obvious changes in normal physiology and behavior. Accurate recognition of these changes requires that research personnel first have some knowledge of normal behavior and physiology for the species they are using.

Signs Associated with Moderate to Severe Pain

- Decreased activity or a reluctance to move
- Abnormal gait or posture
- Rough, greasy-looking coat
- Dark, red material around the eyes and nose in rats
- Decreased appetite
- Excessive licking or chewing of a body part or area
- Aggressiveness when handled
- Eating of bedding material
- Teeth grinding

Signs of Pain Following Abdominal Surgery

- Stretching and back arching

- Abdominal pressing onto the cage floor
- Frequent, sudden short movements

Non-Survival Surgery

In non-survival surgery the animal is *not allowed to regain consciousness* (i.e., euthanized prior to anesthetic recovery). Strict aseptic technique is often not necessary for non-survival procedures. However, at a minimum, the surgical site should be clipped and scrubbed, the surgeon should wear gloves and instruments should be clean. The depth of anesthesia must be monitored throughout the procedure and adjusted as necessary.

IACUC Oversight

The IACUC will review your surgical records every 6 months. The following points will be reviewed at the time of inspection:

- Are the records legible?
- Is the person listed as the surgeon authorized to conduct the procedure?
- Is there a record of analgesic administration?
- Is there a description of the procedure available?
- Is there a record of daily post operative evaluation?

Regulations and Guidelines Regarding Surgery in Research Animals

The Animal Welfare Act, Public Health Service Policy (using the Guide for the Care and Use of Laboratory Animals, 8th ed.) and the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training* (IRAC 1985) govern the use of surgical procedures in research animals in the United States. The following information is compiled from all three sources.

Non-survival surgery: The animal is euthanized following the surgical procedure and *before* it recovers from general anesthesia.

Survival surgery: The animal is allowed to recover from general anesthesia.

Major surgery: A surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. This wording is taken from the *Guide (Guide for the Care and Use of Laboratory Animals, 8th ed.)*. The U.S. Animal Welfare Act has a similar definition for what that document calls a ‘major operative procedure’.

Minor surgery: Procedures that do not expose a body cavity and create little or no physical impairment. The *Guide* includes the following procedures within the category of a minor surgery:

- Superficial wound suturing
- Peripheral vessel cannulation
- Percutaneous biopsy
- Routine agricultural procedures, such as castration
- Most practices routinely done on an “outpatient” basis in veterinary clinical practice

According to the *Guide*, the following factors must be considered when categorizing a particular surgical procedure:

- The potential for pain and other postoperative complications
- The nature of the procedure
- The size and location of the incision(s)

- The duration of the procedure
- The species, health status and age of the animal

Multiple Survival Surgeries

Multiple major survival surgeries on a single animal are acceptable *only* if they meet the following criteria:

- The procedures are included in and essential components of a single research protocol
- The procedures are scientifically justified
- or
- The procedures are necessary for clinical reasons

In addition, the use (in a single animal) of multiple *minor* surgical procedures that cause significant postprocedural pain must be scientifically justified by the investigator.

Training

Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is used. This includes training in the following:

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

Presurgical planning should include input from a veterinarian and specify the requirements for postsurgical monitoring, care and recordkeeping, including the personnel who will perform these duties.

Asepsis

Aseptic (sterile) technique must be used for all survival surgeries. Surgery should also be conducted in “dedicated facilities or spaces” (*Guide*, 8th ed.). Dedicated refers to a specific room or space within a room used *only* for the purpose of surgery during the time the procedure is occurring.

However, for species covered by the Animal Welfare Act, *major surgeries on non-rodents* must be conducted in “dedicated facilities” intended for that purpose. This means a specific room is used only for surgeries. Separate rooms must be used for animal preparation and recovery.

Regardless of the animal species, aseptic technique includes:

- Preparation of the patient, such as hair or feather removal and disinfection of the operative site
- Preparation of the surgeon, such as the provision of appropriate surgical attire, face masks and sterile surgical gloves
- Sterilization of instruments, supplies and implanted materials
- The use of operative techniques to reduce the likelihood of infection

Intraoperative Monitoring

Animals must be monitored during anesthesia and surgery and appropriate records should be maintained.

Monitoring includes evaluation of anesthetic depth and physiologic functions such as:

- Body temperature (Maintenance of normal body temperature is particularly important in [anesthetized] small animals where the high ratio of surface area to body weight quickly leads to hypothermia.)
- Cardiac and respiratory rates and pattern
- Blood pressure
- Hydration

Postoperative Care

Postoperative monitoring and treatment of animals should be documented in a medical record. Animals should be placed in a clean, dry and comfortable area where trained personnel can observe them continuously until they recover from anesthesia. Usually, after the animal is able maintain itself in an upright position on its chest (sternal recumbency), it may be returned to its home cage in the animal room.

After recovery the animal is returned to its home cage continued monitoring is required, until either the surgical wound is healed or the animal is euthanized. Monitoring intensity will vary with the situation, but the animal must be observed a minimum of once a day.

Pain and Distress

Animals may experience significant pain because of surgical procedures. Pain is stressful and, if not relieved, may lead to unacceptable levels of stress and distress (*Guide*, 8th ed.). Investigators have a responsibility to prevent or alleviate pain in their research animals.

The expression of pain varies greatly between animals and humans, different species of animals and individuals within a species. It is usually difficult for humans to understand and recognize the significance of pain, especially if mild to moderate, in animals. Because of this, we use the guiding principle that if a procedure causes pain in humans, it is likely to cause pain in animals (*U.S. Government Principles*, 1985). Investigators are required to use appropriate anesthesia and analgesia when performing procedures expected to cause more than momentary pain. When monitoring animals during and after painful procedures, personnel must have the training and ability to recognize signs of pain/distress in the species they are using.

Research Surgery Resources at Penn State

Veterinary Staff

Animal Resource Program veterinarians have experience with rodent surgery and are available for consultation. The veterinary staff can provide advice and/or training regarding such topics as:

- Anesthesia and patient monitoring
- Relevant surgical anatomy
- Post-operative care
- Principles of aseptic surgery
- Suturing techniques

Web Based Information

- Research Animal Training videos on aseptic technique in rodent surgery (<https://researchanimaltraining.com/article-categories/aseptic-technique/>)
- Animal Resource Program Website <https://www.research.psu.edu/animalresourceprogram/surgery>

Surgery Training Seminar

Surgery training is available online

(<https://www.research.psu.edu/animalresourceprogram/surgery#surgeryclass>) and in-person from the Animal Resource Program veterinary staff. Topics include:

- Anesthesia, analgesia and patient monitoring
- Aseptic technique
- Tissue handling
- Wound closure
- Specific surgical procedures

The emphasis is on fundamental surgical principles and encouraging good techniques for experimental surgery. Participation in the Surgery Training Seminar is voluntary but strongly recommended for those with little or no surgical experience.

Recommendations for Learning a New Surgical Procedure

- Familiarize yourself with the relevant anatomy of the species you will be using.
- Observe someone who is experienced in performing the procedure.
- Develop your technique first using models or cadaver animals.
- Proceed to practicing with anesthetized animals that will be euthanized before recovery from anesthesia.
- Proceed to survival procedures *only after becoming proficient*.
- It is not recommended that you try to obtain experimental data from practice or training animals.

Summary

Requirements for Conducting Rodent Surgery

Research investigators must adhere to the following points when conducting survival surgery on animals at Penn State:

- Consult with an ARP veterinarian during planning and IACUC protocol preparation.
- Use aseptic technique for all survival surgery
- Keep clear written records of the surgery and post operative care
- Monitor the animals until they have recovered from anesthesia and evaluate their condition daily until the surgical wound is healed
- Provide analgesic medication to relieve post procedural pain
- Personnel performing surgery must complete all required training and be competent to perform the procedure(s) listed in their IACUC protocol.

Training Requirements

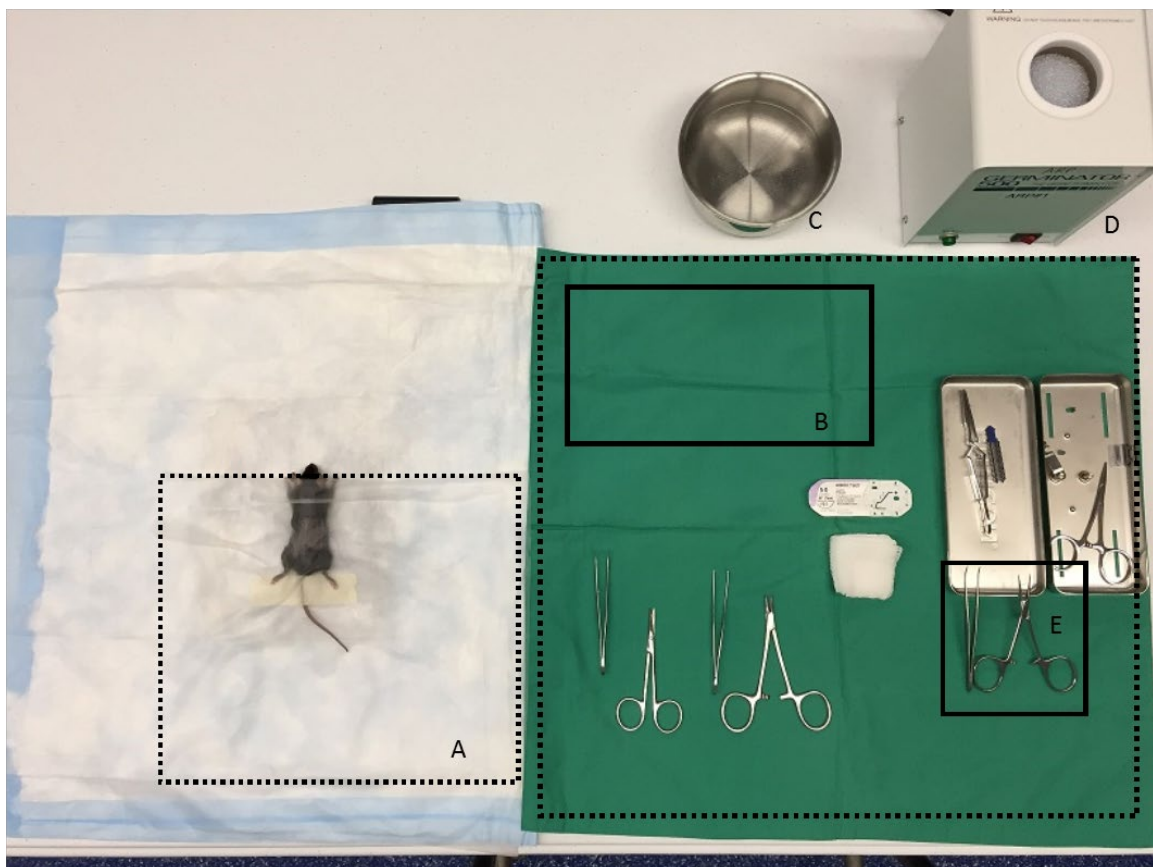
Personnel must complete the following training requirements prior to performing rodent surgical procedures at Penn State:

- Read the IACUC [Rodent Surgery Guidelines](#).
- Complete the online Research Animal Training Aseptic Technique in Rodent Surgery training videos (<https://researchanimaltraining.com/article-categories/aseptic-technique/>). Please note that although PSU does not require rodent surgeons to wear sterile gowns or use sterile towels to dry their hands after washing in preparation for surgery, these materials should be clean and unused.
- Complete the ARP Rodent Surgery Training [online tutorial](#) with associated quiz.
- Obtain additional specific procedural training from ARP or other qualified and experienced personnel as needed, including but not limited to the following:
 - General Aseptic Technique and Surgery training class on the ARP website (highly recommended)
 - Sharps Safety When Working with Research Animals
 - Rodent Surgery Training manual
 - Links to these resources can be found on the ARP website (under IACUC Rodent Surgery Training requirements):
<https://www.research.psu.edu/animalresourceprogram/surgery>

Appendix A: Maintaining Asepsis During Surgery

Surgeons and support staff must work together to maintain sterility during the surgical procedure. Sterile articles must touch only sterile surfaces and all sterile surfaces should be kept as dry as possible. If contamination of surgical gloves or equipment is suspected, new sterile gloves or equipment should be substituted for the contaminated material.

- It is highly recommended that a non-sterile assistant be available during the procedure.
- Place sterile instruments on a sterile surface when not in use.
- Do not touch non-sterile surfaces when wearing sterile gloves



In the photo above dotted black outlines the sterile areas.

- A. Sterile clear drape. *Note that in the case of a mouse anesthetized with injectable anesthetic (versus gas anesthetic) the nose must be left uncovered when using plastic drape material to allow the animal to breathe.*
- B. Area used for instruments prior to cleaning for batch surgeries.
- C. Sterile water in sterile container for cleaning instruments prior to placing in glass bead sterilizer.
- D. Glass (hot) bead sterilizer.
- E. Instrument tips cooling after removal from bead sterilizer.

Date _____ IACUC

Surgeon _____
Surgical _____
Procedure _____

Appendix B: Survival Rodent Surgery Checklist

Surgery Preparation ✓

Is the designated surgical surface/apparatus disinfected before surgery?	
Are instruments, suture and other material (cotton swabs, implants, etc.) that will enter the surgical incision site sterile?	
If items outside the sterile field must be touched with sterile gloves (such as knobs and dials of stereotaxic apparatus, microscope, anesthesia machine, etc.), are they covered with Press'n Seal, autoclaved aluminum foil or other sterile item, or are sterile gauze pads available to cover fingers when touching non-sterile surfaces?	
Is a glass bead sterilizer ready to re-sterilize instrument tips between animals?	
Is an external heat source ready to keep the anesthetized animal warm?	
Are the anesthetic drugs and/or isoflurane vaporizer prepared and set up for use?	
Is the oxygen tank turned on?	

Animal Preparation ✓

Has ophthalmic ointment been applied to the eyes of anesthetized animals?	
Does hair removal from the incision site take place in a location other than where surgery will be performed (separation of prep area from surgical area)?	
Is the skin over the incision site scrubbed 3x with germicidal soap followed by 70% alcohol rinse?	
Are warmed fluids administered prior to surgery? Recommended dose: Adult mouse = 0.5-1.0 ml Adult rat = 5-10 ml	
Is a vigorous rear toe pinch (with no response) done before starting incision?	

Surgical Procedure ✓

Is the surgeon wearing a long-sleeved gown or clean lab coat, face mask, head cover and sterile gloves?	
Has the surgeon applied hand sanitizer to the entire hand surface (allow to air dry) prior to putting on surgical gloves?	
Is a vigorous rear toe pinch (with no response) done before starting incision?	
Are surgical instruments cleaned and re-sterilized using a glass bead sterilizer between animals?	
Is anesthetic depth monitored via toe pinch at least every 5 minutes during surgery?	
Is a new sterile set of instruments used after every 4-5 animals?	

Post-Operative Care ✓

Are analgesics and other supportive care administered as per IACUC protocol?	
Is an external heat source provided during recovery?	
Are animals continuously monitored until they regain a righting reflex?	
Are animals checked at least once a day until the wound is healed?	
Are wound clips removed 7-14 days post-operatively?	
Are surgical records filled out with daily post-operative checks and drug administration recorded as completed?	
Are surgical and post-operative records easily accessible to veterinary staff and IACUC members?	

Notes: