

## Video Article

# Principles of Rodent Surgery for the New Surgeon

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## Abstract

For both scientific and animal welfare reasons, training in basic surgical concepts and techniques should be undertaken before ever seeking to perform surgery on a rodent. Students, post-doctoral scholars, and others interested in performing surgery on rodents as part of a research protocol may not have had formal surgical training as part of their required coursework. Surgery itself is a technical skill, and one that will improve with practice. The principles of aseptic technique, however, often remain unexplained or untaught. For most new surgeons, this vital information is presented in piecemeal fashion or learned on the job, neither of which is ideal. It may also make learning how to perform a particular surgery difficult, as the new surgeon is learning both a surgical technique and the principles of asepsis at the same time. This article summarizes and makes recommendations for basic surgical skills and techniques necessary for successful rodent surgery. This article is designed to supplement hands-on training by the user's institution.

## Video Link

The video component of this article can be found at <http://www.jove.com/video/2586/>

## Protocol

### 1. Before Performing Surgery, Be Aware That There Are Legal and Ethical Requirements Around the Use of Animals in Research and Teaching

1. All surgical procedures must be approved by the local ethical or legal authority ( IACUC or Ethics committee or Home Office, etc.).
2. Make sure this approval is in place before beginning.
3. If there is any question of approval for surgery or approval for the use of animals, do not proceed.

### 2. Understanding Aseptic Technique

1. Sterility as a concept
  1. The goal of aseptic technique is to reduce or eliminate where possible the normal bacterial burden present on the animal and in the environment before beginning surgery. This means that instruments are sterilized, the surgical working surface is disinfected, and the animal and the surgeon's hands are disinfected.
  2. Surgery, performed properly, does not need prophylactic antibiotics or any other treatments, other than pain relief.
  3. Even with excellent aseptic technique, bacteria originating from the surgical subject may still be introduced into a surgically-created wound. Proper aseptic technique limits the risk of introduction of infectious agents, primarily bacteria, into surgical sites.
2. Sterile field
  1. The sterile field encompasses the surgical area, the area prepared on the animal, and the front of the surgeon.
3. Breaks in sterility
  1. Breaks in sterility occur when the surgeon touches something outside the sterile field. This may be her face, a light fixture, an unprepared area of the animal, or a non-sterile instrument.
  2. This is the reason prophylactic antibiotics may be used.
  3. Remember, a special activity is taking place. Be calm, thoughtful, and precise.

### 3. Preparing for Surgery

1. Rodent surgery does not require a dedicated surgical suite. Ideally, surgeries should be conducted in a separate area within a lab, such as a hood or laminar flow cabinet. Regardless, the area chosen should be tidy, well-lit, and out of traffic flow. Rodent surgeries are often conducted on a laboratory benchtop. Before arranging the surgical area, clean the benchtop well with a disinfectant.
2. It is helpful for the surgeon to have an assistant. This assistant can help with anesthesia, preparing animals, and monitoring recovery. This allows the surgeon to prepare themselves while the animals are prepared, thus shortening the animal's time under anesthesia, and perhaps allowing for more surgeries to be performed.

3. Arrange the surgical area so that there are three distinct zones: preparation, surgery, and recovery.
4. For the preparation area, assemble the supplies needed:
  1. Lubricating ophthalmic ointment
  2. Clippers or razor
  3. Gauze
  4. Surgical scrub solutions
    1. Surgical scrub solutions commonly used are iodophors or chlorhexidine/
    2. 70% isopropyl alcohol is also used as part of the surgical scrub.
5. For the surgical area, assemble and arrange the supplies needed.
  1. Heat source for keeping the rodent warm
    1. The ideal heat source is a recirculating water heating pad. Electrical heating pads are not acceptable for surgery. Surgical gloves filled with warm water, or chemical hand warmers may also be used. If either of the latter two are used, the temperature of the object must be carefully monitored during surgery.
  2. Surgical platform
    1. This may be a sterile waterproof pad placed over the heat source.
  3. Disposable supplies
    1. These may include: sterile surgical drapes, gauze, cotton applicators, fluids for administration to the rodent, tissue irrigation solution.
  4. Sterile instruments
    1. The complement of instruments will vary depending on the surgery, but generally include scalpels, tissue retractors, forceps, hemostats, and scissors.
    2. Instrument sterilizer or sterilizing solution if the same instruments are to be used on more than one surgical subject
      1. The use of a hot bead sterilizer to sterilize instruments between animals is common.
  5. Material to close the surgical incision.
    1. The materials used will depend on the surgical site and application but may include: Suture and needles, staples and a staple applicator, or tissue glue
  6. Any other special equipment needed (stereotaxic apparatus, implants, operating microscope)

#### 4. Preparing the Instruments for Surgery

1. Instruments should be clean and sterile at the start of surgery. This is often accomplished by creating complete surgical packs that are autoclaved.
2. Instruments may be used for more than one rodent surgery without autoclaving and repacking, but should be cleaned and sterilized between rodents.
  1. If a hot bead sterilizer is used, be sure to allow time for the instruments to cool down after sterilization. Instruments may be cooled by dipping in sterile water.
  2. Chemical sterilants may also be used, but require a longer contact time (~10 minutes-could be 30-60 minutes, require rinsing). Alcohol is not a sterilant.
3. Arrange instruments on sterile waterproof drape in order in which they will be used.

#### 5. Preparing the Animal for Surgery

1. The three things that are administered before surgery are anesthesia, analgesia, and antibiotics.
  1. Anesthesia is necessary for surgery. Anesthetics may be administered via inhalation or injection.
    1. Most common inhalant anesthetic in use is currently isoflurane.
    2. Most common injectable anesthetic used is a combination of ketamine and xylazine, possibly with another sedative added.
    3. Consult your facility veterinary staff for appropriate dosages.
  2. Pre-emptive analgesics are recommended and should be administered before surgery begins.
    1. Commonly used non-steroidal anti-inflammatories include flunixin and carprofen.
    2. Available opioids may include buprenorphine, morphine, or fentanyl.
    3. Consult your facility veterinary staff for appropriate dosages and routes of administration.
  3. With properly conducted surgery, antibiotics are not generally needed, except for some high-risk gastrointestinal surgeries.
    1. If antibiotics are used, used provide peri-operatively (before first incision).
    2. Consult your facility veterinary staff for appropriate antibiotics, dosages, and routes of administration.
2. Surgical preparation of the incision site
  1. Shave the incision site. Generally shave an area approximately 3 times the size of the proposed incision. The size of the shaved and prepared area may be limited by the size of the animal or area on the animal.
  2. The shaved area is disinfected using multiple applications of surgical scrub solution and isopropyl alcohol. Application is typically surgical scrub first, then alcohol. End with a final application of scrub solution.
  3. Surgical scrubbing is performed in a circular pattern, beginning in the center and spiraling outward, or on smaller animals, a unidirectional fashion.
    1. If scrubbing unidirectionally, place sterile gauze at the cranial margin of the shaved area, covering the hair of the animal. Grasp and stabilize the animal gently through the sterile gauze. Apply scrub solution and alcohol unidirectionally away from the sterile gauze.

3. Moving the animal to the surgical area
  1. Move the animal with the prepared surgical site upward, and without contacting the prepared site.
  2. Position the animal on the surgical area so the surgical site is upward, facing the surgeon.
4. Draping the animal
  1. If the animal is draped by the assistant, the assistant must remember that the exterior surface of the drape is considered part of the sterile field and must not be handled by the assistant. Draping is often left to the surgeon, and drape is included in the surgical pack.
  2. A standard waterproof disposable paper drape is generally recommended.

## 6. Preparing the Surgeon for Surgery

1. Preparation of the surgeon should emphasize that a special activity will be taking place, one in which the surgeon must be cognizant of their every move in and out of the sterile field.
2. The hair and face are generally covered by a cap and mask, respectively. These should be new at the start of the surgical session, but do not need to be changed between animals.
3. Surgeons should wear a disposable surgical gown, clean disposable lab coat or at the least a clean lab coat. This does not need to be changed between animals, but should be changed between surgery sessions.
4. Hands should be cleaned well before surgery. Surgical gloves or exam gloves should be used. If exam gloves are used, they should be washed with a surgical disinfectant before being used. Gloves should be changed between animals.

## 7. During the Surgery

1. Maintenance of the sterile field.
  1. Once your hands are sterile and the operating field is sterile, you will have to move carefully and remember not to break sterility. This includes having a sterile field on which you may set down instruments, remembering not to scratch your nose, and only handing the surgical patient through the sterile drape.
2. Tissue handling
  1. Handle tissues gently. Keep tissues moist, especially if surgery is prolonged. Minimize the creation of tissue pockets and unnecessary dissection.
  2. Proper tissue handling reduces opportunity and risk of post-op infections, speeds healing, and reduces post-operative pain.
3. Replace fluids lost
  1. The usual routes of fluid administration in rodents are subcutaneous or intraperitoneal. Use small volumes for small animals-1-2 ml intraperitoneally for mice, for example.
  2. Warmed fluids may help prevent a drop in body temperature. Mouse and rat body temperature is higher than humans, so fluids must be warmer.
4. Maintain warmth
  1. Placement of a heating pad below the animal during surgery and the cage during recovery is a standard way to maintain an animal's body temperature.
    1. Careful with heating pads-use only those marked for surgery. Others may induce thermal injury.
  2. Warmed fluids, given intraperitoneally, may also help warm animals.
5. Operating microscope-may facilitate some procedures (microvascular surgery, older surgeons, etc. Can also be achieved by use of magnifiers like loupes, etc.)

## 8. Post-operative Recovery Considerations

1. Animals should recover on a flat paper bedding (sterile paper towels, etc.) rather than standard animal husbandry bedding.
2. Keep the animal warm. Warmth will aid in speedy recovery. This is often accomplished by placing the recovery cage half-on a heating pad so that animals may choose their preferred temperature as they recover.
3. Do not return animals or cages to the animal holding area until all animals appear normal. Any animal that has had surgery must have regained the ability to right itself in the cage and be able to move about normally before being returned to the holding area.
4. Monitor animals post-operatively for unexpected signs of illness or infection. Animals will generally lose a small amount of weight after surgery, but with proper analgesia and the provision of food, they should regain the weight quickly.

## 9. Post-operative Monitoring is the Final Part of Successful Surgery

1. For five to seven days after surgery, monitor the general condition of the animal.
  1. Post-operatively, animals should be bright, alert, and active. They should be interacting normally with cagemates, eating and drinking, and able to achieve normal species-specific postures.
  2. Animals that are depressed, anorectic, or sluggish should be examined as to the cause of this change. Consider the possibility of untreated pain or infection.
2. Food/fluid intake is also important to recovery.
  1. There will be some drop-off in consumption after surgery. This may be mitigated by making food and water easier to access.
    1. Longer sipper tubes might be useful.
    2. Daily provision of wetted food in the bottom of the cage may also entice animals to eat.
  2. Some analgesics in rats result in pica, so be aware that animals may eat inappropriate items (such as bedding) to injury.
3. Monitor animals for signs of infection at the incision. The danger period is the first 7 days and animals should be checked daily.
  1. Signs of infection include redness, swelling, discharge (purulent or serous), pain, or the opening of the incision (dehiscence).

## 10. After Your Surgery is Finished, the Final Step is Gauging Success of Your Surgery

1. Was the model induced/part removed/functionality restored?
2. Was there as little pain as possible?
3. Was there no infection?
4. Success!

### Discussion

Experimental surgery on rodents is rarely carried out by veterinarians or physicians. Instead, it is often performed by biomedical researchers with little or no formal training in the principles of surgery and aseptic technique. In a research setting, surgery is typically learned from colleagues, themselves usually not formally trained either. Surgery performed in the research laboratory may also be viewed primarily as a method of preparing an animal model with the goal of generating a set of animals for a study. While any particular surgery is usually a matter of hand-eye coordination and technical skill, both of which can be acquired via practice, principles and skills associated with the concept of aseptic technique (and thus surgery without infection) cannot be acquired solely via practice and are often not fully conveyed to researchers.

The absence of formal training in surgical principles can lead to a lack of understanding of the equal significance of both technically correct surgery and pre- and post-operative care of animals. Consideration of both the surgery itself and the peri-operative care of animals will result in an increase in animal welfare and the quality of data obtained from the surgical model. For example, subclinical post-surgical infection can confound results, as can changes in behavior and physiology seen with pain. As prey species, most rodents are surprisingly stoic and although suffering from post-surgical pain or infection may show few clinical signs. The subtle signs of pain or distress in rodents are further obscured by a general lack of training of biomedical researchers in recognition of such signs in rodents. Additionally, rodents are most active during the dark cycle, when staff are generally not available to view clinical and behavioral signs of pain following surgery. This video does not substitute for proper surgical training, which would ideally be both hands-on and provided by their institution, but is designed to give researchers an overview of common concerns and visual demonstration of accepted aseptic procedures and techniques required to develop a minimal competency in performance of survival surgery in rodents.

### Disclosures

All authors work for Charles River, a major supplier of surgically-altered animals for research.

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