GUIDELINE ON RODENT SURVIVAL SURGERY

Surgical Facilities

For most rodent surgery, a facility may be compact and simple, such as a dedicated space within a laboratory that is appropriately managed to minimize contamination from other activities in the room during surgery. In general, a rodent surgery facility should have the following components:

- A place for cages of rodents awaiting surgery.
- An animal preparation area, away from the surgery area, for hair removal and initial skin preparation.
- A surgery area that is uncluttered and easily sanitized.
- A holding and recovery area in a quiet location where the animals can be continuously observed until fully recovered from anesthesia.
- Be limited to access only for people involved in performing the procedure.

Aseptic Procedures

The basic concept of aseptic surgery is to ensure that anything that comes in contact with the surgical site is sterile. The procedures outlined here are to ensure that sterility is achieved when beginning the procedure and maintained throughout the procedure.

Instruments

- Instruments, supplies and devices must be sterilized (includes: catheters, osmotic pumps, telemetry transmitters, trocars, etc). See Table 1.
- Often rodent surgeries are done on multiple animals in a single session; instruments must be sterilized between animals. Two sets of sterile instruments facilitate re-sterilizing instruments between animals.
- Segregation of instruments according to function helps maintain aseptic technique (i.e., instruments used on skin should not be used within the abdominal cavity).
- Instruments should be wiped clean of blood and tissues with sterile gauze and then sterilized either chemically or mechanically. See Table 1.
- Do not use toothed or crushing instruments if it is not necessary.
- Close surgical wounds using appropriate techniques and materials (see Table 2)

Table 1

<table>
<thead>
<tr>
<th>RECOMMENDED INSTRUMENT STERILANTS</th>
<th>(always follow manufacturers’ instructions)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agents</strong></td>
<td><strong>Examples</strong></td>
</tr>
<tr>
<td>Physical: Steam Sterilization</td>
<td>Autoclave</td>
</tr>
<tr>
<td>(moist heat)</td>
<td></td>
</tr>
<tr>
<td>Dry Heat</td>
<td>Hot Bead sterilizer</td>
</tr>
<tr>
<td></td>
<td>Dry Chamber</td>
</tr>
</tbody>
</table>
Ionizing Radiation | Gamma radiation | Requires special equipment.
---|---|---
Chemical (Gas sterilization) | Ethylene Oxide | Requires 30% or greater relative humidity for effectiveness against spores. Requires safe airing time.
Chlorine | Chlorine Dioxide | Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes | Cidex ® | Requires many hours of contact time for sterilization. Corrosive and irritating. Must rinse equipment with sterile water before use.

**Table 2**

| **SUTURE* SELECTION** |
|---|---|
| **Suture** | **Properties** |
| Vicryl ®(polyglactin 910)  
Dexon® (polyglycolic acid) | Absorbable; 60-90 days. |
| PDS® (polydioxanone),  
Maxon® (polyglyconate) | Absorbable; 6 months. |
| Prolene® (polypropylene) | Nonabsorbable, Inert |
| Ethilon® (nylon) | Nonabsorbable, Inert |
| Silk | Nonabsorbable. (Caution: Tissue reactive and may wick microorganisms into the wound). |
| Chromic Gut | Absorbable. Versatile material. |
| Stainless Steel Wound Clips, Staples | Nonabsorbable. Requires special instrument to remove. |
| Surgical Adhesive, Vet Bond | Area needs to be dry and free of blood. |

* Suture material or staples used for skin closure must be removed by 7-10 days post-surgery.

**Preparation of Surgical Site**

- Prepare animal in a location separate (bench or room) from the surgical area
- Remove hair using electric clippers with a #40 blade. Avoid skin abrasions and thermal injuries. (Brand names of electric clippers are Oster, Andis, etc.) Dispose of removed hair (e.g., vacuum) to avoid hair from becoming airborne and potentially contaminating the surgical site.
- Sterile gauze / Q-tips soaked with skin disinfectant can be used for scrubbing. See Table 3.
- Do not wet large areas of animal, particularly with ethanol or alcohol based scrub; it will exacerbate hypothermia.
  - An alternative to ethanol is to use sterile water or saline solution, at room temp or slightly warmer; this will reduce the risk of hypothermia that ethanol may cause as it evaporates from rodent skin.
- Begin along the incision line with disinfectant soaked Q-tip or gauze and extend outward in a circular pattern. Never from outward (dirty) towards the center (clean). Do not go over the incision site twice with the same gauze/Q-tip.
Table 3

<table>
<thead>
<tr>
<th>NAME</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodophors</td>
<td>Betadine®</td>
<td>Inactivates a wide range of microbes but their activity is reduced in the presence of organic matter.</td>
</tr>
<tr>
<td></td>
<td>Prepodyne®</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wescodyne®</td>
<td></td>
</tr>
<tr>
<td>Cholorhexidine</td>
<td>Hibiclens®</td>
<td>Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.</td>
</tr>
<tr>
<td></td>
<td>Nolvasan®</td>
<td></td>
</tr>
</tbody>
</table>

**SKIN DISINFECTANTS**

An iodophor scrub can be alternated 3 times with room temp sterile saline, followed by a final prep, with an iodophor solution. Chlorhexidine Scrub can be used without an alternate scrub. Scrub 3 times and leave on skin for at least 2 minutes. Remove excess liquid with dry sterile gauze. Please note: alcohols are not endorsed for rodent surgical preparations; they are not adequate for skin disinfection, they are not a sterilant, and they can cause hypothermia due to evaporation from skin; sterile saline/water can be used as an alternative.

**Surgeon Preparation**

The purpose of the surgeon's scrub is to remove dirt, and as much as is possible, decrease the bacterial flora on the surgeon's hands and arms. Some of the agents used during a surgeon's scrub have residual activity which helps decrease bacterial levels under surgical gloves.

Depending on the availability of assistants, the surgeon often opens a sterile towel pack, gown and gloves before scrubbing.

- Put on surgical mask, clean lab coat or scrubs. Surgical cap is recommended.
- Scrub hands with disinfectant scrub
- Put on sterile surgical gloves (these are distinct from laboratory exam gloves out of the box)

**Gloving Procedures**

- Remove outer paper package from gloves,
- Unfold inner paper covering on the gloves; careful not to touch gloves with non-sterile objects (e.g. your fingers)
- Fold the bottom fold (near the cuffs) – under, to prevent it from refolding
- Touch only the folded cuff while putting gloves on
- Do not touch any non-sterile surfaces. If the gloves touch non-sterile surfaces - re-glove.

**Draping**

- Drape the surgical site using sterile material such as towels, stockinet, drapes, gauze, or plastic drape.
- Do not drape the animal such that it is entirely covered; the use of see through draping over the surgical site (e.g. Tegaderm from 3M) provides an appropriate view of the respirations and any potential complications that a full toweled drape will not.

**Maintaining Sterility**

- The surgeon should restrict his/her contact with the surgical site, using only sterilized equipment until the incision is closed.
- The surgeon should not touch anything that is not sterile once gloves are put on.
- ALWAYS put sterile equipment on a sterile surface (sterile surgical tray, sterile towel or drape, or sterile gauze)
when not in use.

- Do not let equipment (instruments, catheters, implants, etc.) become contaminated.
- The tips of sterilized instruments should be used to manipulate and handle tissues. Minimize exteriorizing organs, but if necessary, place on sterile gauze and keep moist with sterile saline.
- Sterile gloves must be changed before touching the animal again, if they come in contact with a non-sterile surface.
- If instruments are being used on multiple animals, a hot-bead sterilizer may be considered for use to sterilize instruments between animals.

**Anesthesia**

- Do not fast rodents unless specifically mandated by the protocol. (The high metabolic rate depletes reserves quickly.)
- Analgesia should be given pre-operatively; see Analgesia section below.
- Following induction of anesthesia, apply ophthalmic ointment to the eyes to prevent corneal drying.
- The animal must be maintained at a surgical plane of anesthesia throughout the procedure. Vital signs of the animal must be monitored throughout the procedure (such as: respiratory rate, skin color, not responsive to toe pinch).
- Anesthetic technique must be on approved Animal Use Form (AUF)
- Gas anesthetics: appropriate scavenging must be used to avoid personnel exposure (Not all hoods provide scavenging; therefore, contact EHS for assistance evaluating your scavenging system)

**Antibiotics**

Prophylactic antibiotics (given immediately prior to surgery in order to maintain a therapeutic blood level during surgery and continued for up to 12 hours post-operatively) decrease wound infection and may be considered for use.

**Analgesia**

Adherence to federal policies and regulations, as well as Russell and Burch’s 3Rs of Reduce, Replace and Refine, necessitate the use of drugs to alleviate or ameliorate pain and distress associated with research procedures. The basic assumption of the US Government Principles (1985) is that if a procedure would be likely to cause pain or distress in a human, then it is likely to also do so in animals. The MSU IACUC has determined that analgesia must be provided in all such cases unless non-administration has been scientifically justified in the animal use protocol and approved by the MSU IACUC. See MSU IACUC guideline IG003: [http://www.iacuc.msu.edu/iacuc/iacuc_index.htm](http://www.iacuc.msu.edu/iacuc/iacuc_index.htm)

Analgesia is most effective if it is administered prior to commencement of the surgery (pre-emptive), followed by additional analgesics given post-operatively. Multi-modal therapy (e.g., use of a local skin anesthetic and a NSAID or opioid) provides broader coverage of pain receptors and is recommended. The level, type and duration of analgesia will depend upon the procedure performed. For post-operative analgesia, minimally invasive procedures need less potent or shorter-acting analgesics and may respond to a single dose of analgesic. More invasive procedures (e.g. laparotomy, orthopedic surgery) may require multiple doses of analgesic for up to 72 hours post-operatively. Specific criteria for evaluating pain and provision of analgesia must be developed through veterinary consult and described in the IACUC Animal Use Form (AUF). It is the responsibility of the investigator and staff to evaluate the animal on a routine basis and determine whether additional treatment is needed based upon the criteria described in the IACUC AUF.

**Local Anesthetics**

These agents (e.g. lidocaine, bupivacaine) have been shown to provide effective analgesia by blocking nerve impulse conduction. They can be used topically (e.g. bathe exposed nerves or cut tissues) or injected locally using a fine needle. For thoracic and abdominal surgery they can be instilled post-operatively. Local anesthetics have not been shown to significantly delay tissue healing. These agents can be cardiotoxic, so it is important that a maximum safe dose should not be exceeded (4 mg/kg lidocaine; 1-2 mg/kg bupivacaine). Bupivacaine has a longer duration of action (4-12 hours) than lidocaine (1-2 hours) but takes slightly longer to take effect (5-10 minutes vs. 1-2 minutes). These agents can be diluted in a balanced electrolyte solution to provide more volume for injection or instillation. CAR recommends the use of bupivacaine (0.25-0.5%) over lidocaine due to its longer duration of action. Local anesthetics should not be used as sole analgesic agents except in minor procedures.
Non-steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs are an alternative class of compounds for pain management. These drugs act by reducing prostaglandin synthesis by inhibiting one or both of the COX isoenzymes. These compounds have analgesic activity both locally and centrally and are synergistic with opioids. In addition to aspirin and acetaminophen, newer compounds used extensively for managing pain include carprofen, ketoprofen, ketorolac and meloxicam. The advantage of the newer compounds is that they are not controlled substances and can be dosed once daily. These drugs can be given post-operatively, pre-operatively or intra-operatively. To avoid renal problems be sure the animal remains hydrated (warmed fluids injected subcutaneously during anesthesia is effective: 5-10ml for rats and 0.5-1.0 ml for mice). Combinations of NSAIDs should not be used because adverse side effects of GI ulceration or renal failure can occur. These compounds may be used as sole analgesic agents in both minor and some major procedures. For rats, CAR is currently recommending the use ketoprofen at a dose of 5 mg/kg subcutaneously once daily (for no longer than 5 days) or meloxicam once daily (0.2 mg/kg IM, IP, SQ). If repeated injections are required, the oral preparation of meloxicam can be provided in drinking water (Mice: 1.2 ug/ml [0.2 mg/kg/day] and rats: 2.0 ug/ml (0.2mg/kg)).

Analgesics can be provided in oral formulation by mixing into palatable foodstuffs, please contact the CAR veterinary staff for more information.

For significantly invasive procedures or procedures which are expected to have severe pain, these agents should be combined with an opioid for maximum effectiveness. For these types of procedures, CAR recommends at least one dose of opioid (either butorphanol or buprenorphine, see below) and 1-5 days of ketoprofen.

Opioids

Long known for their potent analgesic activity, opioids remain the gold standard for analgesic therapy. Opioids are the drug of choice for treatment of moderate to severe pain. Side effects such as respiratory depression and bradycardia are dose-dependent and are reduced with the new mixed agonists and agonist-antagonist compounds (buprenorphine, butorphanol). Pica may be seen with buprenorphine, but again is dose dependent and transient. Metabolic rates for opioids are high for rodents, making several of the opioids (morphine, meperidine and pentazocine) impractical for use, however, buprenorphine is effective for 8-12 hours after administration.

Opioids are ideal for using pre-operatively, which reduces the overall amount of anesthetic needed and allows analgesia to be available when the animal wakes up. While analgesia is excellent with these compounds, there are disadvantages. The primary disadvantage is that all of these drugs are controlled substances and subject to federal regulations for acquisition, record keeping and disposal. Of the opioids, CAR recommends buprenorphine due to its long duration of effectiveness and few side effects. Buprenorphine, however, comes in 1 ml glass ampules and there are problems with proper storage of the drug after opening an ampule. On average, one - 1ml ampule will dose 6 - 250 gram rats once each. The dose for buprenorphine in rats is 0.01-0.05 mg/kg subcutaneously every 8-12 hours (mice - 0.05 – 0.1 mg/kg every 6-12 hours). If combining an opioid with an NSAID, a good combination for short duration opioid is butorphanol (1-4 mg/kg subcutaneously every 4- hours for mice and 2mg/kg every 4 hours for rats) or buprenorphine for longer duration + either meloxicam, ketoprofen, carprofen or flunixin (2.5 mg/kg subcutaneous for both rats and mice). For a multiple dosing alternative, the oral preparation of meloxicam (dose is 0.2mg/kg orally) can be mixed with Jello (strawberry, grape or cherry appear to be preferred flavors) and the dose provided daily in a very small cube per rat or in a very small amount of peanut butter (use the smallest amount possible so that the animal will eat the entire amount at one time). To provide analgesia in food the animal must be singly housed, at least while consuming the food with the medication. Contact a CAR veterinarian for specifics on this mixture.

Monitoring of the animal

Confirmation of Anesthesia Depth

The animal must be maintained at an appropriate depth of anesthesia beginning immediately before the surgical procedure is initiated, through the conclusion of the procedure, and until the post-operative analgesics have taken effect. For most species, the following techniques can be used to ascertain that the animal is appropriately anesthetized.
- Toe pinch. Brief clamping of the web of skin between toes or claws with a hemostat or fingers. Firmly pinching multiple toes should not elicit a withdrawal response from an animal at a surgical depth of anesthesia.
- Palpebral reflex. Gently tapping the medial canthus of the animal’s eye should not elicit a blink or eye flutter. This technique is not always reliable in all animals.
- Corneal reflex. Touching the edge of the cornea with a gauge sponge or cotton swab will produce a good reflex if the patient is too light on anesthesia. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.
- Vital signs. Heart rate and respiratory rate may increase if anesthetic depth becomes too light.

Maintenance of Anesthesia
Each animal responds slightly differently when under anesthesia, therefore it may be necessary to modify your use of anesthetics during the procedure. All routinely used anesthesia options must be described in the IACUC protocol. Anesthetists must be trained in not only delivering the anesthetic to the patient, but also in identifying anesthetic related problems.
- Increases and decreases in vital signs may require modifications in anesthetic dosing.
- If, at any time, an animal begins to respond to pain or attain an anesthetic depth that is too light, stop the procedure and adjust the inhalant anesthetic level or give a supplemental dose of injectable anesthetics. Reassess the animal before resuming work.
- Animals must be continually monitored by the anesthetist providing appropriate anesthesia and life support for the duration of the procedure. Anesthetized animals must NEVER be left alone during the procedure.
- In order to maintain sterility during complex surgical procedures and to properly monitor the animals, it may be necessary to include a second person in the procedure—a surgical assistant or anesthetist.

Monitoring Vital Signs
The anesthetist should continuously monitor the animal patient’s basic physiological function for the duration of the procedure—from induction through recovery. The following vital signs should be monitored during all procedures involving mice, rats, hamsters, and gerbils.
- Respiratory Rate (RR) can be assessed by watching the rising and falling of the chest. Subjective changes should be addressed by altering the depth of anesthesia.
- Body temperature should be maintained near normal during surgical procedures.
- Hypothermia often occurs due to anesthesia-induced vasodilation and from surgery via opened body cavities.
- CAR veterinary staff are always available to assist in choosing an appropriate method for your species.

Heat Loss
- Maintenance of normothermia (a clinically appropriate body temperature) is critical for welfare and recovery of rodents from anesthesia. Warm water recirculating blankets, warmed (body temperature) fluid bags, warming blankets, and warming discs must be used or the animal will become hypothermic.
- When using warming devices, they should be covered such that no animal is placed directly on a warmed surface.
- Electric heating pads should never be used due to inconsistency of heating and history of thermal burns in small animals. As well, microwaveable discs must also be monitored to ensure they are not TOO HOT before exposing an animal to them.
- Heat lamps must be used with caution to avoid overheating rodents.
- No matter the type of heat source provided, ensure that the animals have a non-heated part of the recovery cage which they can access to avoid hyperthermia.

Fluid Loss
- Fluid loss occurs as a result of evaporation from body cavities and blood loss.
- All animals (including humans) lose fluid during anesthesia.
- Rodents are vulnerable to the effects of fluid loss because of their small size.
- Reduce fluid loss by:
  - Irrigating the operative field with warm sterile saline (be careful not to wet drapes).
• Control blood loss.
• Administer warm sterile fluids (up to 1ml intraperitoneally) post procedurally.

Postoperative Care

• Monitor the animal continuously during the procedure and until the animal is conscious and then every 10-15 minutes until fully ambulatory. Body temperature must be maintained. Place animal in a clean, dry, warm area on a padded surface (not animal bedding) and provide warmth with a circulating water blanket, warm water bottle, heat discs, or other method (see 'Heat Loss’ above).
• House rodents individually until they are ambulatory to prevent cannibalism or suffocation. Do not return them to the animal holding room until they are conscious and mobile.
• Post-surgical animals should be seen at least daily by a member of the investigator’s staff or other identified trained individual to ensure that there are no complications.
• If the principle investigator or staff finds that an animal appears ill, or the surgical wound appears abnormal, contact the CAR veterinary staff immediately.
• Animals should be monitored for signs of pain and distress (see below). Administration of analgesics must be consistent with the description provided in the IACUC approved AUF.

Potential signs associated with pain or distress in rodents

• Decreased food and water consumption, weight loss
• Self-imposed isolation/hiding
• Rapid, open mouth breathing
• Biting, aggression
• Increased/decreased movement, twitching, trembling
• Unkempt appearance (rough, dull hair coat)
• Abnormal posture/positioning (hunched back, head-pressing)
• Dehydration, skin tenting, sunken eyes

Medical Records

The IACUC requires that peri-operative records be maintained on all vertebrate animals undergoing survival surgery.

Surgical Records should include the following information:

• Animal identification
• Surgical Procedure and date procedure was performed.
• Name of the person doing the procedure
• Anesthetic agent(s) used and dose
• Analgesic agent(s) used and dose
• Daily observations
• Date sutures removed
• Emergency contact information

Postoperative observations and treatments must be recorded and initialed on each date performed. At a minimum, there must be daily entries as to the animal’s condition for 3 days following the procedure and must also include information describing analgesic drugs as they are administered. The weight of the animal must also be recorded in the daily record if so indicated in the IACUC approved protocol. If the MSU IACUC approved protocol states that food and water consumption will be monitored post-operatively, these observations will be recorded in the daily log. All daily entries must be legible. Sutures or staples must be removed (unless exempted in the IACUC approved protocol), and the date of removal recorded in the record. The surgical records will be maintained as long as the animal is housed in MSU facilities. After the death of the animal, the surgical records for non-USDA covered species will be maintained as the PI deems appropriate. The PI will maintain records for USDA covered species for 3 years after the animals’ termination.
There are two (2) options for maintaining perioperative records.

1. The records may be kept on 3” x 5” cards maintained behind the cage card in the cage cardholder.
2. Records may be maintained in a notebook which is kept in the animal room.

If for some reason option 1 or 2 is not possible, other arrangements must be described in the protocol and approved by the IACUC.

3” x 5” record or notebook format. Cards (see template below) may be obtained from CAR:

<table>
<thead>
<tr>
<th>ULAR Rodent Sx Record</th>
<th>Post Operative Notes and Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt:</td>
<td>Animal ID:</td>
</tr>
<tr>
<td>AUF:</td>
<td>Date of Surgery:</td>
</tr>
<tr>
<td>Procedure:</td>
<td>Name:</td>
</tr>
<tr>
<td>Anesthetic(s):</td>
<td>Ph:</td>
</tr>
<tr>
<td>Analgesics: include drug/ duration/ dose/ route</td>
<td></td>
</tr>
</tbody>
</table>

Include postoperative notes on back

Sutures / Staples: yes □ no □ Date Removed: 

ULAR09102013

REFERENCES

• Atkinson, LJ. 1992. Berry & Kohn’s operating room technique, 7th ed. Mosby, St. Louis, MO.
• Flecknell, PA, et al. 1991. The effects of surgical procedures, halothane anaesthesia, and nalbuphine on locomotor activity and food and water consumption in rats. Laboratory Animals 25: 50-60