Colorado State University
Institutional Animal Care and Use Committee (IACUC)

Guidelines for Rodent Survival Surgery

Purpose
This document explains the current requirements with respect to survival surgery in rodents.

Background
The federal regulations set standards for biomedical research involving live, vertebrate animals[1]. These regulatory standards have been adopted by virtually all public and private funding sources, and are pertinent to all research projects, regardless of funding source.

Requirements for Rodent Survival Surgery
Investigators may conduct rodent survival surgery in their laboratories or in an animal facility procedure room. While there is no requirement for a dedicated surgical facility for rodents, ***there are requirements about how rodent surgery must be conducted***.

Aseptic technique is required. General requirements outlined in the *Guide* include the surgical facility and location, use of sterile instruments, sterile surgical gloves, clean surgical attire and aseptic technique. The balance of this document will expand and clarify the statements in the *Guide*.

1. **Surgery Facilities**: While dedicated surgical facilities are not required for rodent survival surgeries, a rodent surgical area must be easily sanitized.
    a. The immediate surgical area should not be used for other purposes during the time of surgery.
    b. Surgery may be conducted on a clean, uncluttered lab bench or table, in a laminar flow HEPA-filtered hood, or in a glove box or other type of isolator.
    c. It is strongly recommended that surgeries be performed in a HEPA-filtered laminar flow hood to minimize the amount of contamination during surgery and protect the animals from unwanted infections, such as mouse hepatitis virus, rat coronavirus, mouse parvovirus, etc.
2. **Prior to and between surgeries**: The surface upon which surgery will be performed must be cleaned and disinfected, rinsing thoroughly, and followed with an appropriate disinfectant.
    a. Commonly used disinfectants are quaternary ammonium compounds (such as Roccal), household bleach diluted 1 part to 32 parts water, chlorine dioxide-based sterilant (Clidox), chlorhexidine (Nolvasan), or other antimicrobial agent.
    b. Disinfectants must be prepared and used according to the manufacturer's recommendations.

[1] Animal Welfare Act and Regulations, Guide for the Care and Use of Laboratory Animals

3. **Preparation of Surgical Instruments:** ***Surgical instruments must be sterilized*** for use in survival rodent surgery.
    a. Several techniques (steam, dry heat, ethylene oxide, or other chemical agents) can be used to sterilize instruments and other materials that will come in contact with the animal's tissues.
    b. Steam or dry heat (such as bead sterilizer) is the preferred methods to sterilize surgical instruments.
    c. Chemical sterilants (e.g. chlorine dioxide, glutaraldehyde, cetylcide) require instruments be soaked for an appropriate time according to manufacturers' directions after which ***instruments must be rinsed in sterile water or saline*** before use in tissues.
    d. If instruments are to be disinfected in ethanol (70%), they must be immersed for at least 24 hours and rinsed prior to use.
4. **When performing surgeries on multiple animals**:
    a. At least 2 sets of sterile instruments should be available to allow sterilization of instruments between animals. While the first set of instruments is being disinfected, the second set is used.
    b. After using a set of instruments, one must remove all organic material and then immerse the instruments in an appropriate disinfectant (e.g., Sporocidin, Clidox, Cidex, and ethanol) for the correct contact time or a glass bead sterilizer.
    c. The disinfectant must be replaced when contaminated with blood or other body fluids.
    d. A new sterile instrument pack should be used after every 4 or 5 major surgical procedures.
5. **Preparation of the Animals**:
    a. While the animal is under anesthesia and prior to taking the animals to the surgery area, all hair must be removed for at least a centimeter on either side of the surgical site. Hair can be removed by clipping with a #40 clipper blade, shaving with a razor, plucking (in anesthetized rodents), or by using a depilatory cream.
    b. After cutting or plucking, remove loose hair with dry gauze or careful vacuuming.
    c. Lubricating ophthalmic ointment (such as Lacrilube or Tearfair) must be placed in the anesthetized animal's eyes to prevent drying of the cornea if the anesthesia and recovery will last more than five minutes.
6. **Surgical Site:** The surgical site must be cleaned and aseptically prepared. An effective antiseptic surgical scrub solution (Nolvasan, Betadine, etc.) must be used.
    a. The area is scrubbed with a new clean surgical sponge or sterile cotton swab in a gradually enlarging circular pattern from the center of the proposed incision to the periphery.
    b. The sponge or swab should not be brought back from the contaminated periphery to the clean central area.
    c. The scrub is followed with a 70% alcohol (or sterile water)-soaked sponge or sterile cotton swab.
    d. The entire process should be repeated three times.
7. **Thermoregulation of the Animals**: To prevent hypothermia, an attempt should be made not to wet the animal any more than necessary and the animals should be placed

on a heating pad. Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal.

a. The animal should be placed for surgery on a clean absorbent surface which will maintain body temperature (using a circulating water blanket, warm water bottle, bubble wrap, or equivalent external heat source, taking care to not cause thermal burns to the animal's skin).

8. **Preparation of the Surgeon**: Surgeons must wash their hands preferably with a surgical scrub (e.g., Betadine Scrub, Nolvasan Scrub).
    a. Surgeons must wear a mask, sterile gloves, and clean scrub shirt or lab coat.
    b. A new pair of sterile surgical gloves should be used for each animal, or surgeons may wipe their gloves for 30 seconds with a disinfectant (e.g. Nolvasan, Betadine, or Clidox) between animals.
    c. Latex exam gloves may be used if sterilized using the surgical scrub solution.
9. **During Surgery**:
    a. The surgical field must be kept sterile throughout the procedure.
    b. Sterile instruments must be prevented from contacting non-sterile surfaces.
    c. Instruments must be placed on a sterile surface when not in use.
    d. Sterile gloves are required by the *Guide*.
    e. In some cases, the use of sterile drapes is recommended for maintenance of the sterile field.
    f. ***It is necessary to monitor the animal carefully during the surgical procedure.*** Anesthetists should pay close attention to the animal's response to surgical stimulus and respiratory rate. Additionally, heart rate and body temperature may be monitored with specialized equipment.
10. **Postoperative Care:**
    a. Recovering animals should not be placed onto loose bedding material until they are fully awake, as suffocation can result. A paper towel may be placed between the bedding and the animal until it awakens from anesthesia.
    b. Hypothermia may be prevented or treated by placing the recovering animals in a warm cage. If necessary, the cage may be placed on a bedded or padded surface and supplied with supplemental heat as required (such as a circulating hot water pad). Be cautious with supplement heat sources; hyperthermia can be as detrimental as hypothermia.
    c. Dehydration can be ameliorated by the administration of appropriate fluid therapy. Initially this may be done by giving 1 to 2 ml of warm fluids (0.9% sterile NaCl or equivalent) per 100 g of body weight by subcutaneous injection. If blood loss occurred during the surgical procedure, or if the animal is slow to recover from anesthesia, provide additional fluids. Veterinary staff may be consulted for assistance with appropriate fluid therapy.
    d. Analgesia must be administered to control post-surgical pain as described in the IACUC approved protocol. Analgesia should be administered before or during surgery for optimal effect. Veterinary staff may be consulted for appropriate rodent analgesia. For the majority of rodent surgeries a bimodal analgesia regimen should be used which consists of an opioid and NSAID. Buprenorphine, meloxicam or carprofen for at least 3 days are recommended. Sustained release formulations of each of these compounds are available and ensure a constant

therapeutic dose of analgesia and reduce the handling and should be used. Consult the veterinarian for appropriate dosing.

e. Animals should not be returned to the vivarium until they are sternal and clearly awake. To prevent cannibalism or suffocation, rodents should be housed individually until they are ambulatory.
f. Post-surgical animals must be seen at a minimum every day by a member of the investigator's staff or other individual to whom post-operative care has been delegated. Animals should be observed daily until all sutures, wound clips, or other implanted devices have been removed. External wound clips or sutures should be removed 7-14 days after the surgery. The veterinary staff must be notified if post-surgical complications occur.

11. **Records:** Post-operative records are kept in the room where the animals are housed. Having the record in the room accomplishes several functions. See **Attachment 1** for example of a surgical/post-surgical record and **Attachment 2** for example of a cage card observation record.
    a. It explains the condition of the animals to animal care staff (e.g., a sedated animal may otherwise be thought to be ill).
    b. It assures and documents for all involved in the care and use of the animals that proper care is being given to the animals.
    c. It informs animal care staff and veterinary staff how recently the investigator has seen the animal; this knowledge helps them decide whether or not there is a need to contact the investigator to inform him or her of the present condition of the animal.
    d. Although individual records are desirable, the regulations allow a composite post-operative record to be used for a group of rodents (see Attachments). Such a record would have a list of the animal numbers down the side and columns indicating dates. The column entries would include a notation that the animal has been checked, any abnormal observations, and a list of any therapeutics given including drugs, doses, and routes of administration. Records should be kept current during the immediate post-operative period (7-14 days). After all wounds have healed and all sutures/wound clips have been removed, the post-operative record requires no further entries, but should continue to be kept in the area where the animals are housed. When the study involving rodents is completed and the animals are euthanized, the record may either be kept by the investigator or discarded.
12. **Requirements for Non-survival Rodent Surgeries:** While it is not necessary to follow aseptic technique when performing non-survival surgeries in rodents, ***at a minimum*** the surgical site should be clipped, the surgeon should wear gloves, and the instruments should be clean.

Contact the Attending Veterinarian, his delegates, or Laboratory Animal Resources for questions regarding animal health, anesthetic support, surgical wound care, post-operative analgesia, or other questions regarding rodent surgery.

Attachment 1: Example Surgical/Post-Surgical Record

This document is meant to be flexible for both large and small species. Not all criteria included may be appropriate for rodents.

Colorado State University **Anesthesia Record**

Investigator _____

Protocol # _____

Animal # _____

Species _____

Sex _____ Age _____

Weight _____

Housing Site _____

Date:	Surgeons:
	Anesthetists:

Procedures:	Time
1	
2	

PRE-ANESTHETIC DRUGS:

Drug	Dose	Route	Time

ANESTHETIC INDUCTION AND MAITENANCE:

Drug	Dose	Route	Time

Post Anesthetic Recovery:

Time	:00	15	30	45	:00	15	30	45	:00	15	30	45	:00	15	30	45	:00	15	30	45	:00	REMARKS
Procedure completed																						
Body temperature																						
Heart rate																						
Respiratory rate																						
Sternal and ambulatory																						

Post Operative Recovery: Daily observations for normal activity level, evaluation of incision site, and other findings until suture remova

Post Operative Analgesia: Drug Dose Route

Animal identification Day:	1	2	3	4	5	6	7	8	9	10	11	12

Animal identification Day:	1	2	3	4	5	6	7	8	9	10	11	12

REMARKS:

****Report any abnormalities to Laboratory Animal Resources at 566-3414 or 491-7364****

Attachment 2: Example Cage Card Observation Record:

Instructions: The observer dates the card and checks or initials the behavioral score. This give a cage side assessment that others can refer to, and if different individuals are observing the animals, provides a reference for each observer.

Observation Record							
Protocol/CC#:							
Animal ID (if applicable):							
Date:							
Procedure/Disease Type:							
Initial in appropriate score box below:							
Date:	**A**	**B**		**C**			**D**
A=Monitor once daily **B=Increase monitoring to 2x daily** **C=Increase monitoring to 3x daily** **D=Notify to Euthanize**							