



THE RODENT



SEMINAR SERIES

presented by Research Animal Resources (RAR),
the JHU Animal Care and Use Committee (ACUC) Office,
and with support from the Cancer Center's Animal Resources Core

Seminar Details

- GOAL: Enhance education, training, and compliance
- Every 4th Wednesday of the month from 3-4 PM
 - Same Zoom details for the entire seminar series
 - Physical location may vary
- Attendance recorded
- Prizes
 - 3 individual prizes
 - 3 lab prizes

TOPICS INCLUDE:

- ❖ breeding colony management
- ❖ humane endpoints
- ❖ anesthesia, analgesia and euthanasia
- ❖ physiology and behavior
- ❖ common clinical conditions
- ❖ biostatistics

RODENT SURVIVAL SURGERY

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Why should you care?

- **Animal welfare**
- **Quality of science** → Appropriate surgical technique used on animals that are free from pain and distress will provide better data
- **Regulatory compliance** → The information presented will allow you to develop and follow your approved ACUC protocol



Rodent survival surgery basics

- Survival surgery = one or more incisions and the animal wakes up from anesthesia
- PHS, AAALAC, and ACUC regulations and guidelines
- Three main areas to consider:
 - Pre-operative preparation
 - Operating procedures and techniques
 - Post-operative care



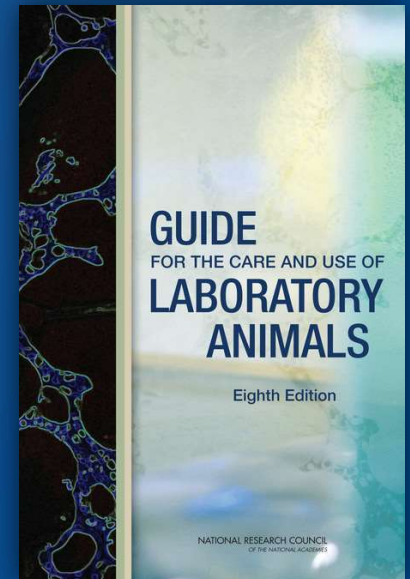
IACUC
Animal Care
and Use
Committee



PROUDLY, AN AAALAC ACCREDITED PROGRAM SINCE 2011

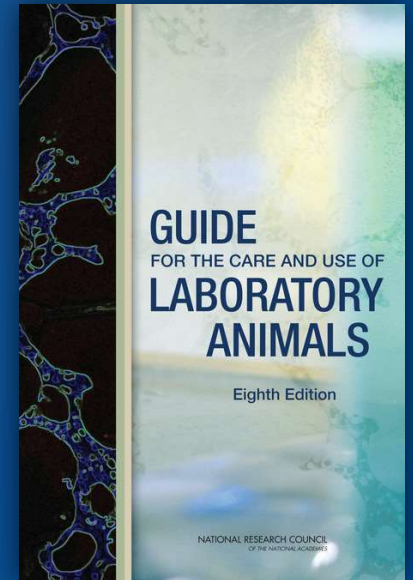
The Guide

- Must be trained in good surgical technique
 - Asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns
- Aseptic surgery should be conducted in dedicated facilities or spaces
 - With sterile gloves, sterile instruments, etc.




The Guide (continued)

- Major vs minor surgeries
 - Major surgeries...
 - 1) penetrate and expose a body cavity OR
 - 2) produce substantial impairment of physical or physiologic functions OR
 - 3) involve extensive tissue dissection or transection
 - Ex. laparotomy, limb amputation
 - Minor surgeries...
 - Do none of the above
 - Ex. subcutaneous tumor implantation



JHU Animal Care & Use Committee

- Multiple guidance documents
 - Survival Surgery: Major, Minor, and Multiple
 - Use of Anesthetic Gases
 - Animal Surgical Suites
 - Hair Removal on Rodents
 - Rodent Survival Surgery
(currently under revision)



Johns Hopkins University
Animal Care and Use Committee

Survival Surgery: Major, Minor and Multiple


Background:
Survival surgery is defined as any surgery from which the animal recovers consciousness from anesthesia following a surgical procedure. Animal Welfare Act Regulations¹ and Public Health Service Policy (as set forth in the *Guide for the Care and Use of Laboratory Animals*)² require that survival surgeries be performed using aseptic techniques. Major operative procedures on USDA-regulated species must be done in a dedicated surgical facility. Minor operative procedures, surgeries on rodents and other non-regulated species, and surgeries conducted at field sites do not require a dedicated facility. Investigators performing survival surgery must identify where the procedure will take place. Individuals performing these procedures must be appropriately trained and/or supervised to perform them. As with any research or teaching procedures performed on animals, surgical procedures and their justifications must be approved in advance by the Animal Care and Use Committee.

Definitions:¹

- **Major survival surgery:** Any surgical procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiological functions.¹ The *Guide*² adds that if a surgery involves extensive tissue dissection or transection it could be considered a major survival surgery.
- **Multiple survival surgery:** More than one surgical session (major or minor) in which the animal is anesthetized.

Regardless of surgical classification, **pain and distress** should be minimized through use of pre-operative sedation, anesthesia, and analgesia, as well as post-operative monitoring and care appropriate to the procedure performed. Withholding drugs that would prevent or alleviate pain must be justified for scientific reasons.

Complete **records** of the surgical procedure(s) performed and of intra- and post-procedural monitoring for all species must be kept (see sample Surgical Record forms at web.jhu.edu/animalcare). These records should be legible, current, and readily available for inspection.



Johns Hopkins University
Animal Care and Use Committee

Animal Surgical Suites¹

PURPOSE: This document outlines the requirements that are necessary to ensure that surgical facilities and procedures comply with standards set forth in the *Guide*¹.

BACKGROUND: The Animal Welfare Act² and the Public Health Services Policy³ (as stated in the *Guide*) require use of aseptic procedures for survival surgical procedures. Aseptic technique includes preparation of the animal such as hair removal and disinfection of the operative site; preparation of the surgeon such as provision of decontaminated surgical attire, surgical scrub and sterile gloves; sterilization of instruments, supplies and implanted materials; and use of operative techniques to reduce the likelihood of infection. Major operative procedures on non-rodents mammals will be conducted only in facilities intended for that purpose (dedicated surgical facility), which are maintained for use under aseptic conditions. Non-major (minor) operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures. The following criteria should be met for such a facility:

PROCEDURE:
Operating Room

1. The floor, ceiling, and walls must be created by a continuous connection, constructed of materials that are easily sanitizable and must be kept physically clean. Interior surfaces should be constructed of materials that are monolithic and impervious to moisture.
2. Supplies and equipment not relevant to the surgical procedures being performed should not be stored in the room. The operating room cannot be used as an office, laboratory or storage room.
3. A surgical light and an easily sanitizable surgical table must be available.
4. Appropriate scavenging must be provided whenever gas anesthesia is used.
5. The operating room must normally be used only for aseptic surgery. A non-aseptic surgery may be performed, however, provided the operating room is thoroughly decontaminated.

¹ Approved by the IACUC on January 17, 2002, reviewed and refi. updated September 25, 2012; reviewed January 31, 2018
² 9 CFR Chapter 1 Subchapter A, Parts 1.2 and 3
³ *Guide for the Care and Use of Laboratory Animals*, NRC, National Academy Press, 8th ed., 2011

<https://web.jhu.edu/animalcare/policies/index.html>

PRE-OPERATIVE PREPARATION



Area Preparation

- Area should be uncluttered and disinfected
- Separate areas for animal prep and surgery
- Limited access to space by non-essential personnel



Instrument & Equipment Prep

- Sterilize instruments and materials
 - Autoclave or gas sterilizer (ethylene oxide)
 - Alcohol alone is NOT a sterilant
 - TIP: Prepare at least 2 sets of instruments.
- Q-tips and gauze for skin preparation and hemostasis should be sterile.
 - TIP: Autoclave non-sterile Q-tips and/or gauze
- Hot bead sterilizers can be used between animals IF the instruments were sterilized prior to surgery AND handled aseptically during the surgery
 - Remove gross debris prior to placement in the hot bead sterilizer
 - Ensure instruments are cooled down prior to use



Instrument & Equipment Prep Cont.

- Isoflurane vaporizers must be calibrated every 3 years, or annually if extensively used or moved around (e.g., >500 hrs/yr)
- Ensure charcoal canister is upright and has holes that are not blocked
 - Canisters with holes at the bottom should be placed on a rack/stand
 - Follow manufacturer's recommendations



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Animal Care and Use Committee

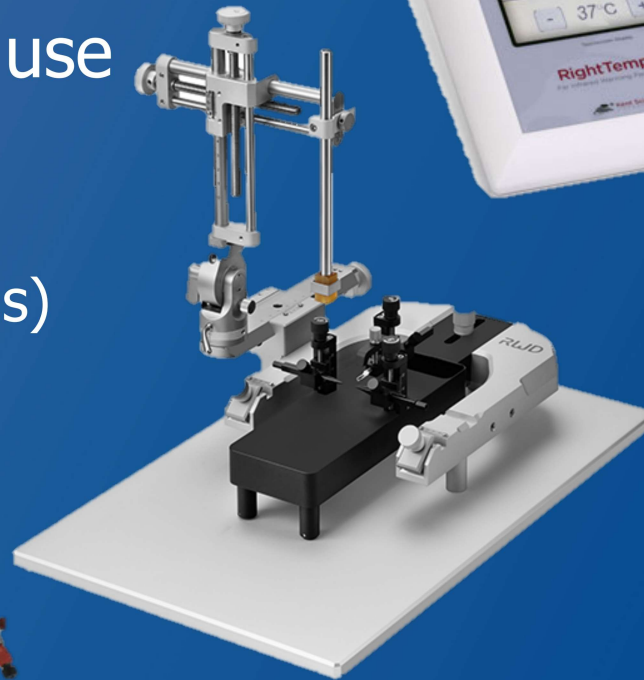
Use of Anesthetic Gases: General Guidelines/Vaporizer Calibration¹

PURPOSE: The Animal Care and Use Committee (ACUC) has developed the following guidelines to control risk of exposure to waste anesthetic gases in the workplace.



Instrument & Equipment Prep Cont.

- Turn on supplemental heat device
- Clean and wipe equipment with disinfectant prior to use
 - Gas anesthetic chambers
 - Specialized equipment (e.g., stereotaxic apparatus)



Heat support tips

- Electric heating blankets can cause thermal burns
- Circulating water blankets provide uniform heat
- Reflective foil can increase warming device effectiveness

Surgeon Preparation

- Wash hands!
- Surgeons must wear:
 - Sterile gloves
 - Clean gown or lab coat
 - Hair cap or net*
 - Mask*
- Other personnel in the vicinity must wear:
 - Hair cap or net*
 - Facemask*
 - Clean garments



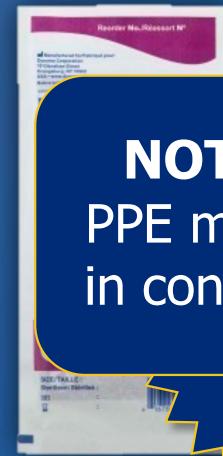
Did you know?
You can sterilize
exam gloves for
surgery



*not necessary when working
in a laminar flow hood

Surgeon Preparation

- Wash hands!
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 - Sterile gloves
 - Clean gown or lab coat
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 - Facemask*
 - Clean garments



NOTE: Additional PPE may be required in containment areas

surgery



*not necessary when working in a laminar flow hood

Animal Preparation

- Animals should be acclimated ~2 days prior to use
- Pre-operative fasting is NOT required in rabbits and small rodents as vomiting does not occur during induction in these species and small animals are prone to hypoglycemia.
- Use healthy animals! → perform a clinical evaluation



Animal Preparation: Anesthesia

- Anesthesia Stages

1) Pre-medication

2) induction

3) maintenance

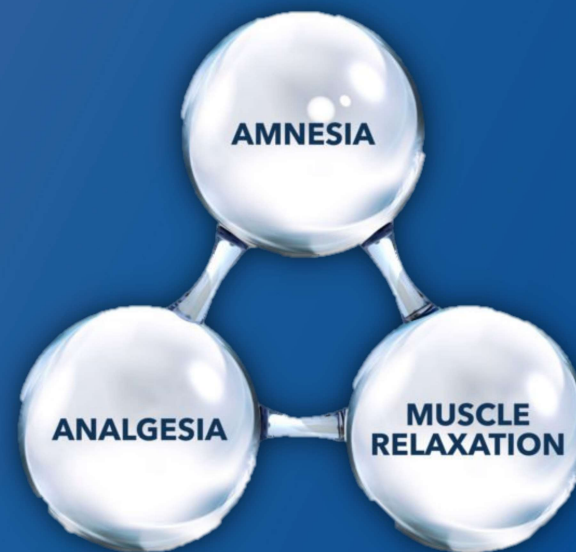
- Anesthesia Types

- Inhalants

- Preferred
- Can control depth
- No analgesia

- Injectables

- Ex. ketamine/xylazine
- Little control over depth
- Possible analgesia depending on agent(s)



Animal Preparation: Analgesia

- Pre-emptive pain medication
 - Give pain medication as soon as animal is anesthetized, prior to start of surgery
 - Pain medication will work **BETTER**
 - Prevents wind-up pain
 - Standard of care for animals & people
- Common pain medications
 - Buprenorphine/Buprenorphine SR
 - Meloxicam



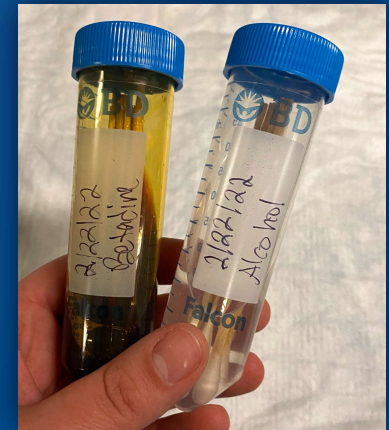
Animal Preparation

Once anesthetized...

1. Provide pain medication
2. Apply sterile eye ointment
3. Remove hair at surgery site
 - Use clippers or apply depilatory cream (ex. Nair) for 30-60 sec



-
-
-
4. Aseptically prepare surgical site
 - Use betadine or chlorhexidine, then alcohol, x 3
 - TIP: Area should 2-3 times the length of your incision squared
5. Check anesthetic depth (ex. toe pinch reflex)



INTRA-OPERATIVE ANIMAL MONITORING



Intra-op Animal Monitoring

- Anesthetic depth stages:
 - I. Stage 1: Voluntary movement
 - II. Stage 2: Delirium or involuntary movement
 - III. Stage 3: Surgical anesthesia
 - Light, medium, and deep
 - IV. Stage 4: Extreme CNS depression/death





Stage 2: thoracic breathing only, rapid breaths. Too light.



Stage 4: abdominal breathing only, slow breaths. Too deep.



Stage 3: thoracic and abdominal breathing. Steady breaths. Appropriate depth.

Intra-op Animal Monitoring

- Heat support should be provided during prep and surgery → rodents prone to hypothermia
- Physiologic parameters
 - Temperature, respiratory rate, heart rate, blood pressure, etc.
 - Not required, but helpful for certain procedures
- Anesthetic depth checked every 10-15 minutes
 - Toe pinch → surgeon can do this through drape
 - Watch respiratory rate
 - Check mucous membranes → should be pink, not pale or grey



Intra-op Animal Monitoring

- If you think an animal is “light” and is starting to feel what you are doing...

STOP IMMEDIATELY

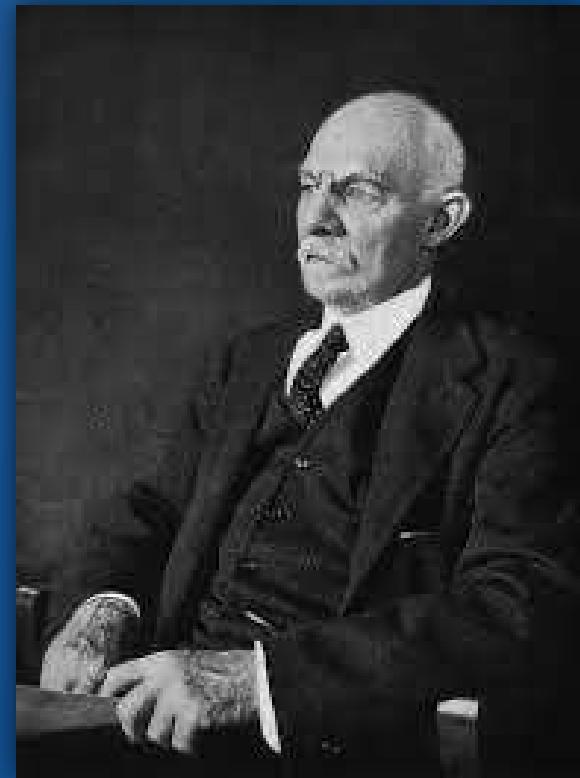
- Increase inhalant rate or give more injectable anesthetic

OPERATING PROCEDURES AND TECHNIQUES



Surgical Technique

- **Halstead's principles**
 1. Strict asepsis
 2. Gentle tissue handling
 3. Meticulous hemostasis
 4. Preservation of blood supply
 5. Minimal tension
 6. Accurate tissue apposition
 7. Obliteration of dead space



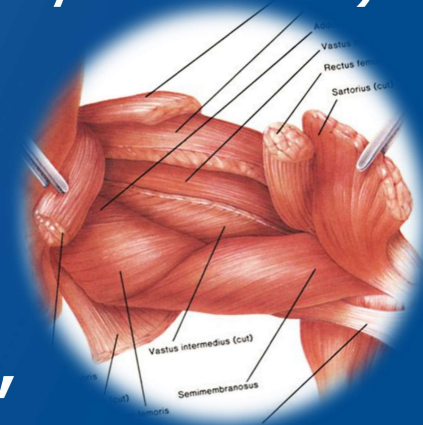
Aseptic Technique

- MUST BE USED
- Sterile only touches sterile
- Sterile draping strongly recommended for all procedures, required for some
 - Animals should be draped for major survival surgeries
 - Draping is required when using suture to prevent contamination



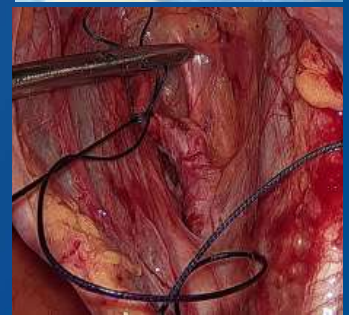
Gentle Tissue Handling

- Avoid unnecessary tissue damage
 - Ex. clamping a hemostat on tissue that is staying in the body and should remain functional
- Prevent drying out: use sterile saline (preferably warmed)
- Minimal dissection
- Cut/dissect along fascial planes
- Complications if not done:
 - **Tissue ischemia, delayed healing, dehiscence, seroma/abscess, pain, wound contamination**



Hemostasis

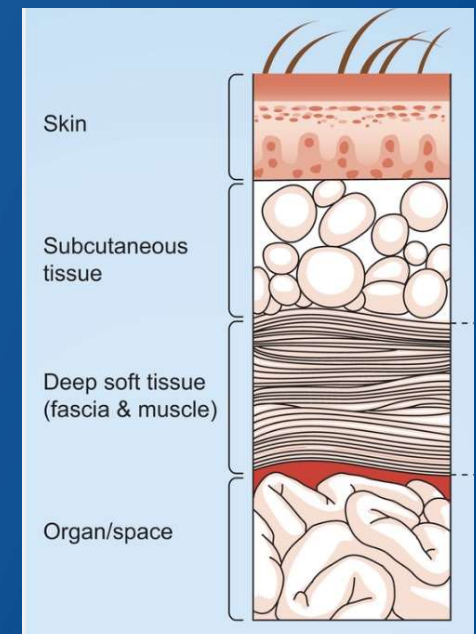
- Hemostasis = stopping blood flow
- Blood loss can result in dehydration, anemia, increased complication rates, and death
- Ways to provide hemostasis:
 - 1) Pressure
 - 2) Ligation
 - 3) Cauterization
- Make sure there is no bleeding prior to closing a body cavity
 - TIP: Use sterile Q-tips to check the body cavities
- There should never be active bleeding when an animal is returned to their cage



Closure

Close each layer individually (as applicable)

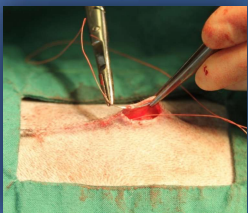
1. Muscle/abdominal wall
 - Absorbable suture
 - Simple continuous or simple interrupted pattern
2. Subcutaneous space (if present)
 - Absorbable suture
 - Simple continuous or simple interrupted pattern
3. Skin
 - Absorbable suture, non-absorbable suture, or wound clips
 - Non-absorbable suture and staples **MUST** be removed 10-14 days after surgery
 - Skin glue should rarely be used on a regular basis → irritating



Closure (continued)

Suture

- Different types → make sure you are using what is appropriate
- Non-absorbable
 - Examples: Ethilon, Prolene
 - Can use for ligation of vessels, skin sutures (must be removed)
- Absorbable
 - Examples: Dexon, Biosyn, Vicryl, PDS
 - Can use for body wall closure, skin closure



Wound Clips

- Use for skin closure
- Various sizes → use what is appropriate
- Must be removed 10-14d after surgery
- Good option if lab member has minimal suturing experience and incision is small



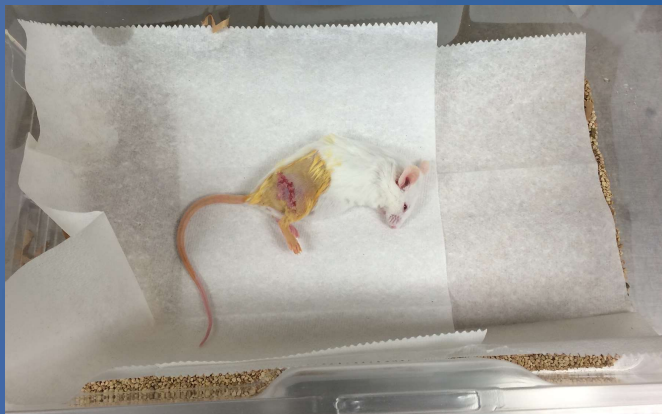
Monitor all incisions for dehiscence, regardless of closure type!

POST OPERATIVE CARE



Anesthetic Recovery

- Place animals in a clean cage
 - TIP: Place animal in sternal recumbency, if possible, to prevent positional atelectasis
- Monitor animals until they can right themselves, can move around the cage, and are responsive to light touch.
- Heat support should be provided until animals are fully recovered



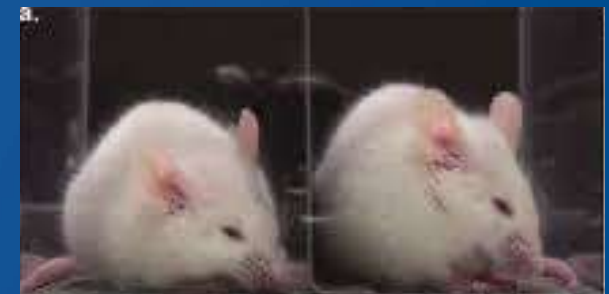
Anesthetic Recovery (continued)

- Administer analgesic (if due) and check for signs of discomfort or pain
- Administer fluids (SQ or IP) when surgery is long, if there has been severe blood loss, or if procedure will prevent animal from drinking/eating. Give 20-30 ml/kg (600-1000 ul/mouse).
- Monitor food and water intake. Provide fluid and nutritional support, if needed
 - Examples: food in the cage floor, hydrogel, nutrigel, ensure



Post-op analgesia

- Give pain medication when due following pre-surgical administration
 - May not correlate with end of surgery
 - Pain medication duration of action should be in your protocol if you are unsure
- Perform monitoring and additional analgesic administration as per protocol



REMINDER: Rodents are a prey species. They do not show signs of pain unless they are significant. If something is painful in people, assume it is painful in your animals!

Complications and How to Fix Them

NOTE: Any complication repair must be in your protocol for you to perform. If it is not, you must seek veterinary approval before performing the repair

Complications and How to Fix Them

- Surgical site dehiscence
 - 1-2 days post operatively, no complications just dehisced skin:
 - Anesthetize animal and provide pain medication
 - Prepare area with betadine and sterile saline x 3 → safe to use on open wounds
 - Reclose using suture or staples after freshening incision edges
 - After 1-2 days, skin has begun healing and closure is more complicated
 - Recommend seeking veterinary help unless proficient in animal wound healing and repair
 - This should be an uncommon occurrence



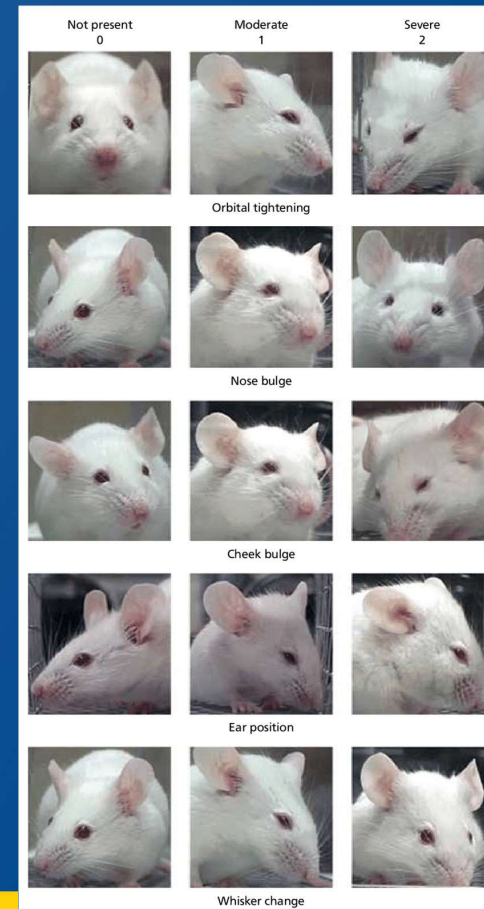
Complications and How to Fix Them

- Surgical site infection
 - Signs of infection
 - Redness, discharge, swelling, poor general health, dehiscence
 - **If you suspect your surgical site is infected, reach out to veterinary staff immediately**
 - We can work with you on treatment if possible, such as cleaning the site and antibiotic administration
 - Rarely included in protocols as expected complication for surgery
 - This should be an uncommon occurrence



Complications and How to Fix Them

- Animal showing signs of pain and distress
 - If your animals are showing signs of pain and/or distress and are not category E → **MUST** give pain medicine or euthanize
 - Follow your protocol for administering analgesia
 - If you have an animal showing signs of pain, and you do not have additional pain meds approved, reach out to veterinary staff and we can help
 - Unexpected pain and distress should be an uncommon occurrence



Common theme: these complications should not be a routine occurrence!

- If you notice an increased incidence of unexpected outcomes, please reach out to veterinary staff → we can help!
- Expected adverse outcomes must be discussed in your protocol
 - We understand some adverse outcomes are scientifically justified, they just need to be described in the protocol

If you need help with unexpected outcomes:
Email Dr. Villano (jvillano@jhmi.edu) or call 5-3273

Other considerations

- Recordkeeping
 - Keep track of your animals → good science!
 - Example records available on ACUC website
 - Surgical records should be kept and available for review
 - If it isn't recorded, it wasn't done!
 - Fraudulent records are not tolerated, and are grounds for loss of animal use privilege and reporting to an ethics committee

POST OPERATIVE EVALUATION

Animal ID _____ Species _____ Protocol _____
 Weight _____ Procedure _____
 Date of Procedure _____
 Surgeon _____ Assistant _____ Lab contact # _____

| DATE | | | | | | | |
|---|--|--|--|--|--|--|--|
| TIME | | | | | | | |
| OBSERVATIONS (Y/N) | | | | | | | |
| Alert/Responsive | | | | | | | |
| Eating/Drinking | | | | | | | |
| Feces/Urine | | | | | | | |
| PHYSICAL EXAM | | | | | | | |
| Breathing Normal? | | | | | | | |
| Gait/Posture Normal? | | | | | | | |
| SURGERY SITE | | | | | | | |
| Bandage clean/dry? | | | | | | | |
| Bleeding from site? | | | | | | | |
| Drainage from incision? | | | | | | | |
| PAIN ASSESSMENT | | | | | | | |
| Pain score (0-4) | | | | | | | |
| Medication given? | | | | | | | |
| Agent _____ Dose _____ Freq _____ | | | | | | | |
| Dialysis performed | | | | | | | |
| OBSERVER INITIALS | | | | | | | |
| Veterinary Assessments (for veterinary use only) | | | | | | | |

PAIN ASSESSMENT SCORING
 0-normal behavior and physiology
 1-mild behavior and physiological changes (decreased food/water consumption, slightly depressed, minor guarding of incision site)
 2-moderate pain (group 1 plus swelling/redness/discharge at surgical site, reluctance to move, guarding with vocalization or aggression)
 3-severe pain/distress (groups 1 and 2 plus, immobility, dehiscence of incision, profound dehydration/weight loss)
 4-Moribund/comatose

****Please contact a member of the veterinary staff for group 2 or higher animals or if you have questions about analgesia or postoperative monitoring.**
 * N=normal, L=labored, R=rapid, S=shallow

Summary of Key Points

- Use aseptic technique
- Give appropriate pain medication
- Monitor your animals
- Follow surgical principles
- Everything done **must** be in protocol

We are here for you!
If you need help with...

- Writing your protocol or an amendment
- Training on general or specific techniques
- Questions about using your animals
- Troubleshooting problems with your animal model
- Other animal concerns

Please reach out to Dr. Villano
(jvillano@jhmi.edu) or call 5-3273

Questions so far?



SURVIVAL SURGERY IN ACTION





outbreaks ☹️
Mites Rack 1 + Rack 5
done 11/16/21
p testing *negative*
12A EDIM → confirmatory
pending

ULINE
INSULATED SUPPLY
RUSH!
PERISHABLE
INSULATED
UP

Reference Laboratories
of
Shelton, CT

WIPAC
WIPAC
WIPAC



What was wrong with that video?

- Cluttered benchtop
- Isoflurane on 5% after induction
- Did not lubricate eyes
- Did not appropriately clean/prepare skin
- Non-sterile instruments
- No sterile gloves
- No clean gown or hair net
- No pain meds



**Don't be
that guy!**

Resource Packet

Includes ACUC resources:

- Survival Surgery: Major, Minor, and Multiple
- Use of Anesthetic Gases: General Guidelines/Vaporizer Calibration
- Use of Anesthetic Gases: Drop Method
- Animal Surgical Suites
- Hair Removal on Rodents
- Rodent Survival Surgery
- Surgery and Anesthesia Form

Additional resources can be found:

- <https://web.jhu.edu/animalcare/>
- <https://researchanimalresources.jhu.edu/>
- NOTE: We are revising the current description of survival surgery procedures for rodents in the ACUC website.

Rodent Survival Surgery Seminar Documents

JHU ACUC Website: <https://web.jhu.edu/animalcare/>

1. Protocol Forms & Guidelines
2. Sample Surgery and Anesthesia Form
3. Sample Post-Operative Evaluation Form
4. FAQs

RAR Website: <https://researchanimalresources.jhu.edu/>

ACUC Guidelines: <https://web.jhu.edu/animalcare/policies/index.html>

5. Survival Surgery: Major, Minor, and Multiple
6. Use of Anesthetic Gases: General Guidelines/Vaporizer Calibration
7. Use of Anesthetic Gases: "Drop method"
8. Animal Surgical Suites
9. Hair Removal on Rodents
10. Rodent Survival Surgery (*Currently Under Revisions*)

Other Useful Information:

11. *Guide for the Care and Use of Laboratory Animals 8th Edition (2011)*
<https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth>
12. FLSC Rodent Dehydration and Treatment Policy (Notre Dame)
https://freimann.nd.edu/assets/263457/fullsize/ap.ro_dehydration_treatment_pol17_1_.pdf
13. Helpful Information Sheet: Sterile Gloves for Rodent Surgery (University of Michigan)
<https://az.research.umich.edu/animalcare/informational/helpful-information-sheet-sterile-gloves-rodent-surgery>

References:

- https://freimann.nd.edu/assets/263457/fullsize/ap.ro_dehydration_treatment_pol17_1_.pdf
- <https://az.research.umich.edu/animalcare/informational/helpful-information-sheet-sterile-gloves-rodent-surgery>
- <https://researchanimalresources.jhu.edu/>
- <https://web.jhu.edu/animalcare/policies/index.html>
- <https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth>
- <https://nc3rs.org.uk/3rs-resources/grimace-scales>
- <https://azanimal.sites.uofmhosting.net/animalcare/guidelines/guidelines-performance-surgery-rodents>
- https://oacu.oir.nih.gov/system/files/media/file/2021-02/b6_survival_rodent_surgery.pdf
- <https://www.jove.com/v/2586/principles-of-rodent-surgery-for-the-new-surgeon>
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6935704/>

Final questions?

Please feel free to email:

- Dr. Jason Villano (jvillano@jhmi.edu)
- Dr. Jessica Plunkard (jplunka1@jhmi.edu)
- Jonathan Harrold (jharold1@jhmi.edu)
- The ACUC (acuc@jhmi.edu)

Next seminar:

Getting Back to the Basics of Colony Management

March 23rd, Wed, 3-4 PM
Sponsored by Transnetyx



Now what you've all been
waiting for...



**RAFFLE
RESULTS**

Thank you all for coming!

Please fill out the evaluation form:
https://jhmi.co1.qualtrics.com/jfe/form/SV_1Y0yjqdTeuMYgyW

