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
- biomethodology
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
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)

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

[Image of a medical device]
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

*not necessary when working in a laminar flow hood

Did you know?
You can sterilize exam gloves for surgery
Surgeon Preparation

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NOTE: Additional PPE may be required in containment areas
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
     → **TIP:** Area should 2-3 times the length of your incision squared
4. Aseptically prepare surgical site
   - Use betadine or chlorhexidine, then alcohol, x 3
     → **TIP:** Prior to the surgery, place Q-tips in Falcon tubes with betadine or chlorhexidine, and alcohol
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 \( \rightarrow \) 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
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
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
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.
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://nc3rs.org.uk/3rs-resources/grimace-scales
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:
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