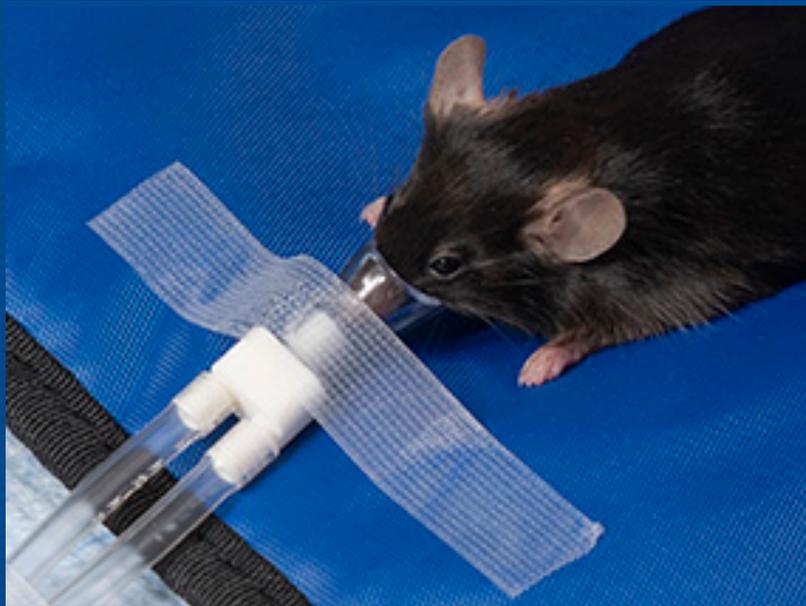


THE RODENT SEMINAR SERIES

**presented by the Research Animal Resources (RAR) and
the JHU Animal Care and Use Committee (ACUC) Office**



JOHNS HOPKINS
M E D I C I N E

Anesthesia, Analgesia, & Euthanasia

Ryan Navarro, DVM, MS

Rodent Seminar Series

Department of Molecular and Comparative Pathobiology

3/27/24



Outline

- Anesthesia
 - Choosing an anesthetic plan
 - Common anesthetic protocols in rodents
- Analgesia
 - Importance of pre-emptive and post-operative analgesia
 - Common analgesic agents in rodents
 - Multimodal pain control
- The Anesthetic Event
 - Monitoring the anesthetized patient
 - Anesthetic recovery & post-operative care
- Euthanasia
 - Acceptable and unacceptable methods
- The ACUC protocol & amendments
- Resource list

*Applications &
Take Home Messages
for investigators and
lab members*



*See JHU ACUC
Guidelines!*



Animal Care and Use Committee

Search

CONTACT

- Protocol Schedule
- Forms
- Guidelines
- Training
- Announcements
- Links
- Animal Welfare Concerns
- Emergency Information



Animal Care and Use Committee



Definitions

- Anesthesia: a drug-induced, reversible condition that induces unconsciousness and depresses sensory and motor responses
- Analgesia: lack of response to noxious/painful stimuli; absence/relief from pain
- Euthanasia: intentional ending of life to end/prevent pain and/or suffering

“Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia.”

- PHS Policy (2015)

“An adequate veterinary care program consists of assessment of animal well-being and effective management of...

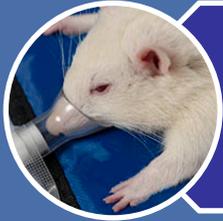
- *Surgery and perioperative care*
- *Pain and distress*
- *Anesthesia and analgesia*
- *Euthanasia*

- Guide for the Care and Use of Laboratory Animals (2011)



ANESTHESIA

Anesthesia and sedation



General anesthesia: a drug-induced, reversible condition that induces unconsciousness.



Deep sedation: depression of consciousness to allow for brief/minimally invasive procedures; patient typically maintains oxygenation and cardiovascular function



Locoregional anesthesia: blocks sensation of pain/stimulation along certain pathways (e.g. line block, epidural)

- Often used in conjunction with sedation/general anesthesia

4 Functions of General Anesthesia



UNCONSCIOUSNESS
(NOT AWAKE)



AMNESIA
(NO MEMORY)



ANALGESIA
(NO PAIN)



IMMOBILITY
(NO MOVEMENT)

*Not all anesthetic agents accomplish
all of these functions perfectly;
multimodal anesthesia is ideal!*

Components of the anesthetic event

- Premedication
 - Injectable drugs that have rapid onset, reduce stress or induce calm, and facilitate rapid and uneventful induction
 - Decreases the amount of general anesthesia needed
- Induction
 - Places animal on appropriate anesthetic plane
 - Inhalants or injectable agents
- Maintenance
 - Allows anesthesia to be maintained throughout the necessary time period
 - Inhalants are most common form of maintenance
- Recovery / post-operative period

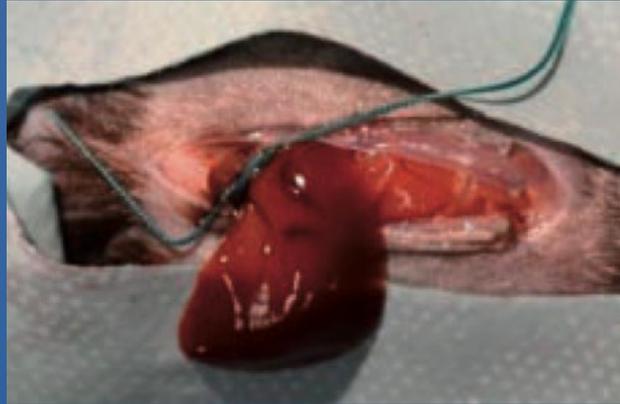
Targeting drugs to each component of the anesthetic event provides more balanced anesthesia



Choosing an anesthetic plan

Procedure-related considerations:

- What organ system?
- How invasive?
- How long?
- Painful?



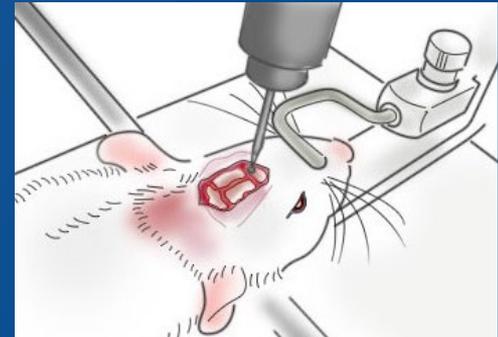
Liver lobectomy



Gut injection



Liver core biopsy



Stereotaxic surgery

Choosing an anesthetic plan

Patient-related factors

- Species, strain/stock
- Health status, age, etc.
- Prior research manipulations

Appropriate patient selection

- Use healthy animals
- Appropriate acclimation period

Side effects of anesthetic agents

- Most anesthetics cause dose-dependent depression of physiologic homeostatic functions; changes vary depending on the agent.
- Some agents have specific side effects – e.g. ketamine can cause tremors



Geriatric rat



Baby chinchilla

Inhalant anesthetics

- Inhalant anesthetics are vapors
- Administered and metabolized through the lungs
 - Predictable speed of onset
- Most common inhalant anesthetics in veterinary medicine = isoflurane and sevoflurane
- Saturated vapor pressure – amount of inhalant liquid that will vaporize into air; much higher than the amount needed for anesthesia
- Delivered via vaporizer (preferred) or drop method
- **Scavenging system** collects waste vapors (dedicated exhaust; hard-ducted biosafety cabinet; fume hood; CO2 canister)



Do not use cage changing stations and Class II A2 biosafety cabinets when using drop-jar method.

Dosing inhalants using vaporizer

- Safest for patient and personnel
- Mixes the saturated vapor with oxygen to dilute it to a clinically useful concentration
- Allows fine adjustment of inhalant %
- Rapid adjustment of anesthetic depth
- Vaporizer calibration is required (every 3 years for isoflurane, or more frequently as needed)

See JHU ACUC
Guidelines!



Johns Hopkins University 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.

B. PROCEDURES TO REDUCE EXPOSURE

Equipment and system maintenance for anesthesia machines and vaporizers

1. All anesthetic vaporizers must undergo calibration verification by a professional service technician as recommended by the manufacturer and be serviced if necessary.
2. If no such recommendation exists, the following schedules apply:
 - Halothane vaporizers-** Calibration verification must be performed *annually* because halothane's properties lead to increased clogging of internal vaporizer components.
 - Isoflurane vaporizers-** Calibration verification must be performed at least *every 3 years*. If the machine is subject to extensive use (e.g., > 500 hrs/year) or is frequently moved to different locations, then verification must be performed annually.
3. A copy of the manufacturer's guidelines for calibration verification must be available in the laboratory to assist with oversight by the ACUC of proper maintenance of anesthetic equipment.
4. Documentation of equipment validation must be affixed to each anesthesia machine or vaporizer that is in service.

Dosing inhalants using vaporizer

MICE

Inhalant Anesthesia

Agent(s)	Dose	Comments/Reference(s)
Isoflurane	3-5% Induction 1-3% Maintenance	Administer via precision vaporizer and compressed oxygen or drop method

- Minimum Alveolar Concentration (MAC) – the amount of inhalant in the lungs that produces a lack of purposeful movement in response to a noxious stimulus, in 50% of the population
- Surgical MAC = 1.3-1.5x MAC – produces lack of purposeful movement in 95% of the population
- Every animal is different – titrate to effect

Use of inhalants

- Induction
 - Inhalant-only induction
 - Induction box
 - Odor is unpleasant, can be irritating to upper respiratory tract
 - Animals may struggle
 - Induction after premedication with injectable agent
 - Nose cone
- Maintenance
 - Nose cone
 - Intubation



Induction box



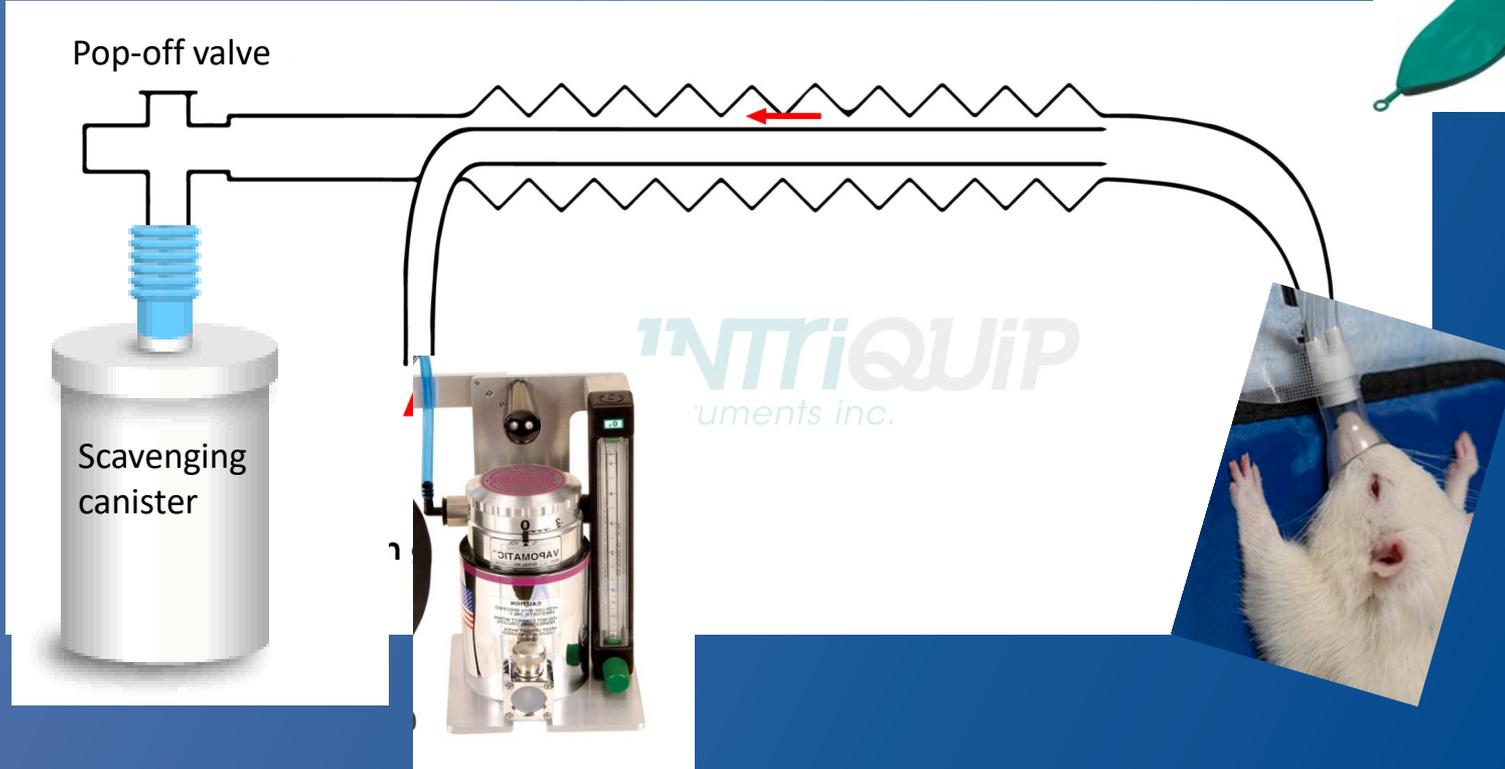
Nose cone



Device to prepare for intubation

*You can borrow
anesthetic machines
from RAR.*

Non-rebreathing anesthetic circuit



Somnosuite

- All-in-one low-flow anesthesia system
- Precision syringe pump
- Integrated digital vaporizer – uses room air or compressed gas
- Less waste anesthetic vapors
- Ideal when space is limited
- Designed specifically for mice and rats
- Can be purchased from Kent Scientific



Somnosuite low-flow anesthesia system; Voziyanov et al. 2016

Drop Method

See JHU ACUC
Guidelines!

- Drop method = permitting anesthetic liquid to vaporize in proximity to animal
- Wet gauze/cotton with isoflurane mixture
 - Mice: 20% v/v isoflurane in propylene glycol
 - Rats: 30% v/v isoflurane in propylene glycol
- Inhalant anesthetics can irritate skin upon contact!
 - Place absorbent material far away from the skin/nose or in a barrier like a tissue cassette
- Difficult to adjust and monitor anesthetic depth
- Occupational safety – perform in a fume hood or hard-ducted BSC to collect waste vapors



Nose cone

Do not allow anesthetic liquid to come in contact with the animal's skin!

Drop Method

See JHU ACUC
Guidelines!

- Induction – chamber
 - Provides <1 minute of surgical plane anesthesia once animal is removed from the chamber
- Maintenance – nose cone
 - Up to ~8 minutes of anesthesia
 - 15 mL conical tube for mice
 - 50 mL conical tube for rats
 - Adjust depth of anesthesia by moving nostrils closer or further away from end of the cone
 - Do not create a vacuum between nose and tube!
 - Must allow oxygen flow



Induction chamber



Nose cone

Side effects of inhalant anesthetics

- Respiratory depression
- Hypotension (due to vasodilation)
- Hypothermia
- Side effects of inhalants can be reduced by employing multimodal anesthesia and by giving analgesics
 - “MAC-sparing” effects

Common injectable anesthetic agents in rodents

Drug	Class/MoA	Notes
Ketamine	NMDA Antagonist; dissociative anesthetic	<ul style="list-style-type: none">- Tremors/seizures can occur- Provides some analgesia – bone pain
Xylazine	Alpha-2 adrenergic agonist	<ul style="list-style-type: none">- Provides some analgesia – visceral pain- Muscle relaxation- Reversible with yohimbine
Acepromazine	Dopamine receptor antagonist	<ul style="list-style-type: none">- Does not provide analgesia- Muscle relaxation
Midazolam	Benzodiazepine (GABA agonist)	<ul style="list-style-type: none">- Causes sedation and amnesia; does not cause loss of consciousness when used alone- Does not provide analgesia- Muscle relaxation- Reversible with flumazenil
Pentobarbital	Barbiturate	<ul style="list-style-type: none">- Does not provide analgesia- Muscle relaxation
Avertin (tribromoethanol)	Alcohol compound	<ul style="list-style-type: none">- Does not provide analgesia- Severe peritonitis; only administer IP <u>once</u>- <u>Not pharmaceutical grade</u>



Pharmaceutical-Grade Drugs

See JHU ACUC
Guidelines!



- Pharmaceutical grade: any drug approved, conditionally approved, or indexed by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written or established by a recognized compendia
- Must use pharmaceutical-grade drugs unless:
 - Pharmaceutical-grade alternative is not available
 - Valid scientific justification
 - Cost savings alone is not a valid justification
 - Specifically reviewed and approved by the ACUC
- ACUC protocol: “will all drugs used for sedation, anesthesia, analgesia, or euthanasia be “pharmaceutical grade”?”
 - Check “No” if you are going to mix drugs before administering!
 - Once diluted or mixed, no longer considered pharmaceutical grade
 - Mixing anesthetic drugs to reduce the number of injections is a valid justification!

***Mixing anesthetic
drugs into one syringe
renders them non-
pharmaceutical grade***

Example: Tribromoethanol

A non-pharmaceutical grade drug

*See JHU ACUC
Guidelines!*



- Historically used for many procedures
- NO analgesia
- Many risks associated with Avertin use
 - Toxic by-products due to light exposure and storage at incorrect temperature
 - Potent GI irritants → fibrinous peritonitis, ileus and fatalities
 - Variable concentration by batch and over time
- Effects on research unknown
- Better alternatives available that are safer and pharmaceutical grade

Controlled Drugs

- Federal regulations (Controlled Substances Act) – Schedules I-V depending on potential for abuse and whether they have a medical use
- Examples: fentanyl, buprenorphine, ketamine, midazolam, pentobarbital
- **Labs must have their own DEA license to obtain** (except buprenorphine-SR)
- Must be securely stored (2 locks)

See JHU ACUC Guidelines!



Controlled Drugs

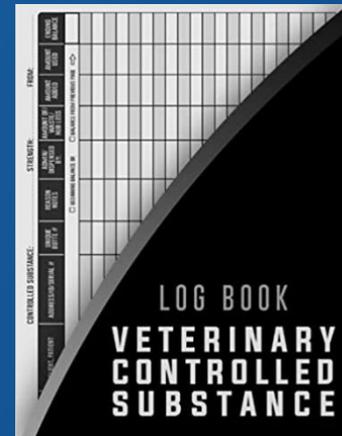
- You MUST keep record of their use
 - Bound notebook dedicated to this purpose
 - Date of use
 - Volume obtained / used
 - Animal ID & purpose
 - Balance
 - Your initials
- Drugs withdrawn and not used must be “wasted”
 - Rendering substances “non-retrievable” under witness, and documenting this in the controlled drug log
- Expired drugs
 - Record on DEA Form 41 and dispose of according to DEA instructions
- Drug logs are reviewed at semiannual ACUC inspections and deficiencies must be corrected

See JHU ACUC Guidelines!



PAGE 1 OF _____
DRUG NAME: Buprenorphine SR BOTTLE #: 22-7
CONCENTRATION: 1 mg/mL LOT #: BSRLAB1-221003
EXPIRATION DATE: 2/28/2023 ADJUSTED EXPIRATION DATE*: _____

STARTING BALANCE**			WASTED					
DATE	USED	BALANCE	PI	ANIMAL ID	INITIALS	AMOUNT	INITIALS	WITNESS
		5						



Every use of all controlled substances must be logged!

Routes for injectable drugs

- Intraperitoneal (IP)
 - Simple, very commonly used
 - Max volume: 2-3 mL (mice), 3-5 mL (rats)
- Subcutaneous (SQ, SC)
 - Max volume: 2-3 mL (mice), 2-5 mL (rats)
- Intravenous (IV)
 - Max volume: 0.5 mL (mice), 1-2 mL (rats)
- Intramuscular (IM)
 - Discouraged in rodents due to limited muscle mass
 - Can be given in epaxial or quadriceps muscles
 - Max volume: up to 0.1 mL divided into 3 sites (mice); up to 0.2 mL divided into two sites (rats)



What drug combination, dose, & route do I select?

- JHU rodent formulary
- Veterinary consultation
- Study-specific needs
- Doses and routes must be specified in the ACUC protocol and strictly observed

Refer to the JHU rodent formulary for ACUC-approved drug combinations

See JHU ACUC Website



MICE

Inhalant Anesthesia

Agent(s)	Dose	Comments/Reference(s)
Isoflurane	3-5% Induction 1-3% Maintenance	Administer via precision vaporizer and compressed oxygen or drop method

Injectable Anesthesia

Agent(s)	Dose	Comments
Ketamine Xylazine	80-100 mg/kg IP 5-10 mg/kg IP	Surgical anesthesia
Ketamine Acepromazine	100 mg/kg IP 5 mg/kg IP	Immobilization/anesthesia
Ketamine Midazolam	100 mg/kg IP 5 mg/kg IP	Immobilization/anesthesia
Ketamine Xylazine Acepromazine	80-100 mg/kg IP 10-20 mg/kg IP 2-3 mg/kg IP	Surgical anesthesia
Pentobarbital	40-60 mg/kg IP	Considerable dose variation by strain, gender, genetic modifications etc. Starting at low end of dose range is advisable. Note: Euthanasia dose is 90-100 mg/kg or greater
Tribromoethanol (Avertin)	200-500 mg/kg IP	Non-pharmaceutical grade; special preparation and storage required; Adverse effects likely with repeat dosing

Calculating injectable drug doses

- Doses typically given in mg/kg (milligrams drug per kg body weight)
 - 1 kilogram = 1000 grams
 - Typical mouse = 25 grams (0.025 kg)
 - Typical rat = 300-500 grams (0.3-0.5 kg)
- Concentration of drug typically given in mg/mL (milligrams of drug dissolved in 1 mL of liquid)
- Formula = $(\text{Dose} \times \text{Body Weight}) / \text{Concentration}$
- Example: $(50 \text{ mg/kg} \times 0.5 \text{ kg}) / 100 \text{ mg/mL} = 0.25 \text{ mL}$

Neuromuscular blocking agents

See JHU ACUC
Guidelines!

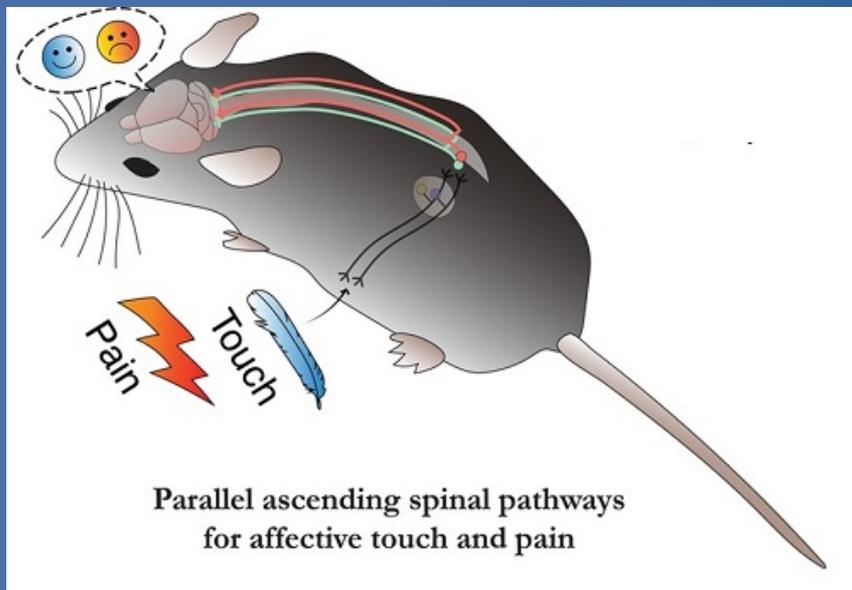


- Cause immobility by paralyzing the animal
- Examples: pancuronium, rocuronium, succinylcholine
- NOT sufficient as a sole agent to immobilize an animal for a procedure
 - *“Procedures that may cause momentary or slight pain or distress to the animal will not include the use of paralytics without anesthesia.” – Animal Welfare Act*
 - *“Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.” – The Guide*
 - *“Acute stress is believed to be a consequence of paralysis in a conscious state and it is known that humans, if conscious, can experience distress when paralyzed with these drugs.” – The Guide*
- Can be used in conjunction with anesthesia
- Use requires justification in the ACUC protocol
- Paralyzed animals cannot breathe spontaneously and must be ventilated



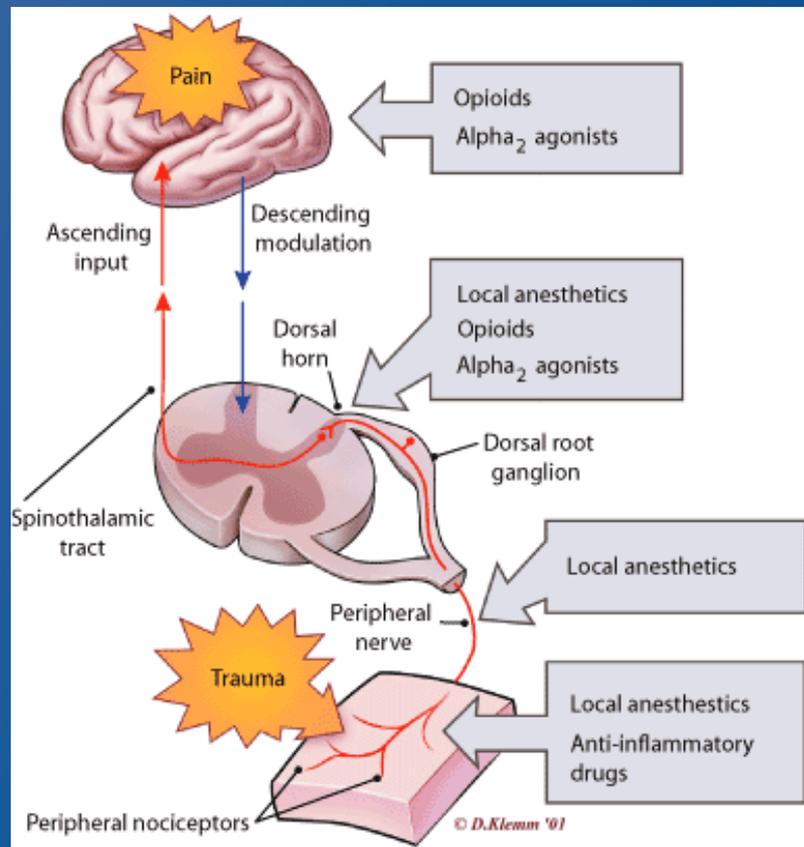
Anesthesia of neonates

- Most commonly used method in mouse and rat neonates up to 7 days of age is cryoanesthesia (aka deep hypothermia)
 - Young altricial rodents cannot regulate body temperature and can be rapidly cooled due to small body surface area (5-10 minutes)
 - Resistant to brain damage associated with circulatory arrest
 - Provides immobilization for 5-15 minutes, and mild analgesia
 - Never place animal in direct contact with ice (potential for skin injury)
- Recovery:
 - Place recovering pups on water circulating heating pad for at least 5 min
 - Complete recovery can take up to 30-60 minutes
 - Pups must be warm, pink, and spontaneously moving before being returned to the dam, to avoid maternal rejection
- Neonates >P7 must be anesthetized with inhalant or injectable anesthetics



Choi et al., 2020.

ANALGESIA



Gottschalk & Smith, 2001 32

Why do we use analgesics?

- *Doesn't general anesthesia cause analgesia?*
 - Anesthetized animals do not have conscious perception of pain = no voluntary response to a toe pinch
 - Some anesthetic agents actually block pain pathways, but many do not
 - If the anesthetic agents are not actually analgesics, then:
 - Autonomic stimulation can still occur (e.g. heart rate jumps up during surgery, even in an unconscious patient)
 - Analgesic effects will not persist after consciousness is regained, but the pain of surgery will still be present!

*“Unless the contrary is established, investigators should consider that **procedures that cause pain or distress in human beings may cause pain or distress in other animals.**”*

- Guide for the Care and Use of Laboratory Animals (2011)



Pre-emptive analgesia

- Given after anesthetic induction and before the first incision is made
- Benefits:
 - Prevents sensitization
 - Once pain signaling pathways begin to fire in response to tissue damage, the nociceptive system is sensitized and pain is harder to treat
 - Reduces acute post-operative pain
 - Can prevent development of chronic pain
- Ideally, therapeutic blood levels of the analgesic agent are reached before animal recovers from anesthesia

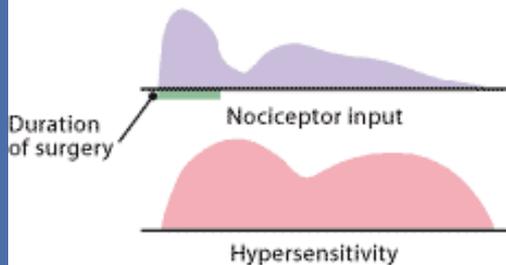
“Preemptive analgesia... enhances intraoperative patient stability and optimizes postoperative care and well-being by reducing postoperative pain. ... Analgesia may be achieved through timely enteral or parenteral administration of analgesic agents as well as by blocking nociceptive signaling via local anesthetics”

- Guide for the Care and Use of Laboratory Animals (2011)

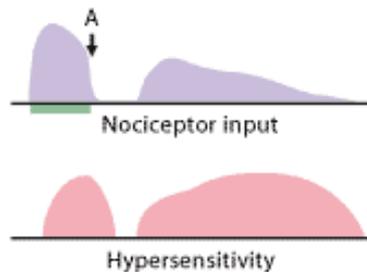
Pre-emptive analgesia can be given right after anesthetic induction and before first incision. Therapeutic blood levels should be reached by the time animal recovers from anesthesia.

Multimodal analgesia

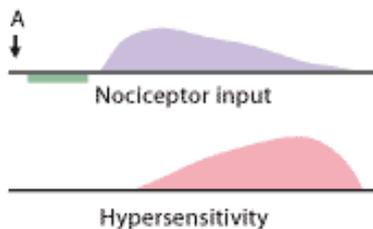
A. Surgical and postsurgical afferent input



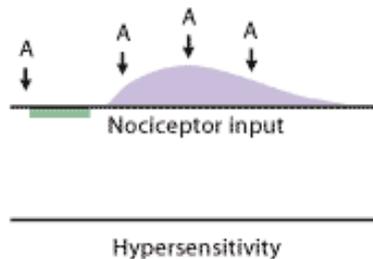
B. Postsurgical analgesia



C. Presurgical analgesia



D. Presurgical and postsurgical analgesia



Pre-emptive and post-operative analgesia work together for the most effective pain control.



Opioids

- Full mu agonists – morphine, oxymorphone, fentanyl, etc.
 - Best pain control
 - Also causes sedation
- Partial mu agonists – **buprenorphine**
 - Moderate pain control
 - Moderate sedation
 - Comes in long-acting formulation
- Kappa agonist, mu antagonist – butorphanol
 - Least pain control
 - Effective for sedation
- Reversible with naloxone (but may require repeated reversal)

Buprenorphine

See JHU ACUC
Guidelines!



- Advantages:
 - Long duration of action = improved analgesia, good compliance
 - Mild depressive effects on cardiovascular and respiratory systems
- Disadvantages:
 - Slow onset of action (~30 minutes) when given SC
 - Decreased food and water intake, decreased GI motility
 - Potential dysphoria
 - Pica in rats

The dose and duration of efficacy of bup-SR differ between rats and mice!

Formulation	Mouse	Rat
Regular release buprenorphine	0.05 - 0.1 mg/kg SC, IP q8-12h	0.01-0.05 mg/kg SC, IP q6-12h
Sustained release (Buprenorphine-SR) (ZooPharm)	0.5-1 mg/kg SC q48h	1-1.2 mg/kg SC q72h

Sustained-release formulations of buprenorphine

See JHU ACUC Guidelines!



- Provides sustained analgesia for 48-72 hours depending on species
- ZooPharm - Buprenorphine-SR
 - Compounded; not pharmaceutical grade
 - Very viscous – need to use large gauge needle to draw up
 - DEA is not required to purchase
 - **Must still be logged in Controlled Drug logs!**

The RAR website has information on drug ordering, especially for procurement of Bup-SR.

Non-steroidal anti-inflammatory drugs (NSAIDs)

- Inhibit prostaglandin synthesis -> anti-inflammatory effects
- Mild analgesic and anti-pyretic activity
- Can be used with opioids for multimodal analgesia
- **Carprofen (Rimadyl):**
 - Mice: 4-5 mg/kg PO, SQ q24h
 - Rats: 5-10 mg/kg PO, SQ q24h
- **Meloxicam (Metacam):**
 - Mice: 1-5 mg/kg PO, SQ q24h
 - Rats: 1-2 mg/kg PO, SQ q24h
- Do not administer two different NSAIDs simultaneously! Risk of gastrointestinal injury or renal failure
- Do not administer in conjunction with steroids – GI injury
- Can confound results of some studies

Carprofen-SR and Meloxicam-SR exist, but also only provide 24 hours of efficacy! (therefore, not recommended)

GUIDELINES ON ANALGESIA FOR RODENT SURGERIES

OBJECTIVE: To establish the minimum required analgesic regimens for rodent survival surgeries.

SCOPE: This applies to all rodents under the Johns Hopkins University animal care and use program. Exemptions to the minimum requirements may be approved with appropriate scientific justification and must be described in the ACUC-approved protocol.

PAIN CATEGORIZATION: The following provides general guidelines in the determination of the severity of pain associated with surgical procedures in rodents.

- **Minimal to mild pain:** Includes procedures that cause momentary pain or pain of low intensity that does not have long-lasting consequences.
- **Mild to moderate pain:** Procedure that cause more than momentary pain, and are known to be painful in humans hours to days after the procedure/surgery is performed. Would cause rodents to be visibly painful by displaying any one of the following behaviors if no analgesics were given (weight loss, decreased grooming, decreased activity, dark red material around the eyes of rats, hunched posture).
- **Moderate to severe pain:** Any procedure that causes intense pain, or a moderate pain that last days to weeks after the procedure is completed. This may include any surgery that induces a chronic pain typically associated with degenerative diseases (e.g., osteoarthritis).

MINIMUM ANALGESIA REQUIREMENTS¹

	Minimal to mild pain	Mild to moderate pain	Moderate to severe pain
Pre-emptive analgesia ²	<p>Single dose of systemic NSAID (e.g. meloxicam or carprofen)</p> <p style="text-align: center;">OR</p> <p>Opioid (e.g. buprenorphine) prior to surgery</p>	<p>Systemic NSAID (e.g. meloxicam or carprofen)</p> <p style="text-align: center;">OR</p> <p>Single dose of buprenorphine SR³ prior to surgery</p>	<p>Systemic NSAID (e.g. meloxicam or carprofen)</p> <p style="text-align: center;">AND</p> <p>Single dose of buprenorphine SR³</p>
Intra-operative analgesia			Lidocaine and bupivacaine
Post-operative analgesia ⁴	PRN	<p>NSAID q 24h for 1 additional day (not necessary if buprenorphine SR³ was administered pre-emptively)</p> <p>PRN after 1 day post-op</p>	<p>NSAID q 24h for 2 additional days</p> <p>PRN after 2 days post-op</p>



THE ANESTHETIC EVENT

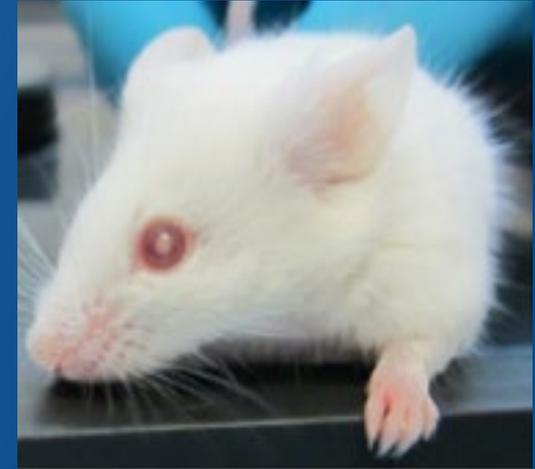
Preparing for anesthetic event

- All necessary drugs are available and not expired
 - Every drug described in the protocol that this patient will or may receive
 - Includes isoflurane and oxygen tanks for anesthesia
- All necessary equipment is available
 - Surgical equipment, gloves, etc. – must be sterile!
 - Anesthetic and monitoring equipment
- Pre-anesthetic fasting not necessary
- Personnel are trained and prepared for emergencies

*Before
anesthetizing an
animal, have
ALL drugs and
equipment
ready!*

Perioperative care

- Corneal lubrication is required in all anesthetized animals!
 - Animals cannot blink when anesthetized
 - Prevents corneal desiccation and ulceration
 - Always lubricate eyes!
- Consider fluid therapy
 - LRS or 0.9% Saline
 - IP or SC
- Individual patient considerations



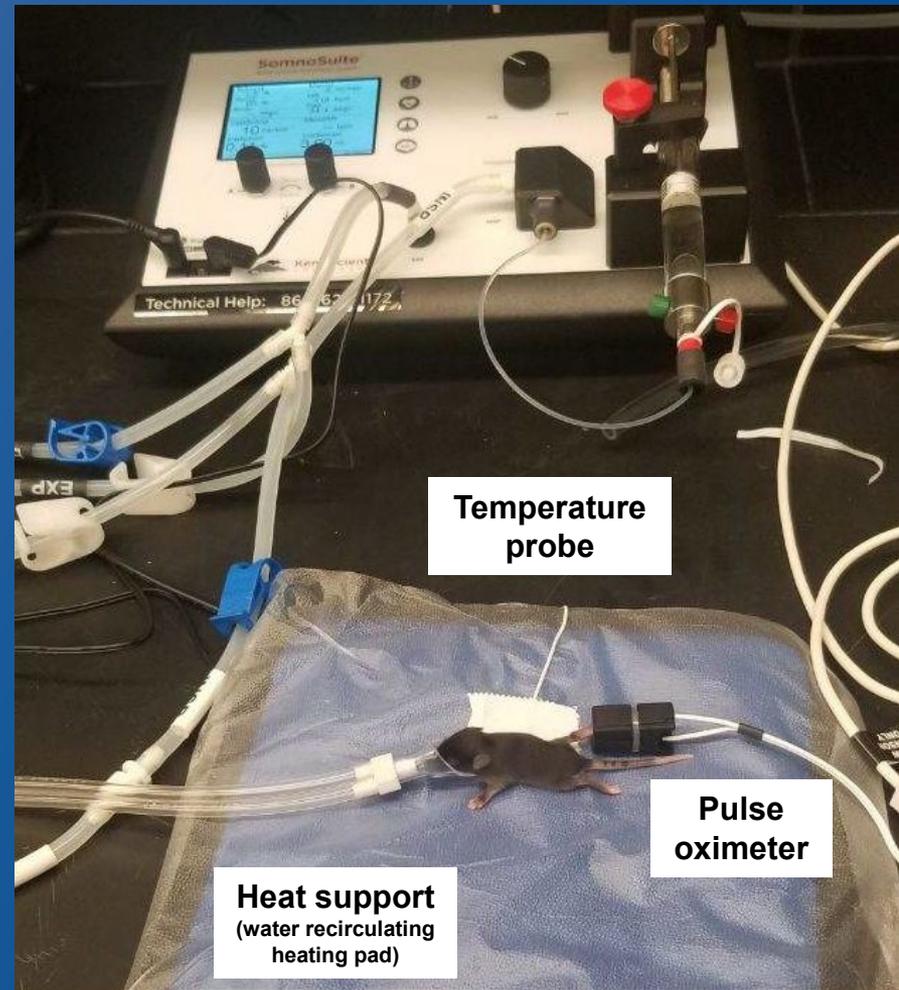
Anesthetic complications

- Anesthesia blunts autonomic responses (cardiovascular, respiratory, thermoregulatory)
- Most common anesthetic complications:
 - Hypothermia
 - Hypotension
 - Bradycardia
 - Hypoventilation

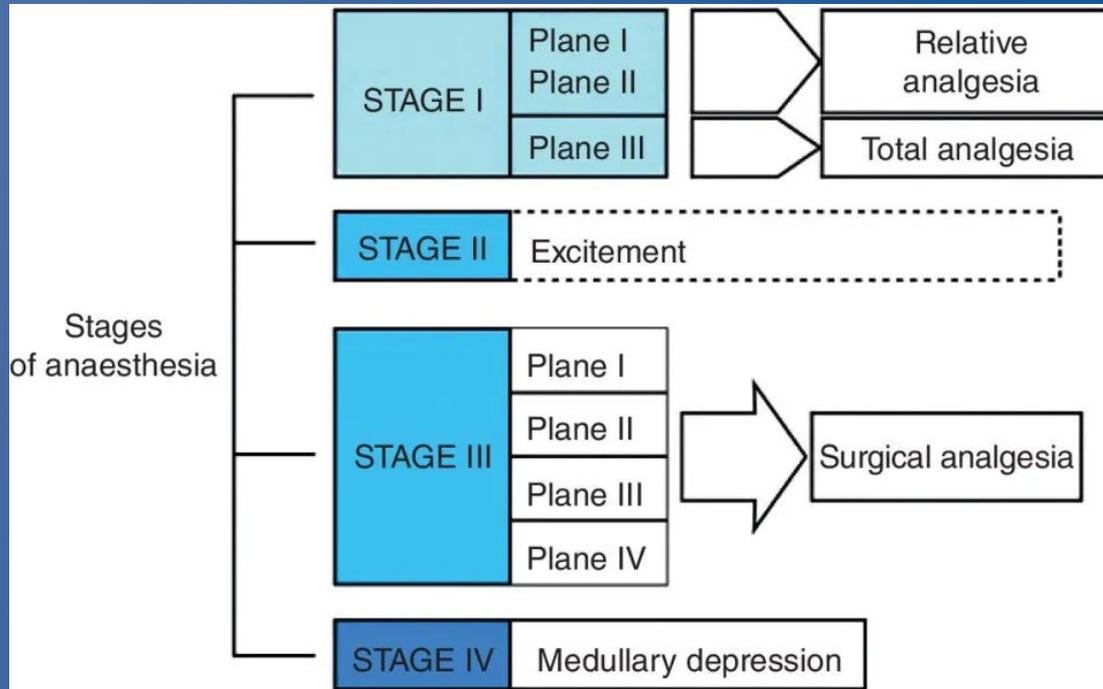


Intraoperative monitoring

- Evaluation of anesthetic depth
- Monitoring of physiologic functions
 - Body temperature
 - Heart rate
 - Respiratory rate and pattern
 - Blood pressure
- Documentation
 - Record vital parameters every 10-15 minutes, or more often if patient is not stable



Anesthetic depth



*Guedel's
stages of
anesthesia*

- **Toe pinch:** pinch toe with fingers (not forceps) – animal at surgical plane of anesthesia cannot consciously perceive noxious stimulus and therefore should not pull the leg away, twitch limb, or vocalize

Cardiovascular Monitoring

- **Pulse:** rate, rhythm, quality
 - Difficult in rodents!
 - Stethoscope, pulse oximeter, ECG
- **Perfusion**
 - Capillary refill time (<2 seconds)
- **Oxygenation**
 - Pulse oximetry
 - Mucous membrane color
- **Blood pressure**
 - Indirect/non-invasive oscillometric blood pressure cuff / Doppler
- Rodent-adapted pulse ox, BP cuffs and ECG are available!



Respiratory Monitoring

- Observe respiration frequency and pattern
 - TOO LIGHT:
 - Thoracic only, fast, shallow
 - Surgical anesthesia plane
 - Abdominal and thoracic, regular pattern
 - TOO DEEP:
 - Abdominal only, slow, gasping
- Capnographs available to monitor breaths and end-tidal CO₂; can be adapted for face masks

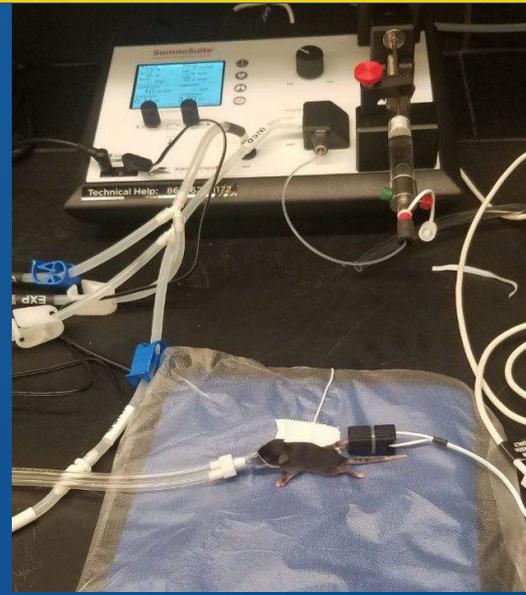


Temperature Monitoring

- Hypothermia risk!
 - Large body surface area to mass ratio
 - Heat dissipates from ears, tail, feet
 - Anesthesia blunts thermoregulation
- Hypothermia prolongs anesthetic recovery
- Thermometer and rectal probes are available for perioperative monitoring

Avoiding hypothermia

- Minimize excessive shaving
- Avoid getting surgical scrub/alcohol on fur outside surgical field
- Minimize surgery time
 - Have all supplies ready before you start
 - Work efficiently
- Minimize length of incision
- Provide heat support during and after surgery
 - Active warming – heating pad (water recirculating pad ideal; electric coil pads can lead to thermal injury!); warm fluid bags
 - Passive warming – bubble pack (relies on animal to generate heat)
- Warm fluids to physiologic temperature



Water recirculating heating pads are preferable because electric coil pads can lead to burns

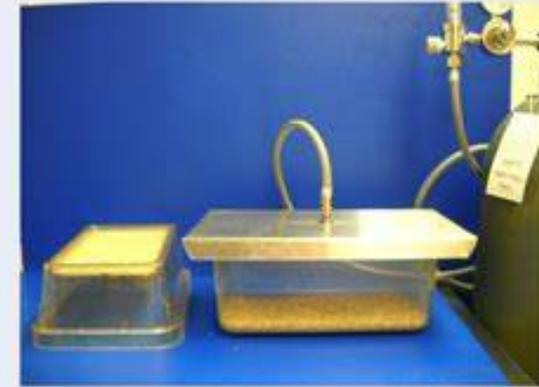
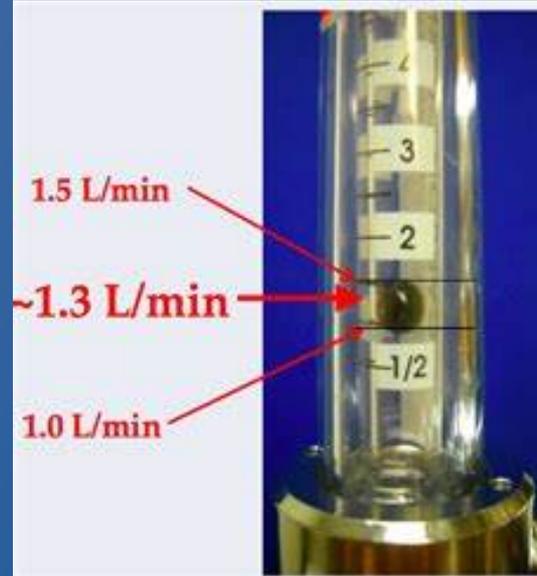
Anesthetic recovery

- Ideally, allow animal to breathe 100% oxygen for a few minutes after discontinuing inhalant anesthesia
- Monitor animal during recovery
 - Can be placed in home cage in procedure room and continue to be monitored
 - Do not recover animal in same cage as unanesthetized cagemates (injury risk)
- Animal must be fully recovered before returning to animal housing facility!
 - Can hold itself upright (sternal) and move spontaneously
 - Returning an animal that is still anesthetized or sedated is dangerous for the animal, and could also result in a clinical call being placed for moribundity
- Ideally, check on animal again at the end of the day before you leave



Post-operative care

- Monitoring:
 - Daily or twice daily in the first 72 hours post-operatively (most post-operative pain occurs during this period)
 - Daily until sutures are removed or incision heals (10-14 days)
- Post-operative pain control
 - Specify in ACUC protocol whether pain control will be prophylactic vs. based on assessment
 - Consider what animal already has on-board from pre-operative analgesia
 - Methods of post-operative pain assessment must be specified in the protocol (see Humane Endpoints seminar)



EUTHANASIA

Euthanasia

- Definitions
- Acceptable euthanasia methods
 - Primary and secondary methods
- Unacceptable euthanasia practices
- Interference of euthanasia methods with certain models
- Death must always be confirmed!

****All methods described in this presentation are specific to Section 2.2 pertaining to small laboratory rodents***

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*

Members of the Panel on Euthanasia

Steven Leary, DVM, DACLAM (Chair); Fidelis Pharmaceuticals, High Ridge, Missouri
Wendy Underwood, DVM (Vice Chair); Indianapolis, Indiana
Raymond Anthony, PhD (Ethicist); University of Alaska Anchorage, Anchorage, Alaska
Samuel Cartner, DVM, MPH, PhD, DACLAM (Lead, Laboratory Animals Working Group);
University of Alabama at Birmingham, Birmingham, Alabama
Temple Grandin, PhD (Lead, Physical Methods Working Group); Colorado State University, Fort Collins, Colorado
Cheryl Greenacre, DVM, DABVP (Lead, Avian Working Group); University of Tennessee, Knoxville, TN
Sharon Gwaltney-Brant, DVM, PhD, DABVT, DABT (Lead, Noninhaled Agents Working Group); Veterinary Information Network, Mahomet, Illinois
Mary Ann McCrackin, DVM, PhD, DACVS, DACLAM (Lead, Companion Animals Working Group);
University of Georgia, Athens, Georgia
Robert Meyer, DVM, DACVAA (Lead, Inhaled Agents Working Group); Mississippi State University, N

S2.2 SMALL LABORATORY AND WILD-CAUGHT RODENTS (MICE, RATS, HAMSTERS, GUINEA PIGS, GERBILS, DEGUS, COTTON RATS, ETC)

All activities related to the euthanasia of rodents deserve consideration equivalent to the euthanasia method itself, and may factor into the choice of meth-

Definitions

- **Euthanasia**: intentionally ending life to end/prevent pain and suffering
 - Greek - eu (good) + Thanatos (death)
- **Methods of euthanasia as defined by AVMA Guidelines:**
 - **Acceptable** - reliably meet requirements of euthanasia
 - **Acceptable with conditions** - reliably meet the conditions of euthanasia, when specified conditions are met
 - **Unacceptable** - do not meet the requirements of euthanasia.
 - **Primary method** - a method of euthanasia utilized on an awake animal; may require subsequent use of a secondary method to ensure death
 - **Adjunctive (aka secondary) method** - a method of assuring death, used after an animal has been made unconscious

Acceptable methods

*See JHU ACUC
Guidelines!*



- Barbiturates and barbituric acid derivatives
 - Pentobarbital / “Euthanasia solution” administered IP
 - Euthanasia dose is typically 3 times the anesthetic dose
- Dissociative agent combinations
 - Ketamine in combination with either alpha-2 adrenergic agonist (xylazine) or benzodiazepine (diazepam)
- These methods can be used as a sole method of euthanasia (death must still be confirmed)

Acceptable with conditions

*See JHU ACUC
Guidelines!*



- Halogenated anesthetics
 - Isoflurane, sevoflurane, etc.
- Carbon dioxide
 - 30-70% vol/min displacement of the chamber
 - Increased distress due to dyspnea at lower flow rates
 - Mucous membrane pain due to higher flow rates
 - Prefilled chambers not recommended (pain upon inhalation)
 - Euthanasia should be conducted in home cage or euthanasia chamber emptied and cleaned between uses



Johns Hopkins University Animal Care and Use Committee

See JHU ACUC
Guidelines!

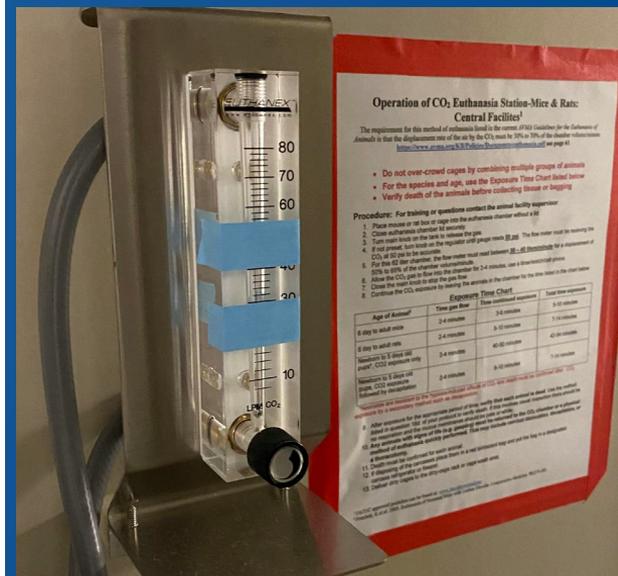


Euthanasia of Mice and Rats Using Carbon Dioxide*

PURPOSE: This document provides guidance on the correct procedures to follow when euthanizing mice and rats using carbon dioxide.

BACKGROUND: Euthanasia of animals at Johns Hopkins University must be carried out according to the most recent guidance of the American Veterinary Medical Association (AVMA).² Carbon dioxide (CO₂) inhalation is a common method of euthanasia used for rats and mice. It is the method that will be used by central facilities staff with mice and rats identified for euthanasia by researchers. Use of CO₂ euthanasia by researchers must be included in an ACUC-approved protocol. Appropriate technique, equipment, and source of CO₂ must be used. Compressed CO₂ gas in cylinders is the only approved source because the flow of gas to the euthanasia chamber can be regulated precisely. The practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% CO₂, is unacceptable. **CO₂ generated by other means such as dry ice, fire extinguishers, or chemical means (e.g., antacids) is also unacceptable.**

Upon completion of the procedure, death must be confirmed for each animal by one or more of the methods listed below, as approved in the ACUC protocol. It is important to understand that short-term CO₂ exposure produces anesthesia. So failure of the animal to move or show a reflex response is not sufficient to confirm death. **Disposal of an anesthetized, rather than a euthanized, animal is a serious animal welfare concern.** Understanding how to avoid this is the responsibility of anyone carrying out euthanasia with CO₂.



Central facilities euthanasia station with markings for flow meter settings and instructions for use of the station

Changes to the guidelines on the euthanasia of mice and rats using carbon dioxide (CO₂)

Contact Jason Villano, DVM, Director of Rodent Resources
jvillano@jhmi.edu

Chamber	Flow rate (where the bobbin or ball should be)
Mouse cage	2.5-5 liters/min
Rat cage	5-10 liters/min
62-liter box	30-40 liters/min



Results

Using a mouse cage as temporary holding cage for euthanasia?



Using a critter carrier for euthanasia?



Use home cage as much as possible to reduce stress on the animals!

1. Up to **10 compatible mice** may be placed for **up to 30 minutes** and the holding cage is **not left unattended**.

2. If fighting is observed, immediately separate the animals.

3. Adult males \geq 6 weeks old from different cages should not be combined.

4. If only euthanizing pups less than 10 days of age, **up to 2 litters** may be combined in a single cage

Only place any one of the following:

1. One (1) adult mouse, or
2. One (1) litter (before weaning), or
3. Three (3) weanlings.

Acceptable with conditions

What is wrong with this picture?

- Cervical dislocation
 - Applying pressure to the neck to dislocate vertebral column from the skull
 - Loss of cortical function within 5-10 seconds
 - Mice and rats < 200 grams



Cervical dislocation

- Decapitation
 - Swiftly cutting the neck of the animal close to the head, using a **sharp** blade (guillotine, razor blade; scissors for neonates)
 - Loss of cortical function within 5-30 seconds
 - Specialized rodent guillotines are available
 - Can be used for neonates



Guillotine

Proficiency training is required for physical methods of euthanasia

Unacceptable methods

- The following are not acceptable for use as sole methods of euthanasia
- Nitrogen gas – almost impossible to achieve high enough concentration to cause unconsciousness and death
- Argon gas – highly aversive to rats
- Potassium chloride administered IV or IC
- Neuromuscular blocking agents
- Opioids – not rapidly acting, require high doses
- Urethane and alpha-chloralose - can be used as an anesthetic prior to application of an adjunctive method

If you have questions about a euthanasia method that is not covered in this presentation, contact the ACUC for further guidance.



Adjunctive methods (aka secondary methods)

- Should not be used as a sole method of euthanasia
- Acceptable to use to ensure death, when the animal is unconscious
- May be required when certain primary methods are used
- Cervical dislocation
- Bilateral thoracotomy
- Exsanguination
- Perfusion with fixatives
- Immediate harvest of vital organs (i.e., heart, lungs, or brain)
- Others depending on species

Include in the ACUC-approved protocol!

Euthanasia of fetuses and neonates

See JHU ACUC Guidelines!

- Mammalian fetuses are unconscious in utero
 - Low oxygen tension
 - Hormonal influences that suppress consciousness
- Afferent pain pathways in rat and mouse pups not well-developed until P5-7
- Precocial young must be treated as adults!



Mouse pups (altricial)



Chinchilla kit (precocial)



Guinea pig pups (precocial)

Euthanasia of Fetuses and Neonates

- Acceptable methods
 - For fetuses - euthanasia of the dam
 - Injectable agents – same as previously discussed for adults
- Acceptable with conditions
 - Inhalant anesthetics – requires adjunctive method
 - CO2 exposure – **requires prolonged exposure** or adjunctive method once nonresponsive to stimuli
 - Hypothermia – requires barrier between animal and cold source; requires adjunctive method; only appropriate in fetuses and altricial neonates <10 days of age
 - Rapid freezing in liquid N2 – mouse and rat fetuses, altricial neonates <5 days of age
 - Decapitation – altricial neonates
 - Cervical dislocation – fetal and neonatal mice and rats

CO₂ euthanasia exposure time chart

See JHU ACUC
Guidelines!

Exposure Time Chart

Age of Animal	Time of gas flow	Time of continued exposure	Total of time exposure
10 day to adult mice	2-4 minutes	3-6 minutes	5-10 minutes
10 day to adult rats	2-4 minutes	5-10 minutes	7-14 minutes
Newborn to 10 days old pups: CO₂ exposure only*	2-4 minutes	48-50 minutes	50-54 minutes
Newborn to 10 days old pups: CO₂ exposure immediately followed by secondary method.**	2-4 minutes	5-10 minutes	7-14 minutes

***Neonates are resistant to the hypoxia-induced effects of CO₂. Thus, CO₂ exposure time must be considerably longer if a secondary method, such as decapitation, is not performed.**

****Under the AVMA 2020 guidance, secondary methods, such as decapitation, may only be performed after the neonate is nonresponsive to painful stimuli (e.g., a toe pinch).**



Confirmation of Death

- Visual and physical examination
 - Heart has stopped beating
 - Animal is not breathing
 - Mucous membranes should be pale or white
- Observation that the animal fails to recover within 10 minutes after CO₂ exposure ends
- Secondary method can be utilized to ensure death (e.g. thoracotomy) such that prolonged observation is not necessary

Include in the ACUC-approved protocol!



Selecting a euthanasia method

- Consider research objectives in determining method of euthanasia
- Euthanasia methods can lead to metabolic and histologic artifacts
 - Isoflurane may artificially elevate blood glucose
 - IP injection of barbiturates can create artifacts in intestinal tissues
 - Euthanasia by CO₂ inhalation may elevate serum potassium concentrations and could alter metabolites in the brain
- Provide justification for euthanasia methods in the ACUC protocol!



Summary

- Choose an anesthetic plan appropriate for your species and model
- Be fully prepared before anesthetizing your animal!
- Not all anesthetic agents provide adequate analgesia
- Implement appropriate intra-operative and post-operative monitoring
- Implement pre-emptive and post-operative analgesia
- Choose a euthanasia method appropriate for your species and model
- Ensure that all anesthesia, analgesia, and euthanasia practices are detailed in the ACUC protocol and are followed

Links to Resources

- [JHU ACUC Website](#)
 - New / 3rd year renewal protocol form
 - [ACUC Guidelines](#)
- [RAR Website](#)
- [Guide for the Care and Use of Laboratory Animals \(8th Ed\) \(2011\)](#)
- [AVMA Guidelines on Euthanasia, 2020](#)



ACKNOWLEDGEMENTS

- Dr. Jason Villano and Dr. Mitchell Stover
- Kinta Diven and Jonathan Harrold
- Dr. Michael Wallace for preparation of this presentation

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- Dahl JB, Moiniche S. Pre-emptive analgesia. Br. Med. Bull. 2004;13(71):13-27.
- American Veterinary Medicine Association “AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.” 2020.
- NIH Office of Laboratory Animal Welfare. “Public Health Service Policy on Humane Care and Use of Laboratory Animals.” 2015.
- NIH. Institute of Laboratory Animal Resources, National Research Council. “Guide for the Care and Use of Laboratory Animals,” 8th Edition. 2011.
- United States Department of Agriculture, Animal Welfare Act and Animal Welfare Regulations, 2017.



Anesthesia, Analgesia, & Euthanasia: ACUC Office Perspective

Kinta Diven

ACUC Sr. Training & Compliance Specialists

Jon Harrold

8/24/22

Protocol Form: Questions 7, 14, 15, 16



Always obtain current version from ACUC Website

— **UPDATED FORM AVAILABLE WITH JULY 2022 RELEASE DATE**

Johns Hopkins University Animal Care and Use Committee (ACUC)
PROTOCOL FORM
Release Date: July 2022

New Protocol? Yes No
3rd Year Replacement of Protocol#: _____
Be sure to include procedures added by amendments over the last 3 years.
Do you currently have animals housed on this protocol? Yes No
Is this research funded by Department of Defense (DOD): Yes No

PROTOCOL TITLE: _____

For ACUC Use Only
 Original Revision
PROTOCOL #: _____
DATE RECD: _____
EXPIRATION DATE: _____
 Logged Database
BSL: 2 3 ABSL: 2 3

15. SURVIVAL SURGERY: (If more than one type will be performed fill out a separate question 15 for each one.)

Name of Surgical Procedure: _____

15a. MAJOR OPERATIVE PROCEDURE? Will this surgery penetrate and expose a major body cavity or cause substantial impairment of physical or physiologic function? Yes No

15b. Pre-anesthetic agents (e.g. sedatives to permit handling, intubation). *Name, dose, and route.*

15c. Pre-emptive analgesia (i.e., analgesia given prior to the surgical procedure). *Name, dose, and route.*

15d. Anesthesia. *Name, dose, and route.* Also state the method that will be used to assure the animal is anesthetized prior to initial incision and during the surgical procedure.

15e. Will a neuromuscular blocking agent (paralytic) (i.e., one that prevents respiration) be used at any point in the procedure? Yes No If "Yes", state name, dose, route and frequency of administration of paralytic agent(s). What parameter(s) will be used to determine that the animal remains anesthetized?

15f. Describe intra-operative procedures including: intubation, IV fluid delivery, monitoring, surgical procedure, method of wound closure, etc. Must be aseptic technique including assurance regarding the use of gown, gloves, mask, and sterilized instruments. Include name, dose, and route of any intra-operative analgesia, antibiotic or other drug. DO NOT REPEAT INFORMATION ALREADY PROVIDED ABOVE.

14b. WILL ALL DRUGS USED FOR SEDATION, ANESTHESIA, ANALGESIA OR EUTHANASIA NAMED IN THIS PROTOCOL (Questions 14a, 15, 16d) BE "PHARMACEUTICAL GRADE"? ***See "Non-pharmaceutical Grade Drug Policy" at web.jhu.edu/animalcare for full definitions of "pharmaceutical grade" and exceptions and for JHU requirements on preparation and storage.*** Yes No Not applicable. Formulations used for these purposes must be those sold for clinical use (i.e., "pharmaceutical grade") unless an alternative formulation is necessary due to (1) scientific considerations or (2) non-availability of the preferred compound in a clinical use formulation that can be used unaltered (e.g., without dilution). If the answer above is "No", state below the name of any drugs for these purposes that may not be pharmaceutical grade and your reason for using the non-pharmaceutical grade version.

14c. WILL ALL DRUGS/CHEMICALS USED AS RESEARCH TOOLS OR THE SUBJECT OF INVESTIGATION BE "PHARMACEUTICAL GRADE"? Yes No Not applicable. If "No", state below the name or class of the compound and the reason that non-pharmaceutical grade is necessary. Reasons could include: (1) non-availability in a clinical use formulation or in one that could be used unaltered; or clinical use formulation is not suitable for desired mode of delivery (e.g., is a pill); (2) scientific considerations (e.g., lack of suitable vehicle control, presence of preservatives, necessity of manipulating concentration, comparability with previous studies), (3) use of drugs provided by NIH drug supply program. ***See "Non-pharmaceutical Grade Drug Policy" at web.jhu.edu/animalcare for full policy and requirements for preparation and storage.***

Reminder

- Inventory your lab often
- Clearly label items and segregate those expired so they are not inadvertently used

For assistance disposing controlled substances please contact the ACUC Office



JHU ACUC Office Reminder:
Discard
Expired Drugs & Supplies:

**Controlled Substances, Biologicals,
Chemicals, and Materials**

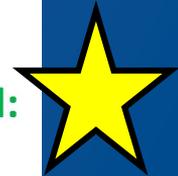
Approved in an Animal Protocol

MUST be disposed of

**When storing prior to disposal clearly label:
“Expired & Not For Animal Use”**

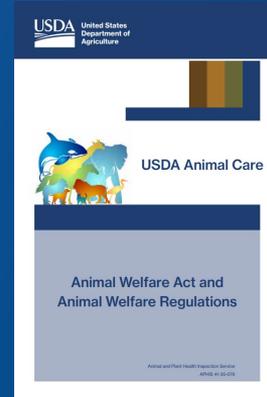
*If necessary, for controlled substances,
contact the ACUC for disposal information*

Email: acuc@jhmi.edu, Voicemail: 443-287-3738



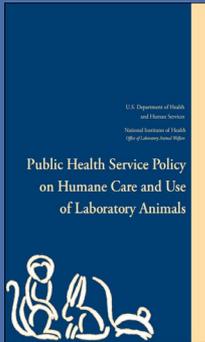
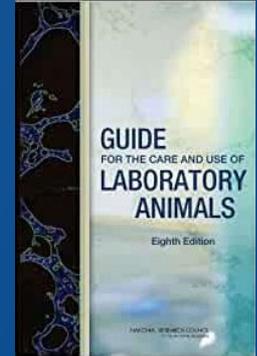
Why Follow the Rules

- **The Question:** Why is it important to have, and to have read, an animal protocol that is approved for what is to be done with your research animals?
- **The Answer:** Many of us work and study in the field of the biological science because it helps our understanding of the world around us. In order to conduct this research, most of us, need funding.
- In most cases funding sources are driven by:
 - Laws, Policies, Guidance:
 - The US Congress enacted the *Animal Welfare Act (AWA)* which is regulated by the USDA.
 - NIH, part of the US dept of Health and Human Services, manages this through the *PHS Policy on the Humane Care and use of Laboratory Animals (PHS Policy)*.
 - Many funding sources require compliance with the *Guide for the Care and Use of Laboratory Animals (the Guide)*.
- All 3 of these documents require that when using live animals in research, teaching and testing an Institutional Animal Care and Use Committee (IACUC) be established to ensure compliance.



Why Follow the Rules

- **The Bottom line:** The protocol will include how any pain or distress will be alleviated or mitigated. Without this information it will not be approved by the IACUC and the research cannot be started.
- Therefore, for each person, approved on a protocol, to know what methods have been approved to alleviate or mitigate pain or distress must have read the protocol. This must also include any amendments that have been approved to add additional procedures relevant to your experiments.



The management of the pain or distress, as described in the protocol, is not optional. To not follow the approved procedures, is not only inhumane but out of compliance with what was approved and out of line with the commitment that was made to the funding source.

Thanks for your attention!

Questions?



Raffle & Prizes

