Pain Management

Anesthesia
Aseptic surgery
Analgesia
Euthanasia
What is Pain?

Normal Behavior  Pain  Analgesics  Altered Behavior
Do Animals Feel Pain?

• Behavioral responses to stimuli
  – Prey species
  – Photoperiod

• Behavioral response to analgesics

• Nociceptive receptors
Definitions

• **Anesthesia:** loss of sensation induced by drug administration.
• **Analgesia:** loss of sensibility to pain.
• **Tranquilizer:** Chemical causing indifference to pain or sensory input.
• **Sedative:** Chemical that calms and promotes sleep.
• **Paralytic (Neuromuscular blocking agent):** chemical that prevents motor function, but not sensory input.
• **Euthanasia:** quiet, painless termination of life.
Health Research Extension Act

- Avoid or minimize discomfort, pain, or distress.
- Appropriate pain management required.
- Sacrifice when pain cannot be alleviated.
  - IACUC Humane Endpoints
  - http://safetyservices.ucdavis.edu/ps/a/IACUC/po/humaneEndpoints
- Vet care is required.
- AVMA Panel on Euthanasia.
- Institutional Animal Care and Use Committee (IACUC)
Controlled Substances

• Legal Classification by the Federal Comprehensive Drug Abuse and Control Act:
  – Schedule I drugs: high potential for abuse, no acceptable use.
  – Schedule II drugs: High potential for abuse, acceptable medical use.
  – Schedule III, IV, and V: progressively less addictive with lower potentials for abuse.
Controlled Substances

• Requirements for use
  – License needed to obtain these drugs
  – Detailed records on
    • Amount received
    • Amount and purpose for use
    • Amount on hand
  – Storage with limited access
  – Subject to unannounced inspections.
Classes of Anesthetics

• Injectables
  – Needles
  – Syringes
  – Sharps container

• Inhalants
  – O₂ source
  – Pressure reduction valve
  – Flow meter
  – Precision vaporizer
  – Non-rebreathing delivery
  – Scavenger

<table>
<thead>
<tr>
<th>Surgeon Prep</th>
<th>Instrument Prep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Prep</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Surgical Arena</td>
</tr>
</tbody>
</table>
Classes of Anesthetics

- **Injectables**
  - Peaks and valleys

- **Inhalants**
  - Steady levels

Injectable

Inhalant

Surgical plane
Classes of Anesthetics

• Injectables
  – Bolus delivery results in peaks and valleys
  – Elimination/Recovery
    • Slow—via circulatory/urinary system
    • 100% metabolized by liver and kidney
    • Possible tissue damage
  – Overdose Tx
    • Antagonistic drugs
  – Rodents—any procedure
  – Rabbits—used for minor procedures or as a premedication for inhalant induction

• Inhalant
  – Continuous delivery results in steady plane of anesthesia.
  – Recovery
    • Fast—via lungs
    • Little or no metabolism
  – Overdose Tx
    • Increase O₂
  – Rodents—induction chamber followed by masking
  – Rabbit surgery generally requires intubation.
Balanced Anesthesia

• Combining drugs causes dose dependent side-effects to decrease.

• Sedatives: Used to calm an animal
  – May be given prior to or with anesthetic
  – Facilitates handling if give prior
  – Lower the dosages of anesthetics required
  – Acepromazine and xylazine

• Muscle relaxants:
  – Muscle tension increases pain and trauma
  – Added to anesthetics that are not good muscle relaxants
  – Xylazine
Injectables

• Ketamine
  – Dissociative Anesthetic
  – Little medullary affect
    • Respiratory
    • Cardiovascular
  – Poor muscle relaxant
    • Mix with xylazine

• Pentobarbital
  – CNS depressant
    • Respiratory depressant
  – Good muscle relaxation
  – Random movement and vocalization common
  – Narrow margin of safety in rodents
Inhalants

- **Isoflurane**
  - Requires a precision vaporizer
  - Rapid induction & recovery (~2 minutes)
    - Rodents are induced in chambers and then masked
    - Rabbits are given an injectable and then intubated
  - Low cardiovascular & respiratory depression
  - 0.17% metabolized
  - Safe for patients and operators
Dosage vs Response

- **ADME:**
  - Absorption
  - Distribution
  - Metabolism
  - Excretion

- Dosages are based on ideal scenarios. Any disruption in these 4 steps change the response.

- Rodents are unpredictable in their response to anesthesia
Individual Response

• Small size:
  – Drugs often must be diluted
  – IV is often difficult to access; more alternate routes used

• High metabolism rate and oxygen consumption:
  – Require larger relative dosages
  – Clearance rate is faster
  – Decreased tolerance for respiratory depression

• Age
  – Young animals have a faster metabolism but underdeveloped organs
  – Old animals may have failing organs
Individual Responses

• Receptor physiology
  – Number of receptors
  – Speed of breakdown

• Health/research manipulations
  – Hydration level
  – Changes to clearance organs

• Genetics
  – Breeds/stocks/strains/lines do not all respond alike
  – Transgenic and mutations don’t act like background lines

• Environmental Conditions
  – Microsomal liver enzymes effect catabolism speed
Choosing the anesthesia regime

- Look at length of procedure and depth required.
  - Light sedation: Acepromazine alone
    - Clinical techniques that don’t require anesthesia
  - Light anesthesia: Ketamine/Xylazine/Acepromazine
    - Clinical techniques that do require anesthesia
    - Vasodilatation advantageous for blood collection
  - Deep anesthesia: Ketamine/Xylazine
    - Surgical procedures
    - Vasodilatation contraindicated.
Drug Formulary

• Drug Formulary: A document that gives a range of dosages by species
  – Expressed in mg/kg (units to use in the protocol)

Anesthetic Formulary for Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>25 - 40 mg/kg</td>
<td>IM</td>
<td>Light sedation</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>IM</td>
<td>Immobilization</td>
</tr>
<tr>
<td></td>
<td>50 - 100 mg/kg</td>
<td>SC, IP</td>
<td></td>
</tr>
<tr>
<td>Ketamine/Acepromazine</td>
<td>75/2.5 mg/kg</td>
<td>IM</td>
<td></td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
<td>75-95/5 mg/kg</td>
<td>IM, IP</td>
<td>Deep anesthesia</td>
</tr>
<tr>
<td></td>
<td>50/5 mg/kg</td>
<td>SC, IP</td>
<td>Light anesthesia</td>
</tr>
</tbody>
</table>
Drug Concentration

• Concentration: Found on the bottle of the drug
  – Expressed in mg/ml
  – Example: Ketamine 100 mg/kg
  – This can vary; always check.
Calculate Delivery Dose

- Ketamine
  - Formulary dose
    - 50 mg/kg bwt
  - Concentration (invert)
    - 100 mg/ml
  - Calculate delivery dose

\[
\frac{50 \text{ mg}}{1 \text{ kg}} \times \frac{1 \text{ ml}}{100 \text{ mg}} = 0.5 \text{ ml/kg}
\]
Calculating Your Delivery Volume

- Weight the rat and convert to kilograms
  - Kg = g bwt/1000
- Ketamine dose for a 300 g rat
  - 0.5 ml/kg x __________ kg = __________ ml
• Delivery dose calculations
  – Ketamine (100 mg/ml): Anesthetic
    • 50 mg/kg x 1 ml/100 mg = 0.5 ml/kg

  – Xylazine (20 mg/ml): Sedative, analgesic, muscle relaxant
    • 5 mg/kg x 1 ml/20 mg = 0.25 ml/kg

  – Acepromazine (10 mg/ml): Sedative, vasodilator
    • 0.5 mg/kg x 1 ml/10 mg = 0.05 ml/kg
Anesthesia for Clinical Techniques

• Delivery volume calculations--Why we dilute
• Example: 300 g rat

– Ketamine (100 mg/ml): Anesthetic
  • 0.5 ml/kg x 0.3 kg rat = 0.15 ml

– Xylazine (20 mg/ml): Sedative, analgesic, muscle relaxant
  • 0.25 ml/kg x 0.3 kg rat = 0.075 ml

– Acepromazine (10 mg/ml): Sedative, vasodilator
  • 0.05 ml/kg x 0.3 kg rat = 0.015 ml
Cocktails

- Combine drugs used for balanced anesthesia into a single vial with, or without dilution, for ease of delivery.
- You will use 2 cocktails in the lab, both with a delivery dose of 1 ml/kg. This is the cocktail for the clinical techniques lab:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Delivery Dose</th>
<th>Delivery dose for a 1 kg rat</th>
<th>Volume for a 1 ml cocktail</th>
<th>Volume for a 10 ml cocktail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>0.5 ml/kg</td>
<td>0.5 ml</td>
<td>0.50 ml</td>
<td>5.0 ml</td>
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<tr>
<td>Xylazine (20 mg/kg)</td>
<td>0.25 ml/kg</td>
<td>0.25 ml</td>
<td>0.25 ml</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>0.05 ml/kg</td>
<td>0.05 ml</td>
<td>0.05 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td>0.20 ml</td>
<td>2.0 ml</td>
</tr>
</tbody>
</table>
Surgical Cocktail

• For the surgical cocktail
  – Dosages are higher; as is xylazine concentration
    • Ket: 90 mg/kg x 1 ml/100 mg = 0.9 ml/kg
    • Xyl: 9 mg/kg x 1 ml/100 mg = 0.09 ml/kg
  – Water isn’t needed because the ratio is 1:9 and higher dosages negate the need for further dilution

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</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>0.9 ml/kg</td>
<td>0.9 ml</td>
<td>0.9 ml</td>
<td>9.0 ml</td>
</tr>
<tr>
<td>Xylazine</td>
<td>0.09 ml/kg</td>
<td>0.09 ml</td>
<td>0.1 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>(100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Anesthesia

• To re-dose your animal
  – Use ketamine only unless otherwise directed
  – Use 1/3 to ½ of the original dose of ketamine
  – Clinical techniques
    • 50 mg/kg x 1kg/100 ml = 0.5 ml/kg
      – Mild toe pinch: 1/3 x 0.5 ml/kg = 0.15 ml/kg
      – Very responsive: ½ x 0.5 ml/kg = 0.25 ml/kg
  – Anesthesia
    • 90 mg/kg x 1kg/100 ml = 0.9 ml/kg
      – Mild toe pinch: 1/3 x 0.9 ml/kg = 0.3 ml/kg
      – Very responsive: ½ x 0.9 ml/kg = 0.45-0.5 ml/kg
Pre-operative Care

• Fasting:
  – Limiting ridge between esophagus and stomach prevents regurgitation (except GP).
  – High metabolism rate can make fasting risky.
• Rabbit and guinea pig: usually fasted 3 to 6 hours—large cecum can affect bwt.
• Small rodents: not necessary to fast
Peri-operative Maintenance

• Side effects of large surface to mass ratio
  – Dehydration
    • SQ fluids
    • Eye lube
  – Hypothermia
    • Circulating water mat
    • Gel microwavable mat
Evaluation of Anesthetic Level

• Response to stimulation
  – Pedal withdrawal/toe pinch
  – Pinna (for rabbits)
  – Eye blink
  – Purposeful movement or vocalizations

• Muscle tone (jaw tone)

• Color of mucus membranes and eyes

• Breathing patterns
Anesthetic Monitoring

- For non-rodent mammals, measurements are usually recorded.
  - Heart rate
  - Respiratory rate
  - Temperature
  - Possibly blood pressure and circulating oxygen levels
Surgical Categories

- **Major**: Exposing a major body cavity or causing substantial trauma
- **Minor**: Not exposing a major body cavity nor causing substantial trauma
- **Survival**: Patient is expected to wake from anesthesia
- **Terminal**: Patient is humanely euthanized prior to recovery from anesthesia
• Health Research Extension Act requires asepsis for surgery on all vertebrate species.

• Goal of asepsis—to reduce the normal bacterial burden
  – Bacteria entering wound < $10^5$ bacteria/g of tissue or ml of body fluid.

• Link to the campus policy on Survival Surgery Guidelines on Rodents
Operating Area

• Non-rodent mammals require a surgical suite with 2 to 5 rooms
  – Animal prep
  – Human prep
  – Instrument prep
  – Surgery room
  – Recovery room

• Rodents can be done in 1 room
  – Away from human traffic and free of clutter
  – Clean and disinfect surfaces and equipment
  – Separate space within the room for 3 areas: prep, surgery, and recovery.
Instruments

- Clean off tissue, blood, and other proteins.
- Wrap in toweling or commercial packaging
- Sterilize
  - Autoclave
  - Dry heat oven
  - Irradiation
  - Ethylene oxide
  - Chemical bath
- Label and date—shelf life ~8-10 weeks
Instrument Use

• For rodents, instruments may be used for multiple surgeries on the same day if the following guidelines are followed
  – Use instruments for no more than 6 major surgical procedures before cleaning and autoclaving
  – Have a minimum of 2 surgical packs
  – Clean instruments of blood and tissue and sterilize in a glass bead sterilizer between surgeries.
Remove hair

• Prevent contamination by clipping hair away from operating area.
• Use electric clippers, razor, or depilatory
• Clip area larger than fenestration (~1 cm to either side of incision in rat).
• Fur should not show through fenestration, but excess hair removal can cause lost body heat.
• Use a dry gauze or small vacuum to remove loose hair.
Clean site

- Surgical **scrub**—detergent and disinfectant combined.
  - Betadine Scrub (povidone iodine)
  - Nolvasan Scrub (chlorhexidine)
- Scrub pattern must be from center to periphery
- **Rinse** with sterile water, saline, or 70% ethyl alcohol
- Repeat Scrub-Rinse cycle 3 times
- Apply compatible disinfectant without detergent
  - Betadine Solution
  - Nolvasan Solution
Surgeon

- Clean cap and mask
- Put on clean lab coat or sterile gown
- Wash hands and arms with antibacterial soap and scrub brush
- Dry hands and arms on a clean or sterile towel
- Put on sterile gloves
  - For multiple surgeries, only gloves need to be changed between animals.
Sterile Field

• The sterile field is the area above and below the animal and the front of the surgeon.
• Table is disinfected and draped with sterile cloth.
• Drape animal with sterile cloth or adhesive drape leaving only the head and incision site exposed.
  – Fenestration: hole in drape exposing incision. Prevents instruments or viscera from becoming contaminated.
Maintaining the Sterile Field

• Keep on sterile field
  – Instruments and equipment
  – Suture material
  – Gloved hands

• Keep off sterile field
  – Bottles of disinfectant or anesthesia
  – Syringe or suture packaging
  – Animal until scrubbed and prepped
**Surgical Records**

- Surgical records must include:
  - Weight of animal
  - Anesthesia dose (mg/kg), route, and time delivered
  - Procedure times: start and finish
  - Observations every 15 minutes during anesthetic recovery
  - Analgesics given
  - Daily (minimum) monitoring until sutures/wound clips are removed.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>UC Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol #</td>
<td>Campus Veterinary Services</td>
</tr>
<tr>
<td>Animal #</td>
<td>Anesthesia:</td>
</tr>
<tr>
<td>Species</td>
<td>Pre-anesthetic Drugs:</td>
</tr>
<tr>
<td>Sex, Age</td>
<td>ANESTHETIC INDUCTION AND MAINTAINANCE:</td>
</tr>
<tr>
<td>Weight</td>
<td>Post-Anesthetic Recovery:</td>
</tr>
<tr>
<td>Housing Site</td>
<td></td>
</tr>
</tbody>
</table>

**Anesthesia Record**

- Drug  | Date  | Route  |
- Drug  | Date  | Route  |
- Drug  | Date  | Route  |
- Drug  | Date  | Route  |

**Post-Anesthetic Recovery**

- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |

**Post-Operative Anesthesia**

- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |

**Animal Identification**

- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
- Drug  | Time  | Route  |
Post-Operative Recovery

• Post-Anesthesia
  – Maintain body temperature: Offer temperature gradient
  – Bedding: Towel, carpet, etc.
  – Additional hydration can speed recovery
  – Observation: monitor and record every 15 minutes until animals are sternal and clearly awake

• Post-operative (days following surgery)
  – Daily (minimum) checks: Animals must be observed daily for signs of pain or surgical complications.
  – Analgesia administered as specified in protocol
  – Wound clips or sutures removed at 7 to 10 days.
Recognition of Chronic Pain

- Food and water intake
- Weight loss of 10%
- Loss of body conformation
- Activity
- Posture or gait
- Temperament
- Vocalizations
- Localized appearance
- General appearance
Weight vs. Body Condition Scoring

~10% loss of bwt: Treat

~20% loss of bwt: Euthanize

**BC 1**
Mouse is emaciated.
- Skeletal structure extremely prominent; little or no flesh cover.
- Vertebrae distinctly segmented.

**BC 2**
Mouse is underconditioned.
- Segmentation of vertebral column evident.
- Dorsal pelvic bones are readily palpable.

**BC 3**
Mouse is well-conditioned.
- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.

**BC 4**
Mouse is overconditioned.
- Spine is a continuous column.
- Vertebrae palpable only with firm pressure.

**BC 5**
Mouse is obese.
- Mouse is smooth and bulky.
- Bone structure disappears under flesh and subcutaneous fat.

A “+” or a “-” can be added to the body condition score if additional increments are necessary (i.e., ...2+, 2, 2-...)
Analgesic Frequency

- More effective when given before the onset of pain
- Mild pain (minor surgery): 12 to 24 hours. 1 dose is often sufficient with mice.
- Severe Pain (major surgery): 24 to 48 hours
- Intense pain (orthopedic surgery): 3 to 4 days
Morphine (Opioid)

- Most powerful and effective
- Controlled substances (Schedule II)
- Relatively short lasting—need frequent redosing (every 2-4 hours)
- Cause sedation and depresses respiration and gastric motility
- Route: Injection
Buprenorphine (Opioid)

• Not as powerful as morphine
• Also controlled but Schedule V
• Longer lasting—as much as 8 – 12 hours
• Little risk of respiratory or gastric depression
• Route: Injectable (SC), oral (gelatin)
NSAIDS

- NSAIDS (Non-steroidal anti-inflammatory):
  - Mild to moderate relief
  - Not controlled
  - Most are short acting -- ~4 hours
  - Oral route acceptable, but can produce gastric upset
  - Carprofen (Rimadyl): Injectable, long lasting (8-12 hours), fewer gastric side effects
Other Methods of Pain Management

- Immobilization or padding affected area
- Careful tissue handling & wound closure
- Providing easy access to food and water
- Soft bedding/hiding places
- Temporary isolation from cage mates
Euthanasia

- Method must be approved by AVMA (American Veterinary Medical Association)
- Loss of consciousness with little or no pain, distress, or anxiety.
- Assurance of death on an individual basis
  - Physical exam
  - Secondary method
- Other considerations
  - Personnel: skill and acceptance
  - Animal: Age, number, temperament
  - Potential effects: experimental, environmental
Injectable Euthanasia

- **Injectables**
  - Preferred method for rabbits. Can be used with rodents
  - Anesthetic overdose or euthanasia solution
  - Requires no special equipment but does require individual restraint
  - IV: Rapid but difficult
  - IC: Requires prior anesthesia
  - IP: Acceptable in small animals and with non-irritating chemicals
  - IM and SQ are too slow
Inhalant Euthanasia

• CO₂ is most common for rodents
  – Advantages
    • Rapid analgesic effects
    • Minimal handling and restraint
    • Safe for personnel
    • No chemical residue
  – Disadvantages
    • Potential to cause distress
      – Carbonic acid formation
      – Feeling of breathlessness
      – Fear response
    • Neonates resist hypoxia and larger animals take too long
  – Method
    • Fill rate of 10 – 30 liters per minute will take longer
      but will produce loss of consciousness before the onset of pain.
Inhalant Euthanasia

- Anesthetic overdose (Isoflurane is most common)
  - Minimal handling and restraint
  - Long exposure times are required to assure death
  - Rabbits breath-hold with isoflurane and need to be premedicated
Physical Methods

• Hypothermia (no contact with frozen surfaces)
  – Altricial neonatal rodents (≤5 days)

• Decapitation
  – Rodents and small rabbits w/anesthesia
  – Altricial rodents 5 to 14 days old

• Cervical dislocation: w/anesthesia
  – Mice and rats <200 g
  – Rabbits <1 kg.
Protocol Requirement

• All protocols must list an appropriate form of euthanasia.