ANESTHESIA AND OTHER CLINICAL CONSIDERATIONS FOR SMALL ANIMAL IN VIVO IMAGING

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Clinical Laboratory Animal Resident
Research Animal Resources
Johns Hopkins University

Overview
- Key Concepts
- Anesthesia
  - Injectable
  - Inhalant
- Physiologic monitoring
- Supportive care
  - Fasting
  - Heat support
  - Fluid Therapy

Key Concepts
- The principal goal of anesthesia for in vivo imaging is to produce a drug-induced state of immobilization that allows for acquisition of repeatable and reliable data.
- Anesthetic agents should have minimal impact on the systems under study.
- No anesthetic event is benign. Anesthesia should be safe and delivered in an approach that limits morbidity and mortality.

Anesthetic Management
- No single agent or combination is appropriate for all anesthetic events
- What is the anticipated duration of immobilization?
- What are the number of subject(s) to be imaged?
- What route of anesthetic delivery is available?
- What agents can be used safely during the imaging study?
- What physiologic parameter(s) are important to the study?

Anesthetic Selections
Understand your model and your mouse (or rat)!
- Strain sensitivity

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- Genetic modifications (e.g. γ-PKC<sup>−/−</sup>)

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Anesthetic Selections
Understand your model and your mouse!

- Strain sensitivity
- Genetic modifications (e.g. γ-PKC<sup>−/−</sup>)
- Co-morbidities
  - Organ dysfunction (e.g. DCM, hepatitis, renal tumors)
  - Physiologic changes (e.g. hypertension, bradycardia)

General Anesthesia
1. Loss of consciousness
2. Suppression of muscle activity
3. Analgesia

- Single agents (e.g. inhalants)
- Combinational therapy (e.g. injectables)

All anesthetic agents have side-effects!

Injectable Anesthesia

<table>
<thead>
<tr>
<th>Pros</th>
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<tbody>
<tr>
<td>Rapidity and recovery</td>
<td>Does not require specific equipment</td>
</tr>
<tr>
<td>Titratable effect</td>
<td>Many types/ combinations</td>
</tr>
<tr>
<td>Control over depth</td>
<td>Familiarity</td>
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<td>Respiratory depression</td>
<td>Delayed anesthetic induction and recovery</td>
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<td>Requires specific equipment (e.g. vaporizer, intubation or nose cone)</td>
<td>Variable side-effects</td>
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<td>Renal/hepatic metabolism</td>
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<td>Often blind administration</td>
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Inhalant Anesthesia

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Injectable Anesthesia

- Pentobarbital: potentiates GABAergic neurotransmission
  - Frequently used agent: hypnosis → anesthesia
  - Cardiopulmonary depression is dose-dependent
  - May require ventilatory support with severe respiratory depression
  - Administration: Mice 50 – 90 mg/kg; Rats 30 – 45 mg/kg (IP)

- 2-2-2 Tribromoethanol (Avertin®):
  - Short-acting 15-30 minutes of anesthesia
  - Good muscle relaxation, mild respiratory depression
  - Purchased as a chemical grade product and requires reconstitution
    - Light and temperature sensitive: degraded by-products can cause delayed nephro/hepatotoxicity and gastrointestinal stasis
  - Increased fatalities with multiple IP administrations (best for single survival/terminal/acute use)
Injectable Agents

- **Ketamine (Ketaset®)**: NMDA receptor antagonist
  - Not recommended as sole anesthetic (increased muscle tone, tremors, occasional seizures)
  - Typically used in combination with sedatives, tranquilizers, and opioid analgesics
  - Indirect sympathomimetic agent
    - Increased HR, CO, MAP
    - Increased cerebral blood flow and metabolic rate
    - Increased intracranial and intraocular pressure

- **Xylazine (Rompun®)**: α₂ adrenergic receptor agonist
  - Commonly used in combination with ketamine
  - Induces a deep plane of anesthesia for 25 – 40 minutes
  - Sedation, muscle relaxation, analgesic properties
  - Reversible agent (e.g. yohimbine or atipamazole)

- **Medetomidine (Domitor®)**: α₂ adrenergic receptor agonist
  - More potent and longer lasting when compared to xylazine
  - Side effects:
    - Moderate to severe cardiopulmonary depression (bradycardia, occasional 1st or 2nd degree AV-block, peripheral vasoconstriction)
    - Increased stroke volume as compensation
    - Caution with vascular perfusion and cardiac studies

Inhalant Anesthesia

- **Stages of Anesthesia:**
  1. Induction
  2. Maintenance: know the MAC
  3. Recovery

- **Delivery:** Precision calibrated vaporizer
  - Nose-cone
  - Inhalation: airway protection, ventilation
  - Breathing circuits
    - Rebreathing circuits
    - Non-rebreathing circuits (e.g. Bain®)
      - Minimizes dead space and resistance to breathing
      - HIGH fresh gas flow (≥ 150 mL/kg/min)

- **Delivery: “Open drop method”**
  - Closed chamber + gauze + anesthetic + animal
  - Avoid direct animal-anesthetic contact
  - Monitor anesthetic depth, remove once anesthetized
  - Best for very quick (30–45 sec) procedures

  To calculate dose:
  - Assume most inhalants: 1 mL liquid → 200 mL vapor
  - Amount liquid anesthetic (mL) = % for induction X vol. jar / 200 mL vapor/mL

Intubation

- Slant board: ≥45°
- Otoscopy
- Endotracheal tube size:
  - Rats: 22 – 20 G catheter
  - Mice: 24 – 22 G catheter

Inhalants

- Not all inhalants are equal

- **Variable Minimum Alveolar Concentration (MAC)**
  - MAC = the concentration of inhaled gas required to block a response to surgical stimulus in 50% of patients
  - Potency = 1/MAC

- **Variable blood/gas solubility**
  - Solubility 1/g change in the rate of anesthetic depth

- **Common volatile liquid anesthetic agents**
  - Isoflurane (Forane®)
  - Sevoflurane (Ultane®)
  - Desflurane (Tefla®)
  - Cardiac function in general is better maintained with gas (vs injectables)
  - Good muscle relaxation, rapid recovery
Physiologic Monitoring

**Minimal change in homeostasis**
- Challenging in the imaged patient
- Visualization
- Adaptable equipment
- Parameters to monitor and control
  - Heart rate
  - Respiratory rate
  - Temperature
  - %SpO2
  - Cardiac activity (EKG)
  - Blood pressure

**Pulse oximetry:**
- Arterial Hb %SpO2
- NIBP: Doppler
- Temperature

Normal Physiologic Parameters

**Mouse:**
- Temperature: 97.5 – 100.4°F
- Respiratory rate: 150 – 220 bpm
- Heart rate: 550 – 700 bpm

**Rat:**
- Temperature: 96.6 – 99.5°F
- Respiratory rate: 65 – 115 bpm
- Heart rate: 250 – 450 bpm

Fasting

- Depends on your study and imaging modality
- Ex: PET FDG imaging
- Decreases blood glucose
- ~ 6 hrs fast clears stomach, longer can be detrimental
- Rodents are nocturnal feeders

**Length and timing of fast should be documented**

Fluid Therapy

- Prolonged anesthetic events lead to sensible and insensible water losses
- **Cautions** use of fluids in models of cardiovascular disease or hypoproteinemnic subjects (e.g. hepatic, renal or gastrointestinal compromise)
- Administration routes: subcutaneous, intraperitoneal or intravascular
- Compensate for fluid loss and administer daily maintenance requirements (50 – 100ml/kg/day)
- Pre-warmed (37°C) fluids: 0.9% NaCl or LRS

Heat Support

- Rodents have an incredibly large surface area to body mass ratio → rapid hypothermia
- Prolonged anesthetic recovery
- Altered cardiovascular function
- Heat support maintained until ambulation

**Heat options:**
- Self-regulating heating blocks
- Water-circulating heating pads
- Insulation (e.g. saran wrap)
- Pre-warmed subcutaneous fluids
- Hand warmers
- Heat lamps
Heat Support

Rat

Mouse

Health and Biosecurity

- Disinfect anesthetic equipment (e.g. nose-cones, induction chambers, heating elements) between subjects.
- Typically the imaging core is in a barrier facility, but...
  - Once fully recovered (ambulatory), return to facility high risk suite (e.g. JHU BRB 16A &G and 20A).

Questions?

Research Animal Resources
Veterinary Support
Ross 459
410.955.3273
http://www.hopkinsmedicine.org/animalresources/index.html
Research Animal Resources (RAR)
720 Rutland Avenue
Ross Building, Room 459
Baltimore, MD 21205-1191
General information: 410-955-3273 Fax 410-502-506
Clinical calls: 410-955-3273
Animal orders: 410-955-3713
Website: www.hopkinsmedicine.org/animalresources/

Veterinary Consults • Technical assistance • Animal Imports/Exports • Health Certificates • Anesthesia and Surgical Support
RAR is the centralized provider of veterinary medical care and research animal support at Johns Hopkins. The office is open Monday- Friday 8:30 am -5 pm. For emergency veterinary assistance after hours, please call the on-call pager: 410-283-0929.

Johns Hopkins Animal Care and Use Committee
1620 McElderry Street
Reed Hall, Room B122
Baltimore, MD 21205-1911
Phone: 443-287-3738 Fax: 443-287-3747
Email: acuc@jhmi.edu
Website: www.jhu.edu/animalcare

ACUC Policies and Guidelines • Rodent Drug Formulary • JHU “Blue Book” (species specific information) • Animal Welfare Concerns • Protocol Submission Forms
All studies, courses and training programs proposing the use of animals must be approved by the Institutional Animal Care and Use Committee
### MOUSE INJECTABLE ANESTHESIA DOSES

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<tr>
<th>Drug</th>
<th>Dose rate</th>
<th>Effect</th>
<th>Duration of anaesthesia (minutes)</th>
<th>Sleep time (minutes)</th>
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<tr>
<td>Ketamine/xylazine</td>
<td>80-100mg/kg + 10mg/kg</td>
<td>Surgical anaesthesia</td>
<td>30-40</td>
<td>60-120</td>
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<td>Methohexitol</td>
<td>10mg/kg iv</td>
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<td>10</td>
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<td>Metomidate/fentanyl</td>
<td>60mg/kg + 0.06mg/kg</td>
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<td>90-120</td>
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<td>Pentobarbital</td>
<td>40-50mg/kg ip</td>
<td>Immobilization/anaesthesia</td>
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<td>120-180</td>
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<tr>
<td>Propofol</td>
<td>25mg/kg iv</td>
<td>Surgical anaesthesia</td>
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<td>10-15</td>
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<tr>
<td>Thiopental</td>
<td>30-40mg/kg iv</td>
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<td>Telanimex/xylazine</td>
<td>80mg/kg ip</td>
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<tr>
<td>Trihexylenol hydrochloride</td>
<td>240mg/kg ip</td>
<td>Surgical anaesthesia</td>
<td>15-45</td>
<td>60-120</td>
</tr>
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Duration of anaesthesia and sleep time (less of righting reflex) are provided only as a general guide, since considerable between-animal variation occurs. For recommended techniques, see text.

* *Dose is milligrams per kilogram of a mixture of one part ‘Propofol’ plus two parts water for injection, and one part midazolam (5mg/ml initial concentration).*

### RAT INJECTABLE ANESTHESIA DOSES

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**Anesthesia, Analgesia and Monitoring References**

- **Veterinary Anesthesia and Analgesia** 4th edition Tranquilli 2007