Anesthetic regimens for mice, rats and guinea pigs

The intent of the Standard Operating Procedure (SOP) is to describe commonly used methods to anaesthetize rodents. This SOP is intended for use by researchers and Comparative Medicine (CM) staff who anaesthetize rodents. This SOP is approved by the NUS Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC in advance.

TABLE OF CONTENTS

1. Introduction
2. Materials
3. Procedures
4. Safety
5. Contingencies
6. References
7. Appendix

1. INTRODUCTION

Rodents can be anaesthetized with either inhalant gas or injectable drugs. The use of inhalant gas is the preferred method of anesthesia whenever possible. In cases where the use of inhalants is not possible, combinations of injectable anaesthetics for rats and mice can be given.

2. MATERIALS

a. Induction chamber, clear
b. Anesthetic agents
   i. Inhalant option: anesthesia machine, inhalant anesthetic and oxygen supply
   ii. Injectable option:
       1. Ketamine and xylazine
       2. Ketamine and medetomidine
   iii. Crushed ice (for newborns)

c. Support materials:
   i. Species appropriate scale (as applicable)
   ii. Needles and syringes (as applicable)
   iii. Sterile ophthalmic eye lubricant
3. PROCEDURES

a. General considerations:
   i. Heat loss is rapid in anesthetized rodents. Keep animals warm by wrapping/covering
      them (e.g., drape or towel), and/or providing a heat source (e.g. homeothermic heat
      pad, Hot hands hand warmer) until they have fully recovered from anesthesia. Ensure
      that all heat sources are thermostatically controlled and/or carefully monitored so to
      prevent burns while maintaining thermal support.
   ii. Apply sterile ophthalmic lubricant (e.g., Duratears) to eyes once animal is
       anesthetized.
   iii. Ensure adequate depth of anesthesia prior to performing procedures. (e.g., monitor
        Toe pinch reflex (leg withdrawal and eye lid (palpebral) reflexes).
   iv. Ensure animal safety
      1. Minimally, monitor respiration, and skin/mucous membrane color. Respiratory
         rate changes from fast during induction to more slow and rhythmic as the depth
         increases. See appendix B for normal vital parameters.
      2. For long procedures, or procedures that restrict direct observation (e.g. micro CT
         scan) utilize a heart rate monitor or respiratory and/or oxygen saturation monitor
         (e.g. Rodent Pulse Oximeter).
   v. Anesthetized rodents must not be placed in contact with loose bedding or similar
      materials.
   vi. Recover animals in a bare cage or on top of a paper towel (or similar barrier) to
       prevent aspiration of bedding and irritation to the eyes.
   vii. Monitor the rodent until fully recovered from anesthesia i.e. purposeful movements
        and/or gained righting reflex.
   viii. Clearly document all procedures on specific procedure cards (see Appendix C for
        post surgery card) or detailed notes on cage cards:
        1. Type, dose and route of anesthetics
        2. Date and name of surgical procedure
        3. Analgesic and antibiotics drugs
        4. Route and duration of administration

b. Mouse Anesthesia
   i. Isoflurane
      1. Clean and disinfect induction chamber.
      2. Place the animal in the induction chamber.
      3. Adjust the oxygen flowmeter to 1 – 2 L/min.
      4. Adjust the isoflurane vaporizer to 4 – 5 %.
      5. For maintenance, take the rodent out of the chamber and connect to a nose cone or
         mask via a non-rebreathing circuit (e.g. Mappleson E)
      6. Adjust the flowmeter to 1 - 2 L/min and isoflurane 1 - 3%. Please refer to Appendix
         A, Part I for adverse effects and precautions for the use of isoflurane.
   ii. Ketamine-Medetomidine
      1. Dosage Ketamine 75 mg/kg + Medetomidine 1 mg/kg IP
      2. Surgical anesthesia for 20 -30 minutes
      3. Solution preparation:
         a) In a 10 ml sterile vial, aseptically mix 0.75 ml of Ketamine (100 mg/ml) and 1
            ml of Medetomidine (1 mg/ml) in 8.25 ml of 0.9 % Saline for injection. Shake
well before use.

b) Label the bottle:
   - Mice Anesthesia
   - Ketamine 7.5mg/ml
   - Medetomidine 0.1 mg/ml
   - Date prepared: _______ (to be used within 3 days of preparation)
   - "KETAMINE–MEDETOMIDINE FOR MOUSE: 0.1 ml/10 g IP"
   - Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)

c) Store away from light, in a cool place i.e., store below 30°C.
d) Inject 0.1 ml of the Ketamine–Medetomidine solution per 10 g of body weight intraperitoneally (0.1 ml/10 g IP).
e) If the animal is not adequately anesthetized or prolongation of anaesthesia is required, repeat the injection at a dose of 0.05ml/10g (approximately every 30 minutes).
f) Reversal of anaesthetic effects of Medetomidine using Atipamezole.
   i. Dosage 1 mg/kg IP
   ii. In a 10 ml sterile vial mix 0.2 ml of atipamezole (5 mg/ml) in 9.8 ml of 0.9% Saline for injection. Shake well before use.
   iii. Label the bottle:
       - Mice- Reversal for Medetomidine
       - Atipamezole 0.1 mg/ml
       - Date prepared:________ (to be used within 3 days from preparation)
       - "ATIPAMEZOLE FOR MOUSE: 0.1 ml/10 g IP".
       - Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)

c. **Rat Anesthesia**
   
   i. Isoflurane
      1. Clean and disinfect induction chamber.
      2. Place the animal in the induction chamber.
      3. Adjust the oxygen flowmeter to 1 – 2 L/min.
      4. Adjust the isoflurane vaporizer to 4 – 5 %.
      5. For maintenance use a mask connected to a non-rebreathing circuit (e.g. Mapleson E).
      6. Adjust the flowmeter to 1 - 2 L/min and isoflurane 1 - 3%. Please refer to Appendix A, Part I for adverse effects and precautions for the use of isoflurane.

   ii. Ketamine-Medetomidine
      1. Dosage 75 mg/kg + 0.5 mg/kg IP
      2. Surgical anesthesia for 20 – 30 minutes
      3. Solution preparation:
         a) In a 30 ml sterile vial, aseptically mix 7.5 ml of Ketamine (100 mg/ml) and 5 ml of Medetomidine (1 mg/ml) in 7.5 ml of 0.9 % Saline for injection. Shake well before use.
         
         b) Label the bottle:
            - Rat Anesthesia
            - Ketamine  37.5 mg/ml
            - Medetomidine 0.25 mg/ml
iii. Ketamine- Xylazine

1. Dosage Ketamine 75 mg/kg + Xylazine 10 mg/kg IP
2. Surgical anesthesia for 20 – 30 minutes
3. Solution preparation:
   a) In a 30 ml sterile vial, aseptically mix 7.5 ml of Ketamine (100 mg/ml) and 5 ml of Xylazine (20 mg/ml) in 7.5 ml of 0.9 % Saline for injection. Shake well before use.
   
   b) Label the bottle:
      • Rat Anesthesia
      • Ketamine 37.5 mg/ml
      • Xylazine 5mg/ml
      • Date prepared: _______ (To be used within 3 days from preparation date)
      • "KETAMINE–XYLAZINE FOR RAT: 0.2 ml/100 g IP".
      • Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)
   
   c) Store away from light, in a cool place i.e., store below 30°C.
   d) Inject 0.2 ml of the Ketamine–Xylazine solution per 100 g of body weight intraperitoneally (0.2 ml/10 g IP).
   e) Repeat as if the animal is not adequately anesthetized or prolongation of anesthesia is required, using 1/3 to ½ dose at a time (approximately every 30 min).
   f) See Appendix A part II for notes on Xylazine
d. Guinea pig Anaesthesia

i. Isoflurane
1. Place the animal in the induction chamber.
2. Adjust the oxygen flowmeter to 1 – 2 L/min.
3. Adjust the isoflurane vaporizer to 4 – 5 %.
4. For maintenance, take the rodent out of the chamber and connect to use a nose cone or mask via a non-rebreathing circuit (e.g. Mapleson E)
5. Adjust the flowmeter to 1 - 2 L/min and isoflurane 1 - 3%. Please refer to Appendix A, Part I for adverse effects and precautions for the use of isoflurane.

ii. Ketamine-Xylazine
1. Dosage Ketamine 40 mg/kg + Xylazine 5 mg/kg IP
2. Surgical anesthesia ~30 minutes
3. Solution preparation:
   a) In a 10 ml sterile vial, aseptically mix 4 ml of Ketamine (100 mg/ml) and 2.5 ml of Xylazine (20 mg/ml) in 3.5 ml of 0.9 % Saline for injection. Shake well before use.
   b) Label the bottle:
      - Guinea pig Anesthesia
      - Ketamine: 40 mg/ml
      - Xylazine: 5 mg/ml
      - Date prepared: ______ (To be used within 3 days from preparation date)
      - "KETAMINE–XYLAZINE FOR GUINEA PIG: 0.1 ml/100 g".
      - Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)
   c) Store away from light, in a cool place i.e., store below 30°C.
   d) Inject 0.1 ml of the Ketamine– Xylazine solution per 100 g of body weight intraperitoneally (0.1 ml/100 g IP).
   e) Repeat as if the animal is not adequately anesthetized or prolongation of anaesthesia is required, using 1/3 to ½ dose at a time (approximately every 30 min).
   f) See Appendix A part II for notes on Xylazine

iii. Ketamine-Medetomidine
1. Ketamine 40 mg/kg + Medetomidine 0.5 mg/kg IP
2. Moderate anesthesia 30 – 40 minutes
3. Solution preparation:
   a) In a 10 ml sterile vial, aseptically mix 4 ml of Ketamine (100 mg/ml) and 5 ml of Medetomidine (1 mg/ml) in 1 ml of 0.9 % Saline for injection. Shake well before use.
   b) Label the bottle:
      - Guinea pig Anesthesia
      - Ketamine: 40 mg/ml
      - Medetomidine 0.5 mg/ml
      - Date prepared: _______ (to be used within 3 days from preparation date)
      - "KETAMINE–MEDETOMIDINE FOR GUINEA PIG: 0.1 ml/100 g IP".
      - Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)
c) Store away from light, in a cool place i.e., store below 30°C.
d) Inject 0.1 ml of the Ketamine–Medetomidine solution per 100 g of body weight intraperitoneally (0.1 ml/100 g IP).
e) Repeat the injections if the animal is not adequately anesthetized or prolongation of anaesthesia is required, using 1/3 to ½ dose at a time (approximately every 30 min).
f) Reversal of anaesthetic effects of Medetomidine
   i) Dosage 1 mg/kg IP
   ii) In a 10 ml sterile vial, aseptically mix 0.2 ml of Atipamezole (5 mg/ml) in 9.8 ml of 0.9% Saline for injection. Shake well before use.
   iii) Label the bottle:
       - Guinea pig- Reversal for Medetomindine
       - Atipamezole 1 mg/ml
       - Date prepared: ____ (To be used within 3 days of preparation)
       - "ATIPAMEZOLE FOR GUINEA PIG: 0.1 ml/100 g IP".
       - Initials of person who prepared the drug and department (e.g., Dr. XYZ, Comparative Medicine, MD2)

e. Rodent Neonates

i. Note: This technique may only be used in neonatal, altricial, hairless rodents, generally <10 days old. Time of onset of lethargy may be considerably slower than with other methods. The neonate must be completely dry prior to direct contact with ice; DO NOT USE DRY ICE.
   1. Place the neonate on a bed of crushed ice until anesthesia is noted. Once achieved, anesthesia can be maintained with the addition of a layer of latex or plastic wrap placed between the neonate and the ice. This additional layer allows for maintenance of the neonate on a dry surface.
   2. Monitor the readiness of the animal for a procedure by noting lethargy and immobility. Expect the neonate to remain immobile for up to 10 minutes. If additional time is needed for the procedure, maintain immobility by keeping the neonate on latex covered cold pack.
   3. Illuminate the surgical field by use of a fiber optic light source, as incandescent bulbs may cause inadvertent and uncontrollable warming.
   4. Recover pups and slowly re-warm them in an incubator at 33°C or in nesting material laid on warming devises such as ‘hot hands’ or homeothermic heating blankets. Complete recovery typically requires 30 to 60 minutes.

ii. Isoflurane
   1. Isoflurane could used to anaesthetize neonatal rodent < 10 days of age.
   2. Induction and maintenance was at 2 % and 1.5 % in 1 – 2 L/min of oxygen or air using a mask connected connected to a non-rebreathing circuit (e.g. Mapleson E).

4. SAFETY

a) Ensure that all anesthetic waste gases are properly scavenged.
b) Monitor the use of chemical agents and ensure that product safety recommendations are followed to protect the health and welfare of the humans and animals exposed to the agents.
c) When working with animals, wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks
5. CONTINGENCIES

a) Contact the CM Veterinary Staff for concerns regarding the use of particular anesthetic regimes or additional training.

b) Post contact information for emergency assistance in a conspicuous location within the animal facility.

c) Emergency veterinary care is available at all times including after working hours and on weekends and holidays. Contact 9001 3073

6. REFERENCES

- Cornell University, Institutional Animal Care and Use Committee, Animal Care and Use Procedure 101.02 Rodent Anaesthesia
  [Link](http://www.research.cornell.edu/care/documents/ACUPs/ACUP101.02.pdf)

<table>
<thead>
<tr>
<th>Revision #</th>
<th>Author</th>
<th>IACUC/Approval/Effective Date</th>
<th>SOP #</th>
</tr>
</thead>
<tbody>
<tr>
<td>.01</td>
<td>Enoka Bandularatne</td>
<td>29 October 2012</td>
<td>101.01</td>
</tr>
</tbody>
</table>
7. APPENDIX

**Appendix A Part I**
Isoflurane:
Isoflurane is a vasodilator and its use can result in fatal hypotension. Administer isoflurane with caution (e.g., with hemodynamics monitoring) in animals that may be dehydrated or otherwise at risk for hypotension. Maintaining animals with isoflurane concentrations in excess of the recommended levels may result in death of healthy and compromised animals.

**Appendix A Part II**
Xylazine:
Xylazine use can result in poor tissue perfusion and bradycardia. This drug should be avoided in animals with concurrent organ dysfunction (e.g., renal or heart failure) and in very young or old animals.

**Appendix B: Normal Biological Parameters of Rodents**

**Biological Data of Mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (grams)</td>
<td>Adult male: 20-40</td>
</tr>
<tr>
<td></td>
<td>Adult female: 18-35</td>
</tr>
<tr>
<td></td>
<td>Newborn: 1-1.5</td>
</tr>
<tr>
<td>Normal body temperature</td>
<td>37 - 37.2 °C (98.8-99.3 °F)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>310-840</td>
</tr>
<tr>
<td>Respiration rate (breaths per minute)</td>
<td>84-230</td>
</tr>
<tr>
<td>Life span (years)</td>
<td>1-3</td>
</tr>
<tr>
<td>Daily consumption (per 10 grams body weight)</td>
<td>Feed: 1.2-1.8 grams</td>
</tr>
<tr>
<td></td>
<td>Water: 1.5 ml</td>
</tr>
<tr>
<td>Age at weaning (days)</td>
<td>21-28</td>
</tr>
</tbody>
</table>
### Biological Data of Rats

<table>
<thead>
<tr>
<th></th>
<th>Adult male: 300-500</th>
<th>Adult female: 250-300</th>
<th>Newborn: 5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal body temperature</td>
<td>35.9 - 37.5 °C</td>
<td>(96.6-99.5 °F)</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>250-450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration rate (breaths per minute)</td>
<td>70-115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life span (years)</td>
<td>2.5-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily consumption (per 100 grams body weight)</td>
<td>Feed: 5 grams</td>
<td>Water: 8-11 ml</td>
<td></td>
</tr>
<tr>
<td>Begins eating solid feed (days)</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at weaning (days)</td>
<td>20-21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Biological Data of Guinea Pigs

<table>
<thead>
<tr>
<th></th>
<th>Adult male: 900-1000</th>
<th>Adult female: 700-900</th>
<th>Newborn: 60-115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal body temperature</td>
<td>37.2 - 39.5 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>230-380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration rate (breaths per minute)</td>
<td>42-104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life span (years)</td>
<td>3-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily consumption (per 100 grams body weight)</td>
<td>Feed: 6 grams</td>
<td>Water: 10 ml</td>
<td></td>
</tr>
<tr>
<td>Begins eating solid feed (days)</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at weaning (days)</td>
<td>20-21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Appendix C: Sample Surgery Tag**

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Analgesic</th>
<th>Antibiotic</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/7/12</td>
<td>8AM</td>
<td>✔️</td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>BPM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td>14/7/12</td>
<td>8AM</td>
<td>✔️</td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>BPM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td>15/7/12</td>
<td>8AM</td>
<td>✔️</td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>BPM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td>16/7/12</td>
<td>8AM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>BPM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td>17/7/12</td>
<td>8AM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
<tr>
<td></td>
<td>BPM</td>
<td></td>
<td>✔️</td>
<td>CE</td>
</tr>
</tbody>
</table>

Suture removal date: 21/7/12

Comments: