1. PURPOSE

This Standard Operating Procedure (SOP) describes methods for anesthetizing rats.

2. RESPONSIBILITY

Principal Investigators (PIs) and their research staff, veterinary care staff.

3. INTRODUCTION

3.1. Rats are not routinely fasted prior to anesthesia due to their inability to vomit.

3.2. Rodents can be anesthetized with either inhalant gas or injectable drugs. The use of inhalant gases is the preferred method of anesthesia whenever possible.

3.3. Heat loss is rapid in anesthetized rodents. Keep animals warm by covering them (e.g. gauze pad or towel) and/or providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animal.

3.4. Never leave an anesthetized animal unattended.

4. MATERIALS

4.1. Material or equipment to provide or conserve body heat: heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents.

4.2. Ophthalmic ointment (natural tears)

4.3. Gas anesthesia machine (calibrated within the last 12 months) with adequate gas scavenging system or filter

4.4. Induction chamber constructed of a see-through material (glass, polycarbonate, etc.)

4.5. Rodent anesthesia nosecone or mask

4.6. Isoflurane

4.7. Ketamine (100mg/mL) *Controlled drug

4.8. Xylazine (20mg/mL)

4.9. Acepromazine (10mg/mL)

4.10. Atipamezole (5mg/ml)

4.11. Sterile isotonic saline (0.9% saline) or sterile water for injection

4.12. Crushed ice or ice pack

5. PROCEDURES FOR ADULT RATS

5.1. Isoflurane anesthesia:

5.1.1. Induction:

5.1.1.1. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.

5.1.1.2. Place the animal in the induction chamber

5.1.1.3. Adjust the isoflurane vaporizer to 3% to 5%

5.1.2. Maintenance:

5.1.2.1. Use the nosecone or mask connected to the Bain circuit,

5.1.2.2. Adjust the flowmeter to 0.4 to 0.8 L/min.

5.1.2.3. Adjust the isoflurane vaporizer to 2 to 2.5%.
5.1.2.4. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.

5.1.2.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:
   - 5.1.2.5.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)
   - 5.1.2.5.2. Respiratory rate and breathing pattern
   - 5.1.2.5.3. Mucous membrane color surrounding the nose and mouth (should remain pink)

5.1.3. Recovery:
   - 5.1.3.1. Turn off the isoflurane vaporizer, flush the system and keep the animal on oxygen.
   - 5.1.3.2. Transfer animal to their cage once it begins to move and allow to recover fully (sternal position).
   - 5.1.3.3. Provide supplemental heat during the recovery period.

5.2. Ketamine/Xylazine/Acepromazine anesthesia:
   - 5.2.1. Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.
   - 5.2.2. Contact your veterinarian for advice on the appropriate dose prior to use.
   - 5.2.3. Recommended anesthetic dose: ketamine 50mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg.
   - 5.2.4. When working with a new rat strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, administer the remaining 25% of the recommended dose. An additional 25% of the recommended dose may be administered if pedal withdrawal reflexes remain present after 5 minutes. Do not exceed 125% of the recommended dose.
   - 5.2.5. Prepare the solution the day before or shake it thoroughly before use.
   - 5.2.6. To prepare cocktail, in a sterile vial or bottle with a rubber stopper, mix:
     - 5mL of ketamine (100mg/mL)
     - 2.5mL xylazine (20mg/mL)
     - 1mL acepromazine (10mg/mL)
     - 1.5mL of sterile isotonic saline or sterile water for injection.
   - 5.2.7. Label as “Rodent Cocktail” and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 50mg/mL, xylazine 5mg/mL, acepromazine 1mg/mL.
   - 5.2.8. Mixed cocktail should be protected from light and stored at room temperature.
   - 5.2.9. Administer 0.1mL/100g body weight intramuscularly or intraperitoneally for the recommended dose.
   - 5.2.10. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.
   - 5.2.11. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.
   - 5.2.12. Duration of anesthesia is approximately 30 minutes.
   - 5.2.13. After 30 minutes, a half dose may be administered as needed.
   - 5.2.14. Administer atipamezole to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.
     - 5.2.14.1. Recommended dose: 1-2 mg/kg.
     - 5.2.14.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5mg/mL.
     - 5.2.14.3. Administer 0.2-0.4mL/100g body weight subcutaneously or intraperitoneally.
   - 5.2.15. Provide supplemental heat and monitor until recovery (sternal position).

6. PROCEDURES FOR NEONATAL RATS

6.1. Hypothermia:
   - 6.1.1. Use only in animals less than 7 days of age.
6.1.2. Provides immobilization and mild analgesia for short, minor procedures.

6.1.3. Protect pup in a glove or paper-lined tube to avoid damage to the skin.

6.1.4. Induction:
   
   6.1.4.1. Immerse pup in ice water or crushed ice for 3 to 4 minutes.

6.1.5. Maintenance:
   
   6.1.5.1. Place pup on a paper-covered ice pack.

   6.1.5.2. Use a fiber optic surgical lamp if necessary as incandescent lamps will warm the animal and interfere with anesthesia.

   6.1.5.3. Duration of anesthesia is approximately 10 minutes.

6.1.6. Recovery:
   
   6.1.6.1. Remove animal from ice pack and allow to warm.

   6.1.6.2. Recovery time can be up to 1 hour.

6.1. Isoflurane anesthesia:

   6.1.1. Neonates require higher concentration of isoflurane than adults (maintenance at 3-4%). See section 5.1 for detailed procedure.
### SOP REVISION HISTORY

<table>
<thead>
<tr>
<th>DATE</th>
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<tr>
<td>2016.01.15</td>
<td>Addition of Rodent Procedure Log (annex)</td>
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</table>
| 2016.03.16 | 5.2.1 Injectable anesthetic dose can vary with the sex, the age, the strain, and the body condition of the animal.  
5.2.2 Contact your veterinarian for advice on the appropriate dose prior to use. |
| 2020.05.20 | 3.3. Heat loss is rapid in anesthetized rodents. Keep animals warm by covering them (e.g. gauze pad or towel) and/or providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animal.  
4.5 Rodent anesthesia nosecone  
4.10. Atipamezole (5mg/ml)  
4.12. Crushed ice or ice pack  
5.1.1.1. Place the animal in the induction chamber. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.  
5.1.1.2. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min. Place the animal in the induction chamber.  
5.1.2.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:  
5.1.2.5.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)  
5.1.2.5.2. Respiratory rate and breathing pattern  
5.1.2.5.3. Mucous membrane color surrounding the nose and mouth (should remain pink)  
5.2.11. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex. |
| 2020.05.20 | 5.2.12. Place the animal in the induction chamber. Adjust the oxygen flowmeter to 0.8 to 1.5 L/min.  
5.2.13.1. Recommended dose: 1-2 mg/kg.  
5.2.13.2. Prepare a 1:10 atipamezole solution in sterile isotonic saline or sterile water for injection. The final concentration of the mixture is 0.5mg/mL.  
5.2.13.3. Administer 0.2-0.4mL/100g body weight subcutaneously or intraperitoneally. |
| 2020.05.20 | 5.2.14. Provide supplemental heat and monitor until recovery (sternal position). |
| 2020.05.20 | 5.2.15. Recommended anesthetic dose: ketamine 50mg/kg, xylazine 5mg/kg, acepromazine 1mg/kg.  
5.2.16. When working with a new rat strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, administer the remaining 25% of the recommended dose. An additional 25% of the recommended dose may be administered if pedal withdrawal reflexes remain present after 5 minutes. Do not exceed 125% of the recommended dose. |
| 2020.05.20 | 5.2.17. Prepare the solution the day before or shake it thoroughly before use.  
5.2.18. Label as “Rodent Cocktail” and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 50mg/mL, xylazine 5mg/mL, acepromazine 1mg/mL.  
5.2.19. Mixed cocktail should be protected from light and stored in a cool place at room temperature.  
5.2.20. Administer 0.1mL/100g body weight intramuscularly or intraperitoneally for the recommended dose. |
| 2020.12.07 | 4.1. Material or equipment to provide or conserve body heat: Heating disc, warming pad or warm-water circulating pad. Do not use electric heating pads unless specifically designed for use with laboratory rodents. (e.g. gauze pads, heating disc or pad, warm-water circulating pad)  
5.1.2.5.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:  
5.1.2.5.5.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)  
5.1.2.5.5.2. Respiratory rate and breathing pattern  
5.1.2.5.5.3. Mucous membrane color surrounding the nose and mouth (should remain pink)  
5.1.2.5.6. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex. |
| 2020.12.07 | |
## Rodent Procedure Log

**Instructions:** Complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (e.g., surgeries, experimental infection). Keep the log in the housing room while active and in your files for 3 years for future review by the Quality Assistant and/or the FACC.

### ANALGESIA
- **carprofen:** mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- **buprenorphine:** mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- **lidocaine/bupivacaine** (local analgesic)
- **other:**

### ANESTHESIA
- **isoflurane** 2-2.5%
- **ketamine/xylazine/acepromazine**:
  - mouse: 100 mg/kg (K)- 10 mg/kg (X)- 3 mg/kg (A) IP
  - rat: 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); IP or IM

### OTHER AGENTS ADMINISTERED
- **other:**

### Animal ID | Date | Anesthesia | Analgesia | Other | Heat Source Provided | Recovery time | Comments/observations | Initials
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Comments/footnotes:
*Dose can vary with the sex, the age, the strain, and the body condition of the animal.*

Revised: 2014-01-06
ANALGESIA

- carprofen: mouse: 20mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs
- buprenorphine: mouse: 0.1mg/kg SC or IP every 4-8 hrs; rat: 0.05mg/kg, SC or IP, every 8-12 hrs
- OTHER_________________________________________

Initial the appropriate boxes when completed

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Comments/footnotes: