STANDARD OPERATING PROCEDURE #111

RAT ANESTHESIA

1. PURPOSE

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

2. RESPONSIBILITY

Principal Investigators (PIs) and their research staff, veterinarian, 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. Inhalant anesthesia is recommended due to its wide safety margin, reliability, rapid control of anesthetic depth, and faster recovery.

3.3. When both the subcutaneous and intraperitoneal routes of administration can be used to inject anesthetics or reversal agents, the subcutaneous route is preferred as it is simple to execute and less invasive than the intraperitoneal route which can be associated with pain and peritoneal irritation.

3.4. Heat loss is rapid in anesthetized rodents. Keep animals warm providing a heat source until the animal has recovered from anesthesia. Care should be taken to not overheat or burn the animal; do not place animals directly in contact with the heat source, use a drape or other material as a barrier.

3.5. Monitor animals closely during induction, maintenance, and recovery from general anesthesia. Monitoring must be documented.

- 3.5.1. Never leave an anesthetized animal unattended.
- 3.5.2. Monitor animal every 5 minutes:
  - 3.5.2.1. Anesthetic depth: absence of reflexes, e.g., pedal, absence of movement, muscle relaxation.
  - 3.5.2.2. Respiratory function: respiratory rate, thoracic wall movements. When species-adapted equipment is available include oxygen saturation (SpO_2), end-tidal carbon dioxide ($ETCO_2$).
  - 3.5.2.3. Cardiovascular function (circulation): mucous membrane color, capillary refill time (CRT) when possible. When species-adapted equipment is available include electrocardiography (ECG).
  - 3.5.2.4. Body temperature: rectal temperature when possible. Warming pad probes can also be used.

3.6. Apply ophthalmic ointment to prevent corneal desiccation. Reapply as needed, every 30 minutes at a minimum

3.7. Maintain records of each anesthesia procedure and include:

- 3.7.1. Date and time of procedure
- 3.7.2. Principal investigator and Animal Use Protocol (AUP)
- 3.7.3. Species and animal's identification
- 3.7.4. Animals' weight
- 3.7.5. Name, dose, route, and time of administration of each drug
- 3.7.6. Description of the procedure
- 3.7.7. Time of recovery
- 3.7.8. Observations, i.e., unexpected variations of anesthetic depth, vital signs, or complications during surgery or recovery
- 3.7.9. Name of the individual monitoring the animal and of the surgeon

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. Animal weighing scale

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

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

4.6. Rodent anesthesia nosecone or mask attached to a non-rebreathing circuit with separate tubing for delivery for fresh gas and evacuation of waste gas (Bain circuit).

4.7. Isoflurane

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

4.9. Xylazine (20 mg/mL)

4.10. Acepromazine (10 mg/mL)

4.11. Atipamezole (5 mg/ml)

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

4.13. Crushed ice or ice pack

5. PROCEDURES FOR ADULT RATS

5.1. Isoflurane anesthesia:

5.1.1. Consider premedication in situations where animals are exhibiting signs of distress during induction using an induction chamber. Consult the veterinarian.

5.1.2. Induction:

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

5.1.2.2. Place the animal in the induction chamber

5.1.2.3. Adjust the isoflurane vaporizer to 3%, increase as needed to effect.

5.1.3. Maintenance:

5.1.3.1. Remove the animal from the induction chamber and use the nosecone or mask connected to the Bain circuit.

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

5.1.3.3. Adjust the isoflurane vaporizer to 2 to 2.5%.

5.1.3.4. Apply ophthalmic ointment (natural tears) to both eyes .

5.1.3.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters.

5.1.4. Recovery:

5.1.4.1. Turn off the isoflurane vaporizer, and keep the animal on oxygen.

5.1.4.2. Transfer animal to their cage once it begins to move and allow to recover fully (sternal position).

5.1.4.3. 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. Contact veterinarians if unable to adequately anesthetize animal with administration of 125% of recommended dose.

5.2.5. Prepare the solution the day before use or shake it thoroughly before use.

5.2.6. To prepare cocktail, in a sterile vial or bottle with a rubber stopper, mix:

- 5.0 mL of ketamine (100 mg/mL)
- 2.5 mL xylazine (20 mg/mL)
- 1.0 mL acepromazine (10 mg/mL)
- 1.5 mL of sterile isotonic saline or sterile water for injection.

5.2.7. Label as “Rat Anesthetic Cocktail” and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 50 mg/mL, xylazine 5 mg/mL, acepromazine 1 mg/mL.

5.2.8. Mixed cocktail should be protected from light and stored at room temperature.

5.2.9. Administer 0.1 mL/100g body weight, as calculated from the current bodyweight, subcutaneously.

5.2.10. Apply ophthalmic ointment (natural tears) to both eyes.

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. Monitor animals closely for any signs of movement in response to stimuli and re-dose early to avoid emerging from the surgical plane of anesthesia. A quarter or half dose of ketamine may be administered to prolong anesthesia 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 0.5 mg/mL.

5.2.14.3. Administer 0.2-0.4 mL/100g body weight subcutaneously.

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. Place a thin barrier between the ice and the pup, to protect the animal and avoid damage to the skin. Never place the pup in direct contact with ice.

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.

6.2. Isoflurane anesthesia:

6.2.1. Neonates require higher concentration of isoflurane than adults (maintenance at 3-4%). See section 5.1 for detailed procedure.

7. REFERENCES

7.1. Welberg, L. A., Kinkead, B., Thrivikraman, K., Huerkamp, M. J., Nemeroff, C. B., & Plotsky, P. M. (2006). Ketamine-xylazine-acepromazine anesthesia and postoperative recovery in rats. Journal of the American Association for Laboratory Animal Science : JAALAS, 45(2), 13–20.

7.2. Flecknell, P. 2016. Basic principles of anaesthesia. In Laboratory Animal Anaesthesia , 4th edition. London: Elsevier.

7.3. Struck, M. B., Andrutis, K. A., Ramirez, H. E., & Battles, A. H. (2011). Effect of a short-term fast on ketamine-xylazine anesthesia in rats. Journal of the American Association for Laboratory Animal Science : JAALAS, 50(3), 344–348.

7.4. Taylor, B. J., Orr, S. A., Chapman, J. L., & Fisher, D. E. (2009). Beyond-use dating of extemporaneously compounded ketamine, acepromazine, and xylazine: safety, stability, and efficacy over time. Journal of the American Association for Laboratory Animal Science : JAALAS, 48(6), 718–726.

SOP REVISION HISTORY

DATE	NEW VERSION
2024.03.15	2. RESPONSIBILITY Principal Investigators (PIs) and their research staff, **veterinarian,** veterinary care staff.
2024.03.15	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. **Inhalant anesthesia is recommended due to its wide safety margin, reliability, rapid control of anesthetic depth, and faster recovery.**
2024.03.15	**3.3. When both the subcutaneous and intraperitoneal routes of administration can be used to inject anesthetics or reversal agents, the subcutaneous route is preferred as it is simple to execute and less invasive than the intraperitoneal route which can be associated with pain and peritoneal irritation.**
2024.03.15	3.4. 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 not to overheat or burn the animal**; do not place animals directly in contact with the heat source, use a drape or other material as a barrier**.
2024.03.15	**3.5. Monitor animals closely during induction, maintenance, and recovery from general anesthesia. Monitoring must be documented.** 3.5.1. Never leave an anesthetized animal unattended. **3.5.2. Monitor animal every 5 minutes:** **3.5.2.1. Anesthetic depth: absence of reflexes, e.g., pedal, absence of movement, muscle relaxation.** **3.5.2.2. Respiratory function: respiratory rate, thoracic wall movements. When species-adapted equipment is available include oxygen saturation (SpO_2), end-tidal carbon dioxide ($ETCO_2$).** **3.5.2.3. Cardiovascular function (circulation): mucous membrane color, capillary refill time (CRT) when possible. When species-adapted equipment is available include electrocardiography (ECG).** **3.5.2.4. Body temperature: rectal temperature when possible. Warming pad probes can also be used.**
2024.03.15	**3.6. Apply ophthalmic ointment to prevent corneal desiccation. Reapply as needed, every 30 minutes at a minimum.**
2024.03.15	**3.7. Maintain records of each anesthesia procedure and include:** **3.7.1. Date and time of procedure** **3.7.2. Principal investigator and Animal Use Protocol (AUP)** **3.7.3. Species and animal's identification** **3.7.4. Animals' weight** **3.7.5. Name, dose, route, and time of administration of each drug** **3.7.6. Description of the procedure** **3.7.7. Time of recovery** **3.7.8. Observations, i.e., unexpected variations of anesthetic depth, vital signs, or complications during surgery or recovery.** **3.7.9. Name of the individual monitoring the animal and of the surgeon**
2024.03.15	**4.3. Animal weighing scale**
2024.03.15	4.6. Rodent anesthesia nosecone or mask **attached to a non-rebreathing circuit with separate delivery for fresh gas and evacuation of waste gas (Bain circuit)**
2024.03.15	**5.1.1. Consider premedication in situations where animals are exhibiting signs of distress during induction using an induction chamber. Consult the veterinarian.**
2024.03.15	**5**.1.2.3. Adjust the isoflurane vaporizer to 3%~~ to 5%~~**, increase as needed to effect.**
2024.03.15	5.1.3.1. **Remove the animal from the induction chamber and** use the nosecone or mask connected to the Bain circuit.
2024.03.15	5.1.3.7. Apply ophthalmic ointment (natural tears) to both eyes ~~to prevent dryness and damage to the cornea. Reapply as needed~~.

2024.03.15	5.1.3.8. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters. 5.1.3.8.~~1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)~~ 5.1.3.8.~~2. Respiratory rate and breathing pattern~~ 5.1.3.8.~~3. Mucous membrane color surrounding the nose and mouth (should remain pink)~~
2024.03.15	5.1.3.8. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters. 5.1.3.8.~~1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)~~ 5.1.3.8.~~2. Respiratory rate and breathing pattern~~ 5.1.3.8.~~3. Mucous membrane color surrounding the nose and mouth (should remain pink)~~
2024.03.15	5.1.4.1. Turn off the isoflurane vaporizer, ~~flush the system~~ and keep the animal on oxygen.
2024.03.15	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. **Contact veterinarian if unable to adequately anesthetize animal with administration of 125% of recommended dose.**
2024.03.15	5.2.7. Label as "~~Rodent~~ **Rat Anesthetic** 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.
2024.03.15	5.2.9. Administer 0.1mL/100g body weight**, as calculated from the current bodyweight, subcutaneously**~~ intramuscularly or intraperitoneally for the recommended dose~~.
2024.03.15	5.2.10. Apply ophthalmic ointment (natural tears) to both eyes~~ to prevent dryness and damage to the cornea. Reapply as needed~~.
2024.03.15	5.2.14. **Monitor animals closely for any signs of movement in response to stimuli and re-dose early to avoid emerging from the surgical plane of anesthesia.** ~~After 30 minutes,~~ A **quarter or** half dose **of ketamine** may be administered ~~as needed~~ **to prolong anesthesia as needed**.
2024.03.15	5.2.14.3. Administer 0.2-0.4mL/100g body weight subcutaneously~~ or intraperitoneally~~.
2024.03.15	6.1.3. **Place a thin barrier between the ice and the pup, to** protect ~~pup~~ the animal ~~in a glove or paper-lined tube to~~ **and** avoid damage to the skin. **Never place the pup in direct contact with ice.**
2024.03.15	**7. REFERENCES** **7.1. Welberg, L. A., Kinkead, B., Thrivikraman, K., Huerkamp, M. J., Nemeroff, C. B., & Plotsky, P. M. (2006). Ketamine-xylazine-acepromazine anesthesia and postoperative recovery in rats. Journal of the American Association for Laboratory Animal Science : JAALAS, 45(2), 13–20.** **7.2. Flecknell, P. 2016. Basic principles of anaesthesia. In Laboratory Animal Anaesthesia , 4th edition. London: Elsevier.** **7.3. Struck, M. B., Andrutis, K. A., Ramirez, H. E., & Battles, A. H. (2011). Effect of a short-term fast on ketamine-xylazine anesthesia in rats. Journal of the American Association for Laboratory Animal Science : JAALAS, 50(3), 344–348.** **7.4. Taylor, B. J., Orr, S. A., Chapman, J. L., & Fisher, D. E. (2009). Beyond-use dating of extemporaneously compounded ketamine, acepromazine, and xylazine: safety, stability, and efficacy over time. Journal of the American Association for Laboratory Animal Science : JAALAS, 48(6), 718–726.**