STANDARD OPERATING PROCEDURE #110

MOUSE ANESTHESIA

1. PURPOSE

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

2. RESPONSIBILITY

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

3. INTRODUCTION

3.1. Mice 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 gas 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 by 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) and 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 MICE

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 a 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 the 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. The addition of acepromazine in this anesthetic cocktail increases the percentage of mice that reach a surgical plane of anesthesia.

5.2.2. Injectable anesthetic dose can vary with the sex, the age, the strain, and the general condition of the animal.

5.2.3. Contact your veterinarian for advice on the appropriate dose prior to use.

5.2.4. Recommended anesthetic dose: ketamine 100 mg/kg, xylazine 10 mg/kg, acepromazine 3 mg/kg.

5.2.5. When working with a new mouse 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.

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

5.2.7. To prepare the solution, in a sterile vial or bottle with a rubber stopper, mix:

- 1.0 mL of ketamine (100 mg/mL)
- 0.5 mL xylazine (20 mg/mL)
- 0.3 mL acepromazine (10 mg/mL)
- 8.2 mL of sterile isotonic saline or sterile water for injection.

5.2.8. Label as “Mouse Anesthetic Cocktail” and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL, acepromazine 0.3 mg/mL.

5.2.9. Solution should be protected from light and stored at room temperature.

5.2.10. Administer 0.1 mL/10g body weight, as calculated from the current bodyweight, subcutaneously or intraperitoneally .

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

5.2.12. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.

5.2.13. Duration of anesthesia is approximately 20 minutes.

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. A quarter or half dose of ketamine may be administered to prolong anesthesia as needed.

5.2.15. Administration of atipamezole at the end of the procedure may improve respiration and speed up the recovery. Atipamezole is the antidote for xylazine.

5.2.15.1. Recommended dose: 1-2 mg/kg.

5.2.15.2. 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.

5.2.15.3. Administer 0.02-0.04 mL/10g body weight subcutaneously or intraperitoneally.

5.2.16. Provide supplemental heat and monitor until recovery (sternal position).

5.3. Ketamine/Xylazine anesthesia:

5.3.1. Ketamine/xylazine alone, without the addition of acepromazine or other drugs, may not achieve surgical plane of anesthesia.

5.3.2. Injectable anesthetic dose can vary with the sex, the age, the strain, and the general condition of the animal.

5.3.3. Contact your veterinarian for advice on the appropriate dose prior to use.

5.3.4. Recommended anesthetic dose: ketamine 100 mg/kg, xylazine 10 mg/kg.

5.3.5. When working with a new mouse strain, administer 75% of the recommended dose. If pedal withdrawal reflexes are still present after 5 minutes, adminisemerter 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.

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

5.3.7. To prepare solution, in a sterile vial or bottle with a rubber stopper, mix:

- 1.0 mL of ketamine (100 mg/mL)
- 0.5 mL xylazine (20 mg/mL)
- 8.5 mL of sterile isotonic saline or sterile water for injection.

5.3.8. Label adequately and indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL.

5.3.9. Administer 0.1 mL/10g body weight, as calculated from the current bodyweight, subcutaneously or intraperitoneally .

5.3.10. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.

5.3.11. After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.

5.3.12. Duration of anesthesia is approximately 20 minutes.

5.3.13. 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.

5.3.14. Administration of atipamezole at the end of the procedure may improve respiration and speed up the recovery. Atipamezole is the antidote for xylazine.

5.3.14.1. Recommended dose: 1-2 mg/kg.

5.3.14.2. 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.

5.3.14.3. Administer 0.02-0.04 mL/10g body weight subcutaneously or intraperitoneally.

6. PROCEDURES FOR NEONATAL MICE

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. Never place the pup in direct contact with ice to protect the animal and 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 gradually. Avoid rapid warming, such as with a heating lamp, as this may lead to tissue damage.

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. 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.

7.2. Janssen, C. F., Maiello, P., Wright, M. J., Jr, Kracinovsky, K. B., & Newsome, J. T. (2017). Comparison of Atipamezole with Yohimbine for Antagonism of Xylazine in Mice Anesthetized with Ketamine and Xylazine. *Journal of the American Association for Laboratory Animal Science : JAALAS*, *56*(2), 142–147.

7.3. Levin-Arama, M., Abraham, L., Waner, T., Harmelin, A., Steinberg, D. M., Lahav, T., & Harlev, M. (2016). Subcutaneous Compared with Intraperitoneal KetamineXylazine for Anesthesia of Mice. Journal of the American Association for Laboratory Animal Science : JAALAS, 55(6), 794–800.

7.4. Navarro, K. L., Huss, M., Smith, J. C., Sharp, P., Marx, J. O., & Pacharinsak, C. (2021). Mouse Anesthesia: The Art and Science. ILAR journal, 62(1-2), 238–273. https://doi.org/10.1093/ilar/ilab016

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 gas 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 and~~ 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.
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. ~~Continuously monitor anesthetized animals until fully recovered; sternal and moving in the cage.~~ **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	~~4.12. 2,2,2 Tribromoethanol (Avertin)~~
2024.03.15	~~4.13. Tertiary amyl alcohol~~
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	5.1.3.4. Apply ophthalmic ointment (natural tears) to both eyes ~~to prevent dryness and damage to the cornea. Reapply as needed~~.
2024.03.15	5.1.3.5. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters. ~~5.1.3.5.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)~~ ~~5.1.3.5.2. Respiratory rate and breathing pattern~~ ~~5.1.3.5.3. Mucous membrane color surrounding the nose and mouth (should remain pink)~~

2024.03.15	5.1.4.1. **Turn off the isoflurane vaporizer,** keep the animal on oxygen ~~until it starts to recover~~.
2024.03.15	**5.2.1. The addition of acepromazine in this anesthetic cocktail increases the percentage of mice that reach a surgical plane of anesthesia.**
2024.03.15	5.2.2. Injectable anesthetic dose can vary with the sex, the age, the strain, and the ~~body~~ **general** condition of the animal.
2024.03.15	5.2.5. When working with a new mouse 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	5.2.8. **Label as "Mouse Anesthetic Cocktail" and** indicate expiration date on vial or bottle (maximum 6 months). The final concentration of the mixture is: ketamine 10 mg/mL, xylazine 1 mg/mL, acepromazine 0.3 mg/mL.
2024.03.15	5.2.10. Administer 0.1 mL/10g body weight, **as calculated from the current bodyweight, subcutaneously or** intraperitoneally.
2024.03.15	5.2.11. 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 20 minutes, **a quarter or** half dose **of ketamine** may be administered ~~as needed~~ **to prolong anesthesia** as needed.
2024.03.15	**5.3.1. Ketamine/xylazine alone, without the addition of acepromazine or other drugs, may not achieve surgical plane of anesthesia.**
2024.03.15	5.3.2. Injectable anesthetic dose can vary with the sex, the age, the strain, and the ~~body~~ **general** condition of the animal.
2024.03.15	~~2,2,2 Tribromoethanol (TBE or Avertin) anesthesia~~
2024.03.15	6.1.3. **Place a thin barrier between the ice and the pup. Never place the pup in direct contact with ice to** protect ~~pup~~ the animal ~~in a glove or paper lined tube to~~ **and** avoid damage to the skin.
2024.03.15	6.1.6.1. Remove animal from ice pack and allow to warm **gradually. Avoid rapid warming, such as with a heating lamp, as this may lead to tissue damage.**
2024.03.15	**7.4. Navarro, K. L., Huss, M., Smith, J. C., Sharp, P., Marx, J. O., & Pacharinsak, C. (2021). Mouse Anesthesia: The Art and Science. ILAR journal, 62(1-2), 238–273. https://doi.org/10.1093/ilar/ilab016**

Rodent Procedure Log

McGill

Principal Investigator:	Protocol:
Procedure:	Performed by:
Date:	Species:

Instructions: complete this log for rodent procedures requiring anesthesia, analgesia, or post-procedure care. This log should be readily available for in a format accessible to investigators, veterinarians, animal care personnel, and animal care committees.

ANALGESIA

- ☐ carprofen: mouse: 20 mg/kg, rat: 510 mg/kg, SC, every 24 hrs
- ☐ buprenorphine: (rat) 0.05 mg/kg, SC or IP, every 8-12 hrs
- ☐ buprenorphine SR: 1 mg/kg SC every 72 hours
- ☐ lidocaine/bupivacaine (local analgesic)
- ☐ other: .....

ANESTHESIA

- ☐ isoflurane
- ☐ ketamine/xylazine/acepromazine*: (rat): 50 mg/kg (K)- 5 mg/kg (X)- 1 mg/kg (A); SC
- ☐ .....

OTHER AGENTS ADMINISTERED

- ☐ .....
- ☐ .....
- ☐ .....
- ☐ .....
- ☐ .....

	Animal ID	BW (g)	Anesthesia		Analgesia		Other		Heat Source Provided		Recovery time	Comments/observations
			dose	time	dose	time	dose	time	procedure	recovery		
1									☐	☐		
2									☐	☐		
3									☐	☐		
4									☐	☐		
5									☐	☐		
6									☐	☐		
7									☐	☐		
8									☐	☐		
9									☐	☐		
10									☐	☐		
11									☐	☐		
12									☐	☐		

*Dose can vary with the sex, the age, the strain, and the general condition of the animal.

Rodent Procedure Log

McGill

POST-OPERATIVE CARE

☐ carprofen: mouse: 20 mg/kg, rat: 5-10 mg/kg, SC, every 24 hrs

☐ buprenorphine: mouse: 0.1 mg/kg SC or IP every 4-8 hrs; rat: 0.05 mg/kg, SC or IP, every 8-12 hrs

☐ buprenorphine slow release 1 mg/kg SC

Initial the appropriate boxes when completed

	Animal ID	Date	Analgesia				SC fluids				Wet food				Time				Remove Sutures (Day 7-10)
			Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2	Day 3	
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			

Comments/footnotes: