McGill

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, 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 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.

3.4. Never leave an anesthetized animal unattended.

4. MATERIALS

4.1. Material or equipment to provide or conserve body heat (e.g. gauze pads, heating disc or pad, warm-water circulating pad)

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. Isoflurane

4.6. Ketamine (100mg/mL) *Controlled Drug

4.7. Xylazine (20mg/mL)

4.8. Acepromazine (10mg/mL)

4.9. Atipamezole 5 mg/ml

4.10. 2,2,2-Tribromoethanol (Avertin)

4.11. Tertiary amyl alcohol

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. Induction:

5.1.1.1. Place the animal in the induction chamber

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

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

5.1.2. Maintenance:

5.1.2.1. Use the 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.

5.1.3. Recovery:

5.1.3.1. Turn off the isoflurane vaporizer but 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).

5.2. 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. Anesthetic dose: ketamine 100mg/kg, xylazine 10mg/kg, acepromazine 3mg/kg.

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

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

- 1mL of ketamine (100mg/mL)
- 0.5mL xylazine (20mg/mL)
- 0.3mL acepromazine (10mg/mL)
- 8.2mL of sterile isotonic saline or sterile water for injection.

5.2.6. Label as “Mouse Cocktail” and indicate expiration date on vial or bottle (maximum 6 months).

5.2.7. Mixed cocktail should be protected from light and stored in a cool place.

5.2.8. Administer 0.05-0.1mL/10g body weight intraperitoneally.

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

5.2.10. Duration of anesthesia is approximately 20 minutes.

5.2.11. After 20 minutes, a half dose may be administered as needed.

5.2.12. Administer atipamezole 1-2 mg/kg SC or IP to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.

5.3. 2,2,2-Tribromoethanol (TBE or Avertin) anesthesia:

5.3.1. Anesthetic dose is 250 to 500mg/kg.

5.3.2. Avertin administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion.

5.3.3. Avertin stock solution:

5.3.3.1. In a sterile container add 25g of 2,2,2-tribromoethanol to 15.5mL tertiary amyl alcohol and dissolve by heating to 50°C and stirring until completely dissolved.

5.3.3.2. Store protected from light (wrapped in foil or in an amber container) in the refrigerator or freezer.

5.3.4. Label as “Avertin Stock Solution” and indicate expiration date (up to 1 year).

5.3.5. Avertin working solution:

5.3.5.1. In a sterile container, mix 0.5mL of the stock solution in 39.5mL of sterile isotonic saline.

5.3.5.2. Heat solution to 40°C then shake well until completely dissolved. Mixture should be clear.

5.3.5.3. Filter the working solution through 0.2 micron filter.

5.3.5.4. Store protected from light (wrapped in foil or in an amber container) at 4°C.

5.3.5.5. Label as “Avertin 20mg/mL” and indicate expiration date (maximum 4 months)

5.3.6. Inject 0.1-0.25mL of working solution/10g body weight, intraperitoneally.

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

5.3.8. Duration of anesthesia is approximately 20 minutes.

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. 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:

6.1.5.2. Place pup on a paper-covered ice pack.

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

6.1.5.4. 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.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2016.01.15	(NO TEXT)	**Addition of Rodent Procedure Log (annex)**
2016.03.16	5.3.1 Anesthetic dose is 240mg/kg.	5.3.1 Anesthetic dose is ~~240~~ **250 to 500**mg/kg.
2016.03.16	5.2.1 and 5.2.2 (NO TEXT)	**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.**
2016.03.16	5.2.12 (NO TEXT)	**5.2.12 Administer atipamezole 1-2 mg/kg SC or IP to improve respiration or speed up the recovery if needed. Atipamezole is the antidote for xylazine.**

Rodent Procedure Log

Investigator:	Protocol:
Procedure:	Performed by:

Instructions: complete this log for rodent procedures requiring anesthesia, analgesia or post-procedure care (ex. 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:_____

OTHER AGENTS ADMINISTERED

☐_____

☐_____

☐_____

	Animal ID	Date	Anesthesia		Analgesia		Other _____		Heat Source Provided		Recovery time	Comments/observations	Initials
			dose	time	dose	time	dose	time	procedure	recovery			
1									☐	☐			
2									☐	☐			
3									☐	☐			
4									☐	☐			
5									☐	☐			
6									☐	☐			
7									☐	☐			
8									☐	☐			
9									☐	☐			
10									☐	☐			
11									☐	☐			
12									☐	☐			
13									☐	☐			
14									☐	☐			

Comments/footnotes:

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

Revised: 2014-01-06

Rodent Procedure Log

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

	Animal ID	Date	Analgesia			SC fluids			Wet food			Time			Remove Sutures (Day 7-10)
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															

Comments/footnotes: