Cornell University
Institutional Animal Care and Use Committee

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RODENT ANESTHESIA

1. PURPOSE

1.1. The purpose of this Animal Care and Use Procedure (ACUP) is to describe commonly used methods to anesthetize rodents. This ACUP is approved by the Cornell Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation.

2. SCOPE

2.1. This document applies to personnel who perform rodent anesthesia under Cornell University's animal use program.

3. INTRODUCTION

3.1. This ACUP describes the basic procedures required for the use of anesthesia on rodents. Rodents can be anesthetized 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, recipes for anesthetic cocktails for rats and mice have been given.

3.2. Contact Center for Animal Resources and Education (CARE) at Cornell University by e-mailing care@cornell.edu for more information or for assistance in designing an anesthetic plan.

4. MATERIALS AND EQUIPMENT

4.1. Clear induction chamber

4.2. Anesthetic agents

4.2.1. Inhalant anesthesia option: anesthesia machine, including inhalant (e.g., isoflurane) and oxygen supply.

4.2.2. Injectable anesthesia options:

4.2.2.1. Ketamine and xylazine solution.

4.2.2.2. Tribromoethanol (TBE) in an injectable solution.

4.2.3. Crushed ice anesthesia (for neonates)

4.3. Support materials:

4.3.1. Species appropriate scale (as applicable)
4.3.2. Needles and syringes (as applicable)
4.3.3. Sterile ophthalmic lubricant (e.g., Paralube)
4.3.4. Designated surgical area

5. PROCEDURE

5.1. General Considerations

5.1.1. A designated surgical area that provides separation from other activities is required

5.1.1.1. For rodents, the designated surgical space can be a defined counter top area that is not used for other laboratory activities. Having a designated space reduced the risk of surgical site contamination and eliminates clutter from non-surgical items.

5.1.2. For survival surgical procedures, administer analgesics prior to performing surgery. Exceptions must be scientifically justified and IACUC approved.

5.1.2.1. Effective analgesia necessitates administration prior to tissue injury. Since many analgesics require at least 15 minutes pre-administration prior to effect, administration before anesthesia induction is often necessary.
5.1.2.2. Administration of opioids or other pre-medications is advisable prior to anesthesia since these drugs will alter the amount of general anesthesia required.

5.1.3. Heat loss is rapid in anesthetized rodents. Keep animals warm by wrapping / covering them with a drape or towel, and / or providing a heat source (e.g., surgery warming pad, disposable hand warmer) until they have fully recovered from anesthesia. Ensure that all heat sources do not allow heat in excess of 107° F and / or are carefully monitored to prevent burns.
5.1.4. Apply sterile ophthalmic lubricant to eyes once animals are anesthetized.
5.1.5. Ensure adequate depth of anesthesia prior to performing procedures by testing pedal withdrawal and palpebral reflexes.
5.1.6. Ensure animal safety:

5.1.6.1. Minimally, monitor respiration and skin/mucous membrane color.
5.1.6.2. For long procedures or procedures that restrict direct observation (e.g., CT scan), utilize a heart rate monitor or respiratory and/or oxygen saturation monitor.

5.1.7. Do not place anesthetized rodents in contact with loose bedding or similar materials. Recover animals in a bare cage or on top of a paper towel (or similar barrier) to prevent aspiration of bedding.

5.1.8. Monitor the animal until it has fully recovered from anesthesia.

5.1.9. Clearly document all procedures (including anesthesia) with specific procedure cards, or cage cards. Surgery details can be documented in a laboratory notebook that is readily available to animal care and veterinary staff. See ACUP 542 Maintaining Health and Procedure Records for Research and Teaching Animals for more details.

5.2. Mouse Anesthesia

5.2.1. Isoflurane

5.2.1.1. Place the animal in the induction chamber.

5.2.1.2. Adjust the oxygen flowmeter to approximately 0.5-1.0 L/min.

5.2.1.3. Adjust the isoflurane vaporizer to approximately 3-5% for induction and approximately 1-3% for maintenance.

5.2.1.4. In order to reduce hypothermia related to anesthesia, it is recommended to leave rodent cages off of individually ventilated cage (IVC) racks, away from the rack's airflow, until the animal is fully recovered from anesthesia.

5.2.1.5. See section 9 for adverse effects and precautions associated with the use of isoflurane.

5.2.2. Ketamine-Xylazine

5.2.2.1. Solution preparation:

5.2.2.1.1. In a sterile 10 ml serum collection vial (e.g., red top tube) or 10 ml sterile saline vial, aseptically mix ketamine, xylazine, and sterile physiologic saline (e.g., 0.9% Sodium Chloride) for injection. Shake well before use.

5.2.2.1.2. See section 9.2 for specific directions on how to make cocktail.

5.2.2.2. Write on the bottle:

5.2.2.2.1. "KETAMINE-XYLAZINE FOR MOUSE: 0.1 ml/10 g IP".

5.2.2.2.2. Your initials.

5.2.2.2.3. Expiration date (selecting the earliest expiration date between ketamine or xylazine, for a maximum of 6 months).

5.2.2.3. Store away from light in a cool place.

5.2.2.4. Inject 0.1 ml of the ketamine-xylazine solution per 10 g of body weight intraperitoneally (IP) or subcutaneously (SC) (0.1 ml/10 g IP; ketamine @100 mg/kg, xylazine @10 mg/kg).

5.2.2.5. If the animal is not adequately anesthetized, repeat the injection at a dose of 0.05 ml/10g (approximately every 30 minutes).

5.2.2.6. See section 9.1 for adverse effects and precautions associated with the use of ketamine-xylazine.

5.2.3. Tribromoethanol (a.k.a., TBE or Avertin)

NOTE: This is not a pharmaceutical grade substance and its use must be scientifically justified and approved by the IACUC.

NOTE: TBE administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion. If multiple administrations are required, usage and monitoring for adverse reactions must be explicitly detailed in the approved IACUC protocol.

5.2.3.1. Prepare concentrated “stock solution” by mixing 25 g of tribromoethanol in 15.5 ml of amyl alcohol (2-methyl-2-butanol) in a sterile glass container.

5.2.3.2. Write on the bottle:

5.2.3.2.1. "50X TRIBROMOETHANOL"

5.2.3.2.2. Your initials

5.2.3.2.3. Expiration date (1 year from mixing date)

5.2.3.3. Store in freezer (-20°C), away from light.

5.2.3.4. Prepare solution for injection:

5.2.3.4.1. Mix 1 ml of the concentrated solution in 50 ml of 0.9% sterile saline for injection in a sterile container.

5.2.3.4.2. Heat the solution to 40°C, allow to sit at room temperature until dissolved (while maintaining sterility).

5.2.3.4.3. Shake well manually or by vortex.

5.2.3.4.4. Label the container (date, contents, concentration-32 mg/ml, expiration date [4 months from time of dilution], and initials) and store in a dark environment at 4 - 6° C.

5.2.3.5. Inject the TBE solution through a 0.2 micron filter prior to use.

5.2.3.6. Inject 0.15 ml/10 g of body weight IP. Dosage may vary depending on the strain/genotype of mice.

5.2.3.6.1. Repeat dosing may cause death. If repeat dosing is necessary, using inhalant anesthesia is preferable.

5.2.3.7. See section 9.1 for adverse effects and precautions associated with the use of TBE.

5.3. Rat Anesthesia

5.3.1. Isoflurane- follow instructions is section 5.2.1 above.

5.3.2. Ketamine-Xylazine

5.3.2.1.1. In a sterile 10 ml serum collection vial (e.g., red top tube), aseptically mix ketamine and xylazine for injection. Shake well before use.

5.3.2.1.2. See section 9.2 for specific directions on how to make cocktail.

5.3.2.1.3. Write on the bottle:

5.3.2.1.3.1. "KETAMINE-XYLAZINE FOR RAT: 0.05–0.10 ml/100 g IP".
5.3.2.1.3.2. Your initials.
5.3.2.1.3.3. Expiration date (selecting the earliest expiration date between ketamine or xylazine, for a maximum of 6 months).

5.3.2.1.4. Store away from light, in a cool place.
5.3.2.1.5. Administer 0.05–0.10 ml/100 g of body weight IP (ketamine @44-87.5 mg/kg, xylazine @6.25-12.5 mg/kg).
5.3.2.1.6. Repeat as required with 1/3 to 1/2 dose at a time (approximately every 30 minutes).

5.3.2.2. See section 9.1 for adverse effects and precautions associated with the use of ketamine-xylazine.

5.4. Hamster Anesthesia

5.4.1. Isoflurane

5.4.1.1. Follow instructions in section 5.2.1 above

5.4.2. Ketamine-Xylazine

5.4.2.1.1. In a sterile 10 ml serum collection vial (e.g., red top tube), aseptically mix ketamine and xylazine for injection. Shake well before use.
5.4.2.1.2. See section 9.2 for specific directions on how to make cocktail.
5.4.2.2. Write on the bottle:

5.4.2.2.1. "KETAMINE- XYLAZINE FOR HAMSTER: 0.10 ml/135 g IP".
5.4.2.2.2. Your initials.
5.4.2.2.3. Expiration date (selecting the earliest expiration date between ketamine or xylazine for a maximum of 6 months).

5.4.2.3. Store away from light, in a cool place.
5.4.2.4. Administer 0.1ml/135 g of body weight IP (ketamine @64.7 mg/kg, xylazine @9.25 mg/kg).
5.4.2.5. Repeat as required with 1/3 to 1/2 dose at a time (approximately every 30 minutes).

5.4.2.5.1. Repeat dosing may cause death. If repeat dosing is necessary, using inhalant anesthesia is preferable.

5.4.2.6. See section 9.1 for adverse effects and precautions associated with the use of ketamine-xylazine.

5.5. Rodent Neonates

NOTE: This technique may only be used in neonatal, altricial, hairless rodents, generally <10 days old. Time to onset of lethargy may be considerably greater than with other methods. Placing a layer of material between the neonate and ice will result in delayed onset of anesthesia; this will increase time that the rodent is not nursing. However, the neonate must be completely dry prior to direct contact with ice; **DO NOT USE DRY ICE**.

5.5.1. Monitor the readiness of the animal for anesthesia. If lethargy or immobility are noted, the animal should not be anesthetized without veterinary consultation.

5.5.2. Place the neonate on a bed of crushed ice until anesthesia is noted. Once fully anesthetized, anesthesia can be maintained with the addition of a thin 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.

5.5.3. Once anesthetized, the neonate will remain immobile for up to 10 minutes after removal from the ice. If additional time is needed for the procedure, maintain immobility by keeping the neonate on a latex or plastic covered cold pack.

5.5.4. Illuminate the surgical field by use of a fiber optic light source, as incandescent bulbs may cause inadvertent and uncontrollable warming.

5.5.5. Recover pups and slowly re-warm them in an incubator at 33° C or in a warm nest with conspecifics. Complete recovery typically occurs in 30 to 60 minutes.

6. PERSONNEL SAFETY

6.1. Medical Emergencies: **CALL 911**.

6.2. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the CARE Occupational Health and Safety webpage for more information.

7. ANIMAL RELATED CONTINGENCIES

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

7.2. Non-emergency veterinary questions and requests for care, email CARE veterinary staff at care@cornell.edu.

7.3. Emergency veterinary care is available at all times including after working hours and on weekends and holidays by calling the CARE pager (1-800-329-2456).

8. REFERENCES

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8.8. USDA Animal Care Policy Manual: http://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf.
8.9. ACUP 542 (Maintaining Health and Procedure Records for Research and Teaching Animals): http://ras.research.cornell.edu/care/documents_k/ACUPs/ACUP542.pdf
8.10. ACUP 712 (Waste Anesthetic Gas Scavenging Systems): http://ras.research.cornell.edu/care/documents_k/ACUPs/ACUP712.pdf
8.11. CARE Occupational Health and Safety webpage: http://ras.research.cornell.edu/care/OHS.html
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9. APPENDIX

9.1. Adverse effects and precautions of anesthetics

9.1.1. Isoflurane

9.1.1.1. Isoflurane is a vasodilator, and its use can result in fatal hypotension. Administer isoflurane with caution 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 both healthy and compromised animals.

9.1.2. Ketamine

9.1.2.1. Ketamine over dosage may cause death. Repeat injections should be avoided if at all possible, particularly at high doses.

9.1.3. Xylazine

9.1.3.1. 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.

9.1.4. Tribromoethanol (TBE)

9.1.4.1. TBE is a non-pharmaceutical grade compound. Prior to use, scientific justification must be reviewed and approved by the IACUC.

9.1.4.2. Proper storage of TBE is critical to prevent formation of irritating degradation products which may cause severe peritonitis. Administration can result in sensitization of the animal; thus, it is recommended to be given only on a single occasion. If multiple administrations are required, usage and monitoring for adverse reactions must be explicitly detailed in the approved IACUC protocol.

9.2. Sample recipes for ketamine-xylazine cocktails

Ketamine / Xylazine Cocktail for Mice				
*1.1 ml can be removed for new sterile saline vial to make room for addition of ketamine and xylazine				
	Stock Drug Concentration	**Volume Added to Cocktail**	**Volume of Cocktail Administered**	**Dose of Cocktail Administered**
Ketamin	100 mg/ml	1.0 ml		100 mg/kg
Xylazine	100 mg/ml	0.1 ml		10 mg/kg
Sterile Saline	0.9%	8.9 ml*		
Cocktail		10.0 ml	0.1 ml/10 g Bodyweight	

Ketamine / Xylazine Cocktail for Rats				
	Stock Drug Concentration	**Volume Added to Cocktail**	**Volume of Cocktail Administered**	**Dose of Cocktail Administered**
Ketamin	100 mg/ml	8.75 ml		44.0-87.5 mg/kg
Xylazine	100 mg/ml	1.25 ml		6.25-12.5 mg/kg
Cocktail		10.0 ml	0.05-0.10 ml/100 g Bodyweight	

Ketamine / Xylazine Cocktail for Hamsters				
	Stock Drug Concentration	**Volume Added to Cocktail**	**Volume of Cocktail Administered**	**Dose of Cocktail Administered**
Ketamin	100 mg/ml	8.75 ml		64.7 mg/kg
Xylazine	100 mg/ml	1.25 ml		9.25 mg/kg
Cocktail		10.0 ml	0.10 ml/135 g Bodyweight	

10. HISTORY

Date:	**Event:**
04 OCT 18	Most Recent Annual Review – Reviewed by: Dr. B. Blank
16 AUG 18	Revised – Revision Author: Dr. R. Labitt
21 APR 16	IACUC Courtesy Review for Clarification – No Revision
17 SEP 15	New Format – Converted by: J. Kirby
01 APR 11	Revised – Revision Author: Dr. S. Bliss
15 FEB 03	New Issued – Original Author: Dr. J. Gourdon