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Mouse-Specific Anesthesia Guidance

I. Purpose:

This document has been designed by the ARC veterinary staff as a guideline for mouse anesthesia. This is not intended to be an inclusive list of all possible drug combinations that can be used in mice. Instead, these guidelines are general recommendations. Consequently, they do not include reference to specific research-associated concerns. If you have questions about the use of anesthetics for your particular situation, please work with your area veterinarian to develop the most effective anesthetic plan.

Anesthesia is the loss of feeling in all or part of the body, with or without loss of consciousness. Animals may be anesthetized for surgery, for non-surgical procedures that may be painful, or for non-painful procedures that require immobilization. Steps must be taken before, during, and after anesthesia to ensure the safety of the animal and efficacy of anesthesia. These are listed in the General Guidelines section below.

Anesthetic drugs can be administered parenterally or by inhalation. Commonly used anesthetic agents are described below. The choice of anesthetic agent will depend on the procedure to be performed, research aims, and other factors such as animal age; there are special considerations for neonatal mice outlined below. Consult your area veterinarian with questions about drug selection.

II. General Guidelines and Considerations for Mouse Anesthesia:

- **Acclimation:** A one week post-shipping acclimation period is recommended for all animals to prevent stress-induced responses. At a minimum, animals require 72 hours to regain basic physiological baselines.
- **Fasting:** Pre-anesthetic fasting is usually not necessary for mice due to their inability to vomit. However, if fasting is employed (e.g., to stabilize body weights before anesthesia) limit to no more than 2-3 hours due to the high metabolic rate of small rodents. **Never restrict water.**
- **Eye protection:** Mouse eyes remain open under anesthesia. This can lead to corneal drying and trauma. Apply ophthalmic ointment (e.g., Paralube® or Lacrilube®) to eyes if:
 - The anesthetic event lasts longer than 5 minutes.
 - Anesthesia is being delivered by facemask.
- **Monitoring:** Regardless of the anesthetic administered, monitor mice under anesthesia to avoid excessive depression of cardiac and respiratory functions, or insufficient anesthesia.

- Parameters that can be monitored in an anesthetized mouse without specialized equipment include:
 - Anesthetic depth – toe pinch
 - Respiratory rate and pattern – normal undisturbed rate = ~180/min, a slow rate drop of 50% is acceptable during anesthesia
 - Mucous membrane color – should be pink not blue or grey
 - Body temperature can be monitored with a rectal thermometer and should be above 97 degrees.
 - Oxygen saturation (>95%) and heart rate (300-800 beats/min) can be monitored using a specialized rodent pulse oximeter.
 - Contact your area veterinarian with questions about monitoring or monitoring equipment.
- Heat support: All species are at risk for hypothermia while under anesthesia. Mice are particularly susceptible due to their high body surface area to body mass ratio. Hypothermia induces a significant physiological stress on animals that can prolong recovery and potentially be fatal.
 - Provide supplemental heat during all anesthetic events.
 - Supplemental heat sources include circulating water blankets, electric heating pads, and commercial products that can be warmed in a microwave or water bath (such as isothermic pads or gel warmers).
 - Never place the animal directly on the heat source.
 - Contact your area veterinarian with questions about using or purchasing supplemental heat sources.
- Fluid support: Consider providing warm SQ or IP fluids, particularly for prolonged anesthetic events or animals that are ill, aged, or debilitated. Consult your veterinarian for additional information.
- Recovery: Continue to monitor animals until they are fully recovered.
 - Recover animals on paper towels (without bedding) in a clean cage. This minimizes the risk of tracheal obstruction or pneumonia.
 - Recover anesthetized animals alone in a cage.
 - Continue to provide supplemental heat during recovery.
 - When the animal is ambulatory, return it to the home cage with immediate access to food and water.
 - Depending on the surgery performed, mice may need to be housed individually in the post-operative period to avoid suture gnawing or incision-site trauma caused by cagemates. Any plans for individual housing should be included in the IACUC protocol so that they can be reviewed and approved.

III. Anesthetic Drugs and Procedures:

1. **Inhaled Anesthetics:** Isoflurane is the preferred inhaled anesthetic. It has rapid and reliable onset and recovery.

- A. Inhalation anesthesia may be delivered by the drop jar method, or by induction chamber, facemask, or endotracheal tube using a precision vaporizer.
- i. Drop jar:
 - a. Do not allow the mouse to come into direct contact with the liquid inhalant anesthetic (which can be achieved by placing an impermeable mesh grid over the cotton/gauze).
 - b. Because the anesthetic concentration cannot be controlled in the chamber and can rapidly reach toxic concentrations, this method is reserved for very short procedures such as tail biopsies, subcutaneous tumor implantation, or induction prior to facemask anesthesia.
 - ii. Induction chambers, facemasks and endotracheal tubes:
 - a. These require gas anesthesia machines with an oxygen source and a precision vaporizer. Some research units may use room air rather than pure oxygen as a gas source which may also be acceptable.
 - b. The vaporizer must be compatible with the specific inhaled anesthetic agent.
 - c. Due to the small respiratory capacity in mice, use a non-rebreathing system.
 - iii. When using inhalant anesthesia, use a fume hood or an anesthetic system equipped with a gas scavenging system to minimize occupational exposure to exiting gases.

Drug	Dose	Oxygen flow rate	Comments
Isoflurane Recommended	4-5% for induction 1-2% for maintenance	300-600 ml/min	Approximately 0.6 mls of liquid isoflurane per liter of chamber volume

- B. Injectable Anesthetics: Combined ketamine/xylazine is the preferred injectable anesthetic in mice. Other commonly used injectable agents are listed below.
- i. Mice vary significantly in their sensitivity to various anesthetics. Age, body composition, strain, health status, genetic manipulation, and sex are just a few of the factors that can contribute to anesthetic sensitivity. The following doses are general guidelines that may vary significantly based on the aforementioned factors. When adding anesthetics to your protocol, it is advisable to provide a range to allow titration for the specific needs of each animal.
 - ii. Intramuscular (IM) injections are not recommended in mice as complications such as tissue irritation, lameness and self-mutilation can result.

Drug	Dose	Ro ute	Duratio n	Comments
Ketamine + Xylazine (Rompun®)	Ketamine: 80-120 mg/kg Xylazine: 5-10 mg/kg	IP SQ	30-45 minutes	Thermal support is crucial. If additional anesthetic is needed, supplement with 1/3 dose of ketamine only. Do not re-dose xylazine. Xylazine (20 mg/ml) can be reversed with 1 - 2 mg/kg yohimbine IP or a volume of atipamezole equal to the volume of Xylazine (20 mg/ml) given (IP)**.
Ketamine+ Xylazine+Acepromazine	Ketamine: 80-100mg/kg Xylazine: 5-10 mg/kg Acepromazine: 3 mg/kg	IP SQ		Thermal support is crucial. To prolong anesthesia, supplement with 1/3 dose of ketamine only. Xylazine can be reversed with 1 - 2 mg/kg yohimbine IP or a volume of atipamezole equal to the volume of Xylazine (20 mg/ml) given (IP)**.
Ketamine + Dexmedetomidine	Ketamine:75-100 mg/kg Dexmed 0.5-1 mg/kg	IP SQ		Thermal support is crucial. If additional anesthetic is needed, supplement with 1/3 dose of ketamine only. Do not re-dose dexmedetomidine. Dexmedetomidine can be reversed with a volume of atipamezole equal to the volume of

				Dexmedetomidine used**
Ketamine + diazepam (Valium®)	Ketamine: 100 mg/kg Diazepam: 5 mg/kg	IP	20-30 minutes	
Pentobarbital (Nembutal®)	30-40 mg/kg (sedation) 40-60 mg/kg (anesthesia)	IP	10-300 minutes	Causes respiratory depression; narrow margin of safety. Use with caution.

*A good starting point is 100 mg/kg Ketamine and 7 mg/kg Xylazine

** Atipamazole (5 mg/ml) is 10X the concentration of Dexmedetomidine (0.5 mg/ml). You need 4-6X as many mg of Atipamazole (5 mg/ml) to reverse Dexmedetomidine. However, it also has a very high safety margin, so giving an equal volume of Atipamazole to Dexmedetomidine gives you 10 X as many mg of Atipamazole, it is safe, and it allows for reversal without calculating a dose if reversal is urgent.. Equal volumes of Atipamazole (5 mg/ml) to reverse small animal Xylazine (20 mg/ml) also works because even though Xylazine has a stronger concentration (20 mg/ml), Atipamazole (5 mg/ml) is cleaner and binds more tightly to the alpha 2 receptor, so equal volumes allows for reversal of xylazine (20 mg/ml) as well.

C. One type of injectable anesthetic is a local anesthetic.

- i. Local anesthetics block nerve impulses by specifically binding the voltage-gated Na⁺ channel in the nerve cell membrane.
- ii. Routes of administration include topical to mucus membranes (nose, eye, etc.) or injected directly into tissues and around nerve bundles.
- iii. Administration of local anesthetics prior to the painful stimulus (eg. incision) would be considered an adjunct analgesic to opioid and NSAID analgesics.
- iv. Use as a primary analgesic is discouraged due to the short duration (hours).

Drug	Dose	Route	Comments
Lidocaine	4 mg/kg (0.4 ml/kg of a 1% solution)	Local infiltration	Do not exceed 7 mg/kg total dose. Rapid onset, short duration
Bupivacaine Recommended	1-2 mg/kg (0.4-0.8 ml/kg of a 0.25% solution)	Local infiltration	Do not exceed 6 mg/kg total dose. Slower onset, longer duration

IV. Special Considerations for Mouse Anesthesia: Neonatal Mouse Anesthesia:

- There are several anesthetic methods currently described in the literature for use in neonatal rodents, including injectable anesthesia, inhalant anesthesia, and hypothermia.
- Parental cannibalism is a problem with neonatal rodent anesthesia. This can be reduced by ensuring that the neonate is fully recovered before returning it to the dam. Additional steps include exposing pups to soiled bedding from the mother's cage, and placing the pup in the middle of the litter.
- Hypothermia anesthesia
 - Hypothermia should only be performed in Neonatal rodents <7 days old and should not be used for procedures lasting longer than 30 minutes.
 - Animals should not be placed directly on ice (use latex glove or another substance between the animal and the underlying ice bath).
 - Animals have reached the proper plane of anesthesia when pedal reflex is lost.
 - Do not use incandescent light during the procedure, as it can warm the surgical field and cause animals to awaken from a surgical plane of anesthesia.
 - Following anesthesia, animal should be re-warmed slowly. Rapid warming can cause tissue damage. Patients can be re-warmed on a circulating water blanket, heating pad (40 °C) or in an incubator (33 °C).
 - Pups can be returned to dam once they are able to move without direct physical stimulation.
- Inhalation Anesthesia
 - Neonatal rodents may have a longer induction time than adult rodents with inhalant anesthesia.
 - Induction in a chamber generally requires 5% isoflurane, and maintenance (chamber or nose cone) requires 1-2% (flow rate = 0.3-0.6 L/min).
- Injectable Anesthesia
 - Ketamine/Xylazine is the recommended injectable anesthetic for mice > 7days of age: inject 50-150 mg/kg Ketamine and 5-10 mg/kg xylazine with a 27g needle IP (max volume = 0.5 ml).

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