Guidelines on Anesthesia and Analgesia in Mice

Last Updated 15 August 2016

1. Purpose
   1. This document has been designed by the ULAM veterinary staff as a guideline for sedation, anesthesia, and analgesia of laboratory mice. This is not intended to be an inclusive tutorial on all possible drug combinations that can be used in mice. The following guidelines are also general recommendations and consequently do not include reference to specific research associated concerns. If you have questions or comments about this document, contact the ULAM veterinary staff (ulam-vets@umich.edu or 734-936-1696). The ULAM training core (ulam-trainingcore@umich.edu or 734-763-8039) can be contacted to provide training in these techniques at no charge.

2. Responsibility
   1. Principal Investigator: Responsible to ensure appropriate anesthesia and/or analgesia is provided for all rodents undergoing painful procedures including rodent survival surgery unless otherwise indicated in the relevant approved protocol.

3. Definitions
   1. Anesthesia: Temporarily induces loss of sensation with or without loss of consciousness.
   3. A/A: Anesthesia and analgesia.
   4. Sedation: A mild degree of central depression in which the patient is awake but calm. This is inadequate for surgery.

4. Procedures
   1. Prior to Anesthetic/Analgesic/Sedative Event
      1. Newly arrived animals should be acclimated at least 3 days prior to anesthesia or sedation.
      2. Age and body weight should be considered when designing an A/A plan.
      3. Preamnesthetic fasting is usually not necessary in rodents. If preanesthetic fasting is required:
         1. The fasting period must be limited to 2-3 hours and no longer due to the higher metabolism in mice.
         2. Water should NEVER be restricted.
      4. Ocular lubrication such as Paralube® must be used to prevent corneal drying during anesthesia or sedation.

   2. Routes of Administration
      1. More detailed information regarding injection techniques and maximum quantities safely administered to mice can be found in Guidelines on Administration of Substances to Laboratory Animals.

   3. Normal Monitoring Parameters
      1. More information on anesthetic/sedation monitoring requirements can be found in Anesthesia and Sedation Monitoring Guidelines.
         1. Respiratory rate should be 55 - 100 breaths/min.
         1. A drop in respiratory rate of 50% can be normal during anesthesia.
         2. Respiratory pattern can be used to monitor anesthesia.
            1. Deep and slow or rapid and shallow.
2. Pulse rate should be 300 - 500 beats/min.
3. Normal temperature ranges while under anesthesia fall between 36.0°C and 38.0°C.
4. Mucus membrane color should be pink. Never pale white or blue.
   1. Normal capillary refill time (CRT) is < 2 seconds.

4. Recovery
   1. Animals must be visibly observed and monitored every 15 minutes during recovery from anesthesia until the animal is ambulatory.
      1. Animals that received alpha-2 agonists (dexmedetomidine, xylazine) as part of anesthetic protocol can receive reversal agents to expedite recovery.
         1. See Table 5 below for recommended doses.
   2. Rodents should be housed individually until completely recovered and ambulating to avoid cannibalism by cage mates.
   3. Recover animals in clean cages without bedding to limit the possibility of tracheal foreign body obstruction or aspiration pneumonia.
   4. Monitoring parameters and thermal supplementation should be continued throughout the recovery period. Refer to Anesthesia and Sedation Monitoring Guidelines and Guidelines on the Performance of Surgery in Rodents for more information on post-operative monitoring and appropriate thermal support devices.
   2. Recover animals in the surgery area so they can be appropriately monitored during the recovery period.
   3. Nutritional support is critical following anesthesia and should be provided as soon as the animal is recovered.
      1. Moist chow, regular chow, or diet gel should be provided on the cage floor to encourage eating as soon as possible.

5. Anesthetics
   1. Detailed information on all approved anesthetics and sedatives can be found in Anesthesia and Analgesia Drug Descriptions.
   2. Anticholinergics
      1. Atropine 0.04 - 0.10 mg/kg Subcutaneous (SC)
      2. Glycopyrrolate 0.01 - 0.02 mg/kg Subcutaneous (SC)
   3. Table 1: Inhalant Anesthetics Used in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane (Forane®, Aerane®)</td>
<td>4-5% for induction 1-2% for maintenance</td>
<td>Requires use of a calibrated vaporizer</td>
</tr>
<tr>
<td>Isoflurane (Forane®, Aerane®)</td>
<td>300µL on gauze placed in a 500ml container</td>
<td>Chamber induction for brief anesthesia for procedures. Gauze must be protected so animal cannot come into contact with isoflurane</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>4-7% for Induction 2-4% for maintenance</td>
<td>Requires use of a calibrated vaporizer</td>
</tr>
</tbody>
</table>

4. Table 2: Injectable Anesthetics Used in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route a</th>
<th>Duration of Anesthesia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dissociatives</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine + xylazine (Rompun®)</td>
<td>80-120mg/kg ket. + 5-10mg/kg xyl. IP</td>
<td>30-45 minutes</td>
<td>Re-dose with 1/3 of Ketamine dose</td>
</tr>
<tr>
<td>Ketamine + xylazine + acepromazine</td>
<td>80-100 mg/kg ket IP + 5-10 mg/kg xyl IP + 1 mg/kg ace IP</td>
<td>40 minutes</td>
<td>Re-dose with 1/2 of Ketamine dose or 1/4 of ketamine dose &amp; 1/4 xylazine dose</td>
</tr>
<tr>
<td>Ketamine + dexmedetomidine</td>
<td>50-75 mg/kg IP + 0.5-5 mg/kg IP</td>
<td>20-30 minutes</td>
<td></td>
</tr>
<tr>
<td><strong>Barbituates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentobarbital (Nembutal®)</td>
<td>30-40mg/kg IP sedation</td>
<td>10-300 minutes</td>
<td>Respiratory depression / poor analgesia</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propofol (Diprivan®)</td>
<td>12-26mg/kg IV</td>
<td>5-7 minutes</td>
<td>Titrate as needed</td>
</tr>
</tbody>
</table>

a Intraperitoneal (IP), Intravenous (IV)

5. Table 3: Injectable Sedatives Used in Mice
Table 4: Injectable Anesthetics Requiring Scientific Justification and IACUC Approval

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route a</th>
<th>Duration of Anesthesia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine + diazepam (Valium®)</td>
<td>100mg/kg ket IP + 5mg/kg dia. IP</td>
<td>20-30 minutes</td>
<td>Sedation / immobilization</td>
</tr>
<tr>
<td>Ketamine + midazolam (Versed®)</td>
<td>100mg/kg ket IP + 5mg/kg mid. IP</td>
<td>20-30 minutes</td>
<td>Immobilization</td>
</tr>
<tr>
<td>Ketamine + acepromazine</td>
<td>100 mg/kg ket IP + 5 mg/kg ace IP</td>
<td>20-30 minutes</td>
<td></td>
</tr>
<tr>
<td>Ketamine (Ketoset®)</td>
<td>100-200mg/kg IP</td>
<td>Unproven</td>
<td>Poor muscle relaxation / mild analgesia</td>
</tr>
</tbody>
</table>

a Intraperitoneal (IP)

6. Table 4: Injectable Anesthetics Requiring Scientific Justification and IACUC Approval

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route a</th>
<th>Duration of Anesthesia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-chloralose</td>
<td>114mg/kg IP</td>
<td>Unproven</td>
<td>5% concentration Non-survival procedures</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>400 mg/kg IP</td>
<td>Unproven</td>
<td>Non-survival procedures</td>
</tr>
<tr>
<td>Tribromoethanol (Avertin®)</td>
<td>125-250 mg/kg IP</td>
<td>30-45 minutes</td>
<td>Avoid re-dosing</td>
</tr>
</tbody>
</table>

a Intraperitoneal (IP)

7. Table 5: Injectable Reversal Agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route a</th>
<th>Reversal Agent For</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atipamezole (Antisedan)</td>
<td>0.5-1.0 mg/kg IP, IM or SC</td>
<td>Dexmedetomidine or Xylazine</td>
<td>Preferred reversal agent for Alpha-2 agonists</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>1.0-2.0 mg/kg IP or SC</td>
<td>Xylazine</td>
<td>Less effective than Atipamezole</td>
</tr>
</tbody>
</table>

a Intraperitoneal (IP), Intramuscular (IM), Subcutaneous (SC)

6. Analgesia

1. Unrelieved pain can have profound negative physiologic consequences, which may alter research results. Mice show a variety of responses to pain, some of which may be fairly subtle and easily missed on casual examination. Pain evaluation in mice consists of evaluating behavioral and physiologic parameters.

2. Table 6: Pain Evaluation Parameters

<table>
<thead>
<tr>
<th>Behavioral Signs</th>
<th>Physiologic Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reluctance to move</td>
<td>Elevated blood pressure</td>
</tr>
<tr>
<td>Hunched posture</td>
<td>Elevated heart rate</td>
</tr>
<tr>
<td>Social isolation</td>
<td>Elevated respiratory rate</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>Changes in body temperature</td>
</tr>
<tr>
<td>Decreased grooming</td>
<td>Dilated pupils</td>
</tr>
<tr>
<td>Aggression</td>
<td></td>
</tr>
<tr>
<td>Self-mutilation</td>
<td></td>
</tr>
<tr>
<td>Decreased nest building (see Appendix A)</td>
<td></td>
</tr>
<tr>
<td>Facial expressions (see Appendix A)</td>
<td></td>
</tr>
</tbody>
</table>

3. Table 7: Analgesics Used in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose a</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine (Buprenex®)</td>
<td>0.05-0.1 mg/kg SC or IP</td>
<td>6-12 hours</td>
</tr>
<tr>
<td>Carprofen (Rimadyl®)</td>
<td>5 mg/kg SC or IP</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
Flunixin (Banamine®) 2.5 mg/kg SC 12-24 hours
Meloxicam (Metacam®) 1-2 mg/kg PO or SC 12-24 hours
Ketoprofen 2-5mg/kg SC 12-24 hours

a Subcutaneous (SC), Intraperitoneal (IP), Oral (PO)

4. UM IACUC requires the use of pre-emptive analgesia (analgesics given prior to the first skin incision) for all surgical procedures. Requirements for analgesic coverage differ depending on the classification of surgery as Type I, II, or III, please consult the UM Policy on Analgesia Use in Animals Undergoing Surgery for updated surgical classifications and analgesia requirements.
   1. It is important to note, the use of buprenorphine as a pre-emptive analgesic may decrease the amount of required anesthetic drugs, due to buprenorphine's sedative and respiratory depressant affects.

7. Local Anesthetics
   1. Lidocaine and bupivacaine are the two most commonly used local anesthetics.

2. Table 8: Local Anesthetics Used in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route</th>
<th>Duration of Anesthesia</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>4mg/kg SC (0.4 ml/kg of a 1% solution)</td>
<td>1.5-2 hours</td>
<td>Rapid onset (1-2 min)</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>1-2 mg/kg (0.4-0.8 ml/kg of a 0.25% solution)</td>
<td>4-12 hours</td>
<td>Slower onset (5-10 min)</td>
</tr>
</tbody>
</table>

3. These doses can be diluted in sterile saline to provide a larger injection volume. These injectable anesthetics are most routinely administered in subcutaneous tissues near the site of the incision to be made.
   1. Administration can be performed in a "line block", in which the subcutaneous tissue proximal to the incision site is infiltrated with anesthetic in a linear fashion.
   2. Administration can be performed in a "ring block", where subcutaneous tissue around the incision site is infiltrated circumferentially.

4. IV administration of lidocaine or bupivacaine can cause cardiovascular effects (e.g., hypotension, dysrhythmias) and central nervous system depression followed by seizures.
   1. To avoid these adverse consequences, each animal should be weighed individually and the maximum safe dose calculated for that individual.
   2. Aspiration of the syringe should always be performed prior to injection to ensure that IV injection is avoided.

8. Neonatal Rodent Anesthesia
   1. A rodent neonate is defined as a mouse or rat < 10 days of age. There are several anesthetic methods currently presented in the literature for use in neonatal rodents. These include injectable, inhalant, and physical methods. Hypothermia is the primary physical method utilized in neonatal rodent anesthesia and it is believed to provide anesthesia/analgesia by decreasing neural conduction and synaptic transmission. However, the cooling process itself may be painful and for this reason direct contact with the cooling agent should be avoided.
   1. Injectable anesthetics have been associated with a high mortality in neonatal rodents.
   2. Neonatal rodents may have a longer induction and recovery time than adult rodents with inhalant anesthetics.
   2. Hypothermia - Can only be performed in neonatal rodents <6 days old and should not be used for procedures lasting longer than 30 min.
      1. Place neonates either on a latex covered bed of crushed ice, in a cut off finger of a latex glove, or in a paper lined test tube in crushed ice.
      2. Check the pup for pedal reflex indicating proper plane of anesthesia.
      3. Remove the pup from the ice bed and place on a chilled cold pack or bed of ice. Place a barrier between the pup and the ice to prevent direct damage to the tissues.
      4. Use fiber optic lighting for the surgical field as incandescent bulbs may warm the pup.
      5. Following hypothermia anesthesia, re-warm the animals slowly. Rapid warming can cause tissue damage. Use of a circulating water heating pad (40 ºC) or in an incubator (33 ºC) is recommended.
      6. Return pups to dam once they are able to crawl.
   3. Parental cannibalism can occur with neonates after anesthesia. The following steps can be used to reduce the occurrence of cannibalism in anesthetized neonatal pups:
      1. Ensure the neonate is fully recovered before returning to the dam.
      2. Smear the pups with soiled bedding from the mother's cage.
      2. Place the pup back in the middle of the litter.

4. Table 9: Inhalant Anesthetics in Neonatal Mice

<table>
<thead>
<tr>
<th>Stage of Anesthesia</th>
<th>Route</th>
<th>Oxygen (L/min)</th>
<th>Isoflurane (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction</td>
<td>Mask or Chamber</td>
<td>0.5-1</td>
<td>4-5</td>
</tr>
</tbody>
</table>
Note: Neonates typically require a higher inhalant anesthetic dose than that observed in adult.

5. **Table 10: Injectable Anesthetics in Neonatal Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage and Route</th>
<th>Duration of Action</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine / xylazine</td>
<td>50-150mg/kg (K) + 5-10mg /kg (K) IP a or SC b</td>
<td>20-40 minutes</td>
<td>Only to be used in mice greater than 7 days old</td>
</tr>
</tbody>
</table>

a Intraperitoneal (IP): 27g needle, 1 ml syringe, maximum volume 0.5ml
b Subcutaneous (SC): 27g needle, 1 ml syringe, maximum volume 1 ml

9. **Neonatal Rodent Analgesia**

1. There is little information regarding the efficacy and dose ranges for the use of analgesics in neonates. Some data suggest that unalleviated pain in neonates can alter responses to pain and stress later in life. It may be worthwhile to investigate the literature on neonatal analgesia when a project involving neonatal surgeries and procedures is started. (LaPrairie and Murphy, 2010; Sternberg et al., 2005; Victoria et al., 2013a,b, 2014; LaPrairie et al., 2008; Walker et al., 2009).

10. **Emergency Resuscitation**

1. Attempts at resuscitating mice that have received an excessive dose of anesthetic or are experiencing cardiac or respiratory arrest for any reason, are generally unrewarding.
   1. Small chest compressions between the thumb and forefinger can be attempted.
   2. Respiratory depression can be treated by the administration of doxapram (Dopram®) 5-10mg/kg IV or IP. If respiratory depression reoccurs, the doxapram should be administered repeatedly at approximately 10-15 minute intervals.
   3. Supportive care for animals which reach too deep a level of anesthesia includes decreasing or discontinuing inhalant anesthetic, stimulating the animal by gentle manipulation of the body, raising the body temperature to normal, providing supplemental oxygen through a facemask or nose-cone
   4. If indicated, administering reversal agents of anesthetic drugs. See Table 5 above.

5. **Related Documents**

1. Guidelines on the Performance of Surgery in Rodents
2. Anesthesia and Sedation Monitoring Guidelines
3. Anesthesia and Analgesia Drug Descriptions
4. Guidelines on Administration of Substances to Laboratory Animals
5. UM Policy on Analgesia Use in Animals Undergoing Surgery
6. EHS Anesthetic Gases in Animal Research

6. **Appendices**

1. **Appendix A: ULAM Assessment of Pain in Mice Handout**

7. **References**

42. Websites regarding neonatal anesthesia:  