Veterinary Guideline

Rodent Anesthesia & Analgesia

Introduction

This guideline discusses rodent anesthesia and analgesia options and gives examples of recommended analgesic plans based on anticipated level of pain from a procedure. Veterinary consultation prior to IACUC protocol submission is strongly encouraged.

Inhalant Anesthesia

- Isoflurane is the preferred general anesthetic agent for most procedures in rodents due to its wide safety margin, ease of administration, rapid titration, and quick recovery after exposure has ended.
- Inhalants are also desirable because they are not DEA controlled substances.
- Inhalant anesthetics should be administered via a calibrated vaporizer and have an appropriate scavenging system for waste gas to minimize personnel exposure.

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Dose (conc.)</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>RECOMMENDED</td>
<td></td>
<td></td>
<td>• Isoflurane machines are available for use within ULAR</td>
</tr>
<tr>
<td></td>
<td>4-5% induction</td>
<td>Continuous</td>
<td>Inhalant</td>
<td>spaces. Oxygen is provided and the machines are</td>
</tr>
<tr>
<td></td>
<td>1-2% maintenance</td>
<td></td>
<td></td>
<td>maintained by ULAR staff.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Isoflurane machines are available for rent through</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><a href="mailto:ulartech@osu.edu">ulartech@osu.edu</a>.</td>
</tr>
</tbody>
</table>

Injectable Anesthesia

- Injectable anesthetic cocktails may provide effective general anesthesia, but the animal's response is often variable based on strain, size, age, sex, and individual animal. Verification of appropriate anesthetic depth is critical throughout the procedure due to the inconsistent drug response.
- When readministering any of the following drug combinations, redosing should consist of ketamine alone to minimize cardiac and respiratory depression and subsequent death.

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Dose (mg/kg)</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine/ Xylocaine</td>
<td>Mouse: 80-110/ 5-10</td>
<td>Once, redose with ½ to ⅓ ketamine dose alone</td>
<td>IP</td>
<td>• Lasts for “20-30 minutes</td>
</tr>
<tr>
<td>RECOMMENDED</td>
<td>Rat: 40-80/ 5-10</td>
<td></td>
<td></td>
<td>• Individual response may vary greatly</td>
</tr>
<tr>
<td>Atropine/ Ketamine/ Xylocaine</td>
<td>Mouse: 0.05/ 80-110/ 5-10</td>
<td>Once, redose with ½ to ⅓ ketamine dose alone</td>
<td>IP</td>
<td>• Atropine may be given to counteract cardiovascular effects of xylazine</td>
</tr>
<tr>
<td></td>
<td>Rat: 0.05/ 40-80/ 5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine/ Xylocaine/ Acepromazine</td>
<td>Mouse: 80-110/ 5-10/ 2-3</td>
<td>Once, redose with ½ to ⅓ ketamine dose alone</td>
<td>IP</td>
<td>• Acepromazine may be added when deeper/longer anesthesia is required (lasts “30-40 minutes)</td>
</tr>
<tr>
<td></td>
<td>Rat: 40-50/ 5-10/ 0.5-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine/ Dexmedetomidine</td>
<td>Mouse: 50-75/ 0.5-1</td>
<td>Once, redose with ½ to ⅓ ketamine dose alone</td>
<td>IP</td>
<td>• Lasts for “20-30 minutes</td>
</tr>
<tr>
<td></td>
<td>Rat: 50-75/ 0.25-0.5</td>
<td></td>
<td></td>
<td>• May not produce a surgical plane of anesthesia</td>
</tr>
</tbody>
</table>
Example preparation

- Mouse ketamine/xyazine dilution
  - **Dilution**: Final concentration 10 mg/ml ketamine / 1 mg/ml xylazine
    - 1.0 ml ketamine (100 mg/ml stock)
    - 0.5 ml xylazine (20 mg/ml stock)
    - 8.5 ml 0.9% sterile saline
  - **Directions**: Mix in sterile vial and administer 0.1 ml per 10g of mouse body weight
  - **Dose**: 100 mg/kg ketamine + 10 mg/kg xylazine

Note: when preparing dilutions, follow appropriate labeling requirements documented in the IACUC Policy, *Use of Pharmaceutical and Non-Pharmaceutical Grade Compounds in Animals and Labeling Expectations*

Anesthetic Reversal Agents

- Reversal drugs are available to reverse alpha-2 agonists such as xylazine. Reverse agents are not available for ketamine or acepromazine.
- Administering reversals when finished with the procedure will lighten depth of anesthesia and hasten post-op recovery.

**Table 3: Rodent anesthetic reversal agents**

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Dose (mg/kg)</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atipamezole</td>
<td>0.5-2 mg/kg</td>
<td>Once</td>
<td>IP, SC</td>
<td>Can be used to reverse xylazine or dexmedetomidine</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>1-2 mg/kg</td>
<td>Once</td>
<td>IP</td>
<td>Can be used to reverse xylazine</td>
</tr>
</tbody>
</table>

Systemic Analgesics

- Analgesics should be given preemptively whenever possible (prior to initiation of a painful stimulus such as a skin incision).
- **Water bottle dosing must be initiated 24-48 hours in advance of the procedure.** Access to other sources of water such as the lixit of the autowatering system must be eliminated while the medicated water bottle is in place. Post-anesthesia and/or post-surgical water consumption may not be consistent.
- When indicated, multimodal analgesia is the standard of care for all laboratory animals including rodents. Multimodal analgesia is defined as the use of two or more different analgesic drugs or techniques targeting different parts of the pain pathway to create a synergistic effect. Drug classes to consider for multimodal analgesia in rodents are NSAIDs, opioids, and local anesthetics.
- **Extended-release formulations of opioids such as Buprenorphine SR and Ethiqa XR® should be used when possible.** These provide more consistent pain control and reduce animal stress caused by repeated handling and injections. The recommended dosing interval for buprenorphine HCl has been shortened to reflect current literature.³

**Table 4: MOUSE systemic analgesic dosing and recommendations**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse Dose</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
</table>
| **Carprofen NSAI**D (e.g., Rimadyl®, OstiFen™, Carprieve®) RECOMMENDED | 5 mg/kg    | Every 12-24 hours          | SC    | Stock solution: 50 mg/ml injectable carprofen
  - Requires refrigeration
  - **0.5 mg/ml dilution**: 0.1 ml carprofen (50 mg/ml) + 9.9 ml sterile saline
  - May store and use at room temperature up to 60d
  - **Dosing**: Give 0.25 ml of the 0.5 mg/ml dilution per 25g body weight
| 5 mg/kg/day           | Change water every 7 days | Water bottle          |       | Stock solution: 50 mg/ml injectable carprofen
  - Requires refrigeration
  - **0.025 mg/ml water bottle**: add 0.2 ml carprofen (50 mg/ml) to 400 ml RO water |
### Drug Mouse Dose Frequency Route Recommendations/Notes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse Dose</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
</table>
| Meloxicam *NSAID* (e.g., Metacam®, Meloxidyl®) | 5 mg/kg | Every 12 hours | SC | - **Stock solution:** 5 mg/ml injectable meloxicam  
- **0.5 mg/ml dilution:** 1.0 ml meloxicam (5 mg/ml) + 9.0 ml sterile saline  
- **Dosing:** Give 0.25 ml of the 0.5 mg/ml dilution per 25g body weight |
| | 5 mg/kg | Every 24 hours | PO | - **Stock solution:** 1.5 mg/ml oral meloxicam suspension  
- **Dosing:** administer 0.08 ml via oral gavage or installation per 25g mouse |
| Ibuprofen *NSAID* (e.g., Children’s Motrin®) | 40 mg/kg/day | Change water 2X per week | Water bottle | - **Stock solution:** 100 mg/ 5 ml oral ibuprofen suspension  
- **0.2 mg/ml water bottle:** add 4.0 ml ibuprofen (100 mg/5 ml) to 400 ml RO water |
| Buprenorphine SR-LAB *Sustained release opioid RECOMMENDED* | 1 mg/kg | Every 48 hours | SC, between shoulder blades | - **Stock solution:** 0.5 mg/ml injectable buprenorphine formulation (compounded by ZooPharm)  
- **Dosing:** Give 0.05 ml of stock formulation per 25g body weight  
- Must be administered by ULAR staff under a veterinary license  
- Please submit the Buprenorphine SR Injection Request Form to OR-ULARSRBup@osu.edu to request administration. |
| Ethia XR® *Sustained release opioid RECOMMENDED* | 3.25 mg/kg | Every 72 hours | SC, between shoulder blades | - **Stock solution:** 1.3 mg/ml injectable suspension  
- **Dosing:** Give 0.05 ml of stock solution per 20g body weight  
- Gently shake before drawing up suspension  
- Manufacturer recommends discarding vial 28 days after puncture; do not dilute  
- Able for purchase and administration directly by investigative staff |
| Buprenorphine HCl *Opioid (e.g., Buprenex®)* | 0.1 mg/kg | Every 4-8 hours | SC | - **Stock solution:** 0.3 mg/ml injectable buprenorphine  
- **0.005 mg/ml dilution:** 0.1 ml buprenorphine (0.3 mg/ml) + 5.9 ml sterile saline  
- **Dosing:** Give 0.5 ml of the 0.005 mg/ml dilution per 25g body weight |

### Table 5: RAT systemic analgesic dosing and recommendations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat Dose</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
</table>
| Carprofen *NSAID* (e.g., Rimadyl®, OstiFen®, Carprieve®) *RECOMMENDED* | 5 mg/kg | Every 24 hours | SC | - **Stock solution:** 50 mg/ml injectable carprofen  
- Requires refrigeration  
- **2.5 mg/ml dilution:** 0.2 ml carprofen (50 mg/ml) + 3.8 ml sterile saline  
- May store and use at room temperature up to 60d11  
- **Dosing:** Give 0.2 ml of the 2.5 mg/ml dilution per 100g body weight |
| | 5 mg/kg/day | Change water every 7 days | Water bottle | - **Stock solution:** 50 mg/ml injectable carprofen  
- Requires refrigeration  
- **0.05 mg/ml water bottle:** add 0.4 ml carprofen (50 mg/ml) to 400 ml RO water |
| Meloxicam *NSAID* (e.g., Metacam®, Meloxidyl®) | 2 mg/kg | Every 24 hours | SC | - **Stock solution:** 5 mg/ml injectable meloxicam  
- **Dosing:** Give 0.04 ml of the 5 mg/ml stock meloxicam per 100g body weight |
| | 2 mg/kg | Every 24 hours | PO | - **Stock solution:** 1.5 mg/ml oral suspension  
- **Dosing:** administer 0.13 ml via oral gavage or installation per 100g body weight  
- Note: Most rats will readily consume the suspension and do not require gavage |
<table>
<thead>
<tr>
<th>Drug</th>
<th>Rat Dose</th>
<th>Frequency</th>
<th>Route</th>
<th>Recommendations/Notes</th>
</tr>
</thead>
</table>
| Ibuprofen NSAID (e.g., Children’s Motrin®) | 20 mg/kg/day | Change water 2X per week | Water bottle | • Stock solution: 100 mg/5 ml oral ibuprofen suspension  
• 0.2 mg/ml water bottle: add 4.0 ml ibuprofen (100 mg/5 ml) to 400 ml RO water |
| Buprenorphine SR-LAB Sustained release opioid RECOMMENDED | 1.2 mg/kg | Every 48-72 hours | SC, dorsal | • Stock solution: 1 mg/ml injectable buprenorphine formulation (compounded by ZooPharm)  
• Dosing: Give 0.12 ml of stock formulation per 100g body weight, round to nearest tenth of a ml  
• Must be administered by ULAR staff under a veterinary license  
• Submit the Buprenorphine SR Injection Request Form to OR-ULARSRBup@osu.edu to request administration. |
| Ethia XR® Sustained release opioid RECOMMENDED | .65 mg/kg | Every 72 hours | SC, dorsal | • Stock solution: 1.3 mg/ml injectable suspension  
• Dosing: Give 0.05 ml of stock solution per 100g body weight  
• Gently shake before drawing up suspension  
• Manufacturer recommends discarding vial 28 days after puncture; do not dilute  
• Able for purchase and administration directly by investigative staff |
| Buprenorphine HCl Opioid (e.g., Buprenex®) | 0.05 mg/kg | Every 6-8 hours | SC | • Stock solution: 0.3 mg/ml injectable buprenorphine  
• 0.03 mg/ml dilution: 0.1 ml buprenorphine (0.3 mg/ml) + 0.9 ml sterile saline  
• Dosing: Give 0.2 ml of the 0.03 mg/ml dilution per 100g body weight |

Local Anesthetics

- Local anesthetics work directly at the surgical site to control pain. They are readily used in conjunction with systemic analgesics to control surgical pain and may reduce the amount of anesthesia needed for a procedure.
- These agents can be injected around a surgical incision or “splashed” onto tissues of interest.
- Most information about local anesthetics in rodents is extrapolated from literature on other species.

Table 6: Rodent local anesthetic dosing and recommendations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rodent Dose</th>
<th>Duration of Action</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Lidocaine (e.g. Xylocaine®) | 2 mg/kg | <1 hour (≈5-minute onset) | • Stock solution: 1-2% (10-20 mg/ml)  
• May need to dilute with sterile saline into working solution for mice  
• Do not exceed 7 mg/kg dose |
| Bupivacaine (e.g. Marcaine®, Senorcaine®) | 1 mg/kg | 4-8 hours (≈20-minute onset) | • Stock solution: 0.25-0.5% (2.5-5 mg/ml)  
• May need to dilute with sterile saline into working solution for mice  
• Do not exceed 8 mg/kg dose |
| Nocita® Sustained release bupivacaine | 5.3 mg/kg | 72 hours | • Stock solution: 13.3 mg/ml  
• May need to dilute with sterile saline into working solution for mice  
• Use bottle within 4 hours of puncture; recent study² shows it may be used up to 4 days when aliquoted aseptically |
Suggested Analgesic Plans

- The following table is intended to guide analgesia selections based on the anticipated level of pain from a procedure.
- Post-procedural pain can vary greatly based on surgical technique and the individual animal. Post-op monitoring and training for identification of pain and distress in rodents is critical for animal welfare. If additional analgesics are indicated beyond what was approved in the protocol, a ULAR veterinarian must be consulted.

Table 7: Minimum analgesic recommendations based on anticipated pain level

<table>
<thead>
<tr>
<th>Level of Pain or Distress</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure examples</td>
<td>Small skin incision with minimal tissue manipulation; skin biopsy</td>
<td>Castration (scrotal approach); intracranial injection; ovariectomy (dorsal approach)</td>
<td>Procedures requiring a thoracotomy (opening the chest cavity) or laparotomy (opening the abdomen)</td>
</tr>
<tr>
<td>Recommended pharmacologic analgesia</td>
<td>1 dose of an NSAID or opioid at the time of the procedure</td>
<td>48 hours of NSAID or opioid analgesia (continue as needed) +/- local anesthetic</td>
<td>48 hours of both NSAID and opioid analgesia (continue as needed) +/- local anesthetic</td>
</tr>
</tbody>
</table>

Non-Pharmaceutical Interventions

- Adjunct (non-pharmaceutical) interventions may reduce pain and distress in the post-operative period and must be considered. Examples of non-pharmaceutical interventions include:
  - Gentle handling of the awake rodents and minimization of stress before a procedure
  - Habituating rodents to handling (particularly rats) so this is less stressful during post-op care
  - Gentle manipulation of tissues intraoperatively to minimize tissue trauma, inflammation, and infections
  - Utilizing appropriate wound closure (e.g., sufficiently spaced wound clips or suture, knots that are secure but not overly tight, selection of non-reactive suture)
  - Providing a warm, dry environment during recovery from anesthesia to prevent hypothermia
  - Maintaining a quiet environment during recovery to minimize external stress
  - Ensuring the animal has easy access to food and water with moistened food pellets in a petri dish (“mash”) or other nutritional support (e.g., Boost or Recovery DietGels®) placed in a dish on the cage floor +/- additional water sources such as a HydroGel® or water bottle
  - Providing warmed subcutaneous fluids as approved in the IACUC protocol
  - Returning to group housing with socially compatible animals following recovery from anesthesia
  - Ensuring standard cage enrichment is present and utilizing soft bedding material for ventral incisions (e.g., Alpha-dri® paper bedding)

Post-Operative Monitoring and Pain Assessment

- Animals should be monitored continuously during anesthesia until recovered and ambulatory (able to move around the cage and access food/water). This is especially critical following injectable or long-acting anesthetics, which may take more time after the procedure has finished to recover. Animals cannot be returned to the rack until they are recovered from anesthesia.
- Maintaining body temperature via an external heat source is important to speed up recovery and minimize post-operative death due to hypothermia. Heat should be supplied to half of the cage to create a warmer and cooler side.
- Ongoing evaluation of the animals must be performed with the analgesic plan to ensure pain/distress is effectively alleviated. Assessing pain in rodents can be difficult as they typically minimize pain-associated behaviors unless the pain is incapacitating. The animal may show “normal” behavior as an inherent response to avoid predation.
- Clinical signs suggestive of pain in rodents include but are not limited to lethargy, rough coat, lack of grooming, lack of nesting, and isolation. Rodent grimace scales® consider assessment of orbit tightening, nose bulge and ear position, and are an additional way to evaluate pain in rodents, although it has limited use at the cage side.
- The veterinary staff should be consulted if an animal exhibits signs of pain/distress which are not alleviated by an approved plan in the IACUC protocol. If pain cannot be relieved, humane euthanasia may be warranted.
- Unless the IACUC has approved withholding of analgesics based on documented interference with the specific model of interest, analgesics must be used to alleviate pain.
References