Anesthesia and Analgesia Recommended for Painful Procedures in Small Animals

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ABSTRACT

The non-invasive but painful and invasive procedures are frequently performed in experimental animal studies, such as blood withdrawal, route of administration, and surgical procedures. Most of these procedures commonly conducted under anesthesia and/or analgesia and should be complied with the ethical requirements. However, pain is difficult to assess in animals, therefore, it is advisable to provide the analgesics pre-emptively or if painful condition is expected. Preanesthetic like tranquilizer is generally used to reduce the stress in animal and further it also aids in the smooth induction of anesthesia and recovery. In view of this, the objective is to demonstrate and discuss the guidelines of anesthesia and analgesia in small animals, especially mouse and rat with principal focus to ethical purview. These guidelines can be applicable to the animal house facility, pharmacology laboratory, and to a large extent for veterinary care.

Keywords: Anesthesia; Analgesia; Pain; Animal Studies; Rodents

Introduction

In preclinical research, conduct of any painful procedures in animals must meet the ethical and legal requirements. The surgical or non-surgical procedures those are painful or non-painful that requires immobilization commonly carried out under deep or light anesthesia, so that the pain and distress can be averted. The surgical procedure and the use of anesthetics, analgesics, antibiotics and/or other medications should be described in the study protocol as well as personnel mentioned in the protocol must be well versed. The protocol must be approved by local ethical committee (IAEC). Anesthesia defines loss of feeling in all or part of the body, with or without loss of consciousness. To produce anaesthesia, drug/anaesthetic can be administered parenterally and/or by inhalation. Usually, while selecting anaesthetic agents, some factors need to be taken into consideration such as the procedure to be employed, research aim, and animal age. The safety and effectiveness of animals can be ensured by monitoring before, during, and after use of anaesthesia. Further, the assessment of pain in animals is difficult. Therefore, analgesics are used whenever a painful procedure is applied or the condition that having presentable pain. It is considered that if there is no evidence, then, it is assumed that something that is painful in humans will also be painful in animals. Usually, analgesia is provided pre-emptively, or if painful procedure is anticipated, irrespective of observing clinical signs of pain.

Materials

Species: Albino Mouse/Albino Rat
Sex: Male / Female
Age/weight range: adult
Drugs: Isoflurane, ketamine, xylazine, Pentobarbital, lidocaine, etc.
Vehicle: 0.9% w/v NaCl is used for dilution.
Equipment: Syringe, needle, Drop jar, Cage, heating pad.

Procedures
Anesthesia is generally performed for surgical or non-surgical procedures, which are painful or non-painful but require immobilization. Anesthetics can be administered parenterally or by inhalation based on the research objective.

General guidelines and considerations for mice/rats anesthesia:

Acclimation
Animals can be acclimated minimum one-week period to avert stress-induced disease.

Fasting
In case of rats, the fasting is not necessary for the induction of anesthesia due to the lack of vomiting centre. If it is required, then, the fasting can be employed without restriction to water for not more than 6 h as the rodents having high metabolic rate.

Preanesthetic medication
Medication such as atropine (0.02 -0.05mg/kg s.c.) can be used for all the species to reduce the salivation, bronchial secretion, and protect from vagal inhibition or to minimize the distress associated with induction and to ensure a smooth recovery from anesthesia.

Eye protection
To prevent corneal drying and trauma under anesthesia, eye ointment (lubricant) is applied if the anesthesia is longer than five min or anesthesia is delivered by facemask.

Monitoring
Rat/Mouse can be monitored under anesthesia to prevent excessive cardiac and respiratory depression, or insufficient anesthesia. Following parameters can be monitored without specialized equipment:
- Toe pinch to assess the depth of anesthesia,
- Respiratory rate: normal rate is ~70-120 breaths/min for rats and ~ 180 breaths /min for mice. During anesthesia, 50% a slow rate drop is acceptable.
- Mucous membrane color: pink not blue or gray.
- Body temperature: A rectal thermometer is measured for rats (between 96.5-99.5°F) and mice (above 97 °F).
- Oxygen saturation and heart rate: Specialized rodent pulse oximeter is used for measurement. Saturation should be greater than 95% and heart rate for rats (between 300-550 beats/min) and mice (300-800 beats/min).

Heat Support
In anesthetized animals, hypothermia is a major risk, which produces physiological distress and could be fatal. Hence,
- Heating pad can be used to supplement the heat.
- Direct heat source can be avoided.

Fluid support
Whenever there is prolongation of anesthesia event or debilitating condition, fluid can be given SQ or IP.

Recovery
Animals should be monitored continuously till they are fully recovered from anesthesia. For recovery, following guidelines can be used:
- Paper towels (without bedding) in a clean cage so that the risk of tracheal obstruction or pneumonia reduces.
- Animals left alone in a cage.
- Heat can be supplemented continuously.
- Once animal ambulatory, can be returned to home cage with access to food and water immediately.

Anesthesia Procedure and Anesthetics

A. Inhaled Anesthetic/s
Liquid anesthesia commonly isoflurane is recommended for rodents because of its wide safety margin, reliability, ease of administration, and rapid recovery after end of exposure. It is delivered by:

Drop Jar: In a drop jar anesthesia procedure, cotton gauze is kept in a jar and the liquid anesthetic can be put on the gauze. Then, the mesh grid can be kept over it. The animal can be placed immediately on mesh grid without allowing direct contact with the liquid inhalant to prevent toxic concentration, which could be uncontrolled in chamber. The drop jar method can be used for short procedures, e.g. Tumor implantation subcutaneously or Induction prior to facemask anesthesia.

Induction chamber and facemask: In these methods, anesthesia machine with an oxygen source and a precision vaporizer can be used for the induction of anesthesia. Due to the small respiratory capacity in rats, use a non-rebreathing system. Anesthesia can be induced under fume hood or system equipped with a gas scavenging system so that the occupational exposure to existing gas/es can be minimized. For chamber or nose cone: flow rate=0.5-1 L/min. oxygen flow rate= 300-600 ml/min.
Rodent Anesthesia Machine

gas inlet

latex membrane

gas inlet

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Isoflurane – Commonly Recommended

<table>
<thead>
<tr>
<th>Dose</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/Mouse</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; option: procedures involve invasive or surgical - Jugular blood collection in rat, retro orbital, cardiac puncture, surgery, etc.</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; option: procedures involve minimal invasiveness - Lateral tail vein blood collection, injection by lateral tail vein, etc.</td>
</tr>
<tr>
<td></td>
<td>Anesthetic/s recommendation</td>
</tr>
<tr>
<td></td>
<td>Non-painful procedure: isoflurane</td>
</tr>
<tr>
<td></td>
<td>Minor surgical procedure: isoflurane +/- local anesthetic (Bupivacaine)</td>
</tr>
<tr>
<td></td>
<td>Major surgical procedure: isoflurane + Bupivacaine</td>
</tr>
</tbody>
</table>

Other inhalants:
Ether: 15-20% induction conc., Maintenance conc. 5%
Halothane: 4-5% induction conc., Maintenance conc. 1-2%

B. Injectable Anesthetics

The preferable combined injectable anesthetics used in rats can be ketamine + xylazine/ Dexmedetomidine.

Ketamine + Xylazine – mostly recommended

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong>: Ketamine: 75-90 mg/kg + Xylazine: 5-10 mg/kg + Dexmedetomidine: 0.5-0.75 mg/kg (in same syringe)</td>
<td><strong>Dose</strong>: Ketamine: 80-120 mg/kg + Xylazine/ Dexmedetomidine: 10-20 mg/kg/0.5-1 mg/kg (in same syringe)</td>
</tr>
<tr>
<td>Route: IP/SQ</td>
<td>Route: IP/SQ</td>
</tr>
<tr>
<td>Duration: 45-90 min (medium duration)</td>
<td>Duration: 30-60 min (medium duration)</td>
</tr>
</tbody>
</table>

**Comments**: Heating pad is used for thermal support. 1/3<sup>rd</sup> dose of ketamine only supplement as additional. 1 - 2 mg/kg yohimbine IP or 0.1 - 1.0 mg/kg atipamezole IP/SQ X can be used for reversal of xylazine. dexmedetomidine reverse with 0.1 - 1.0 mg/kg atipamezole IP/SQ

**1<sup>st</sup> option**: Procedures involve minimal invasiveness - Lateral tail vein blood collection, injection by lateral tail vein, etc.

**2<sup>nd</sup> option**: Procedures involve invasive or surgical - Jugular blood collection in rat, retro orbital, cardiac puncture, surgery, etc.

Ketamine (K) + Xylazine (X) + Acepromazine (A)

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>K: 75-90 mg/kg + X: 5-10 mg/kg + A: 1-2.5 mg/kg IP or SQ (in same syringe)</td>
<td>K: 75-100 mg/kg + X: 16-20 mg/kg + A: 3mg/kg IP or SQ (in same syringe)</td>
</tr>
<tr>
<td>Duration: 60-120 min</td>
<td></td>
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</tbody>
</table>
### Anesthetics Recommendation

- **Non-painful procedure:** Ketamine + Xylazine
- **Minor surgical procedure:** Ketamine + Xylazine + local anesthetic (Bupivacaine)
- **Major surgical procedure:** Ketamine + Xylazine + Acepromazine + Bupivacaine

### Ketamine + Diazepam

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: Ketamine: 40-80 mg/Kg + Diazepam: 5-10 mg/kg</td>
<td>Dose: Ketamine: 100 mg/Kg + Diazepam: 5 mg/kg</td>
</tr>
<tr>
<td>Route: IP</td>
<td>Route: IP</td>
</tr>
<tr>
<td>Duration: 20-60 min (medium duration)</td>
<td>Duration: 20-60 min (medium duration)</td>
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</tbody>
</table>

### Pentobarbital

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: 40-55 mg/Kg</td>
<td>Dose: 30-40 mg/Kg (sedation); 40-60 mg/kg (anesthesia)</td>
</tr>
<tr>
<td>Route: IP</td>
<td>Route: IP</td>
</tr>
<tr>
<td>Duration: 20-60 min (medium duration)</td>
<td>Duration: 20-60 min (medium duration)</td>
</tr>
</tbody>
</table>

**Comments:** A dose which produces surgical anesthesia may cause severe respiratory depression and death. It should be administered in diluted form in saline (<10 mg/ml). Buprenorphine and Pentobarbital should not be co-administered as it can result in cardiorespiratory depression. Buprenorphine should be administered after full recovery. Analgesic (opioid or NSAID) should be supplemented for invasive procedures, especially when used on a survival basis.

**Comments:** It may cause respiratory depression; the margin of safety is narrow. Use with caution.

### Alpha-chloralose – not recommended

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: 55-65 mg/kg</td>
<td>Dose: 50-100 mg/kg</td>
</tr>
<tr>
<td>Route: IP</td>
<td>Route: IP</td>
</tr>
<tr>
<td>Duration of anesthesia: Unproven</td>
<td>Duration: Unproven</td>
</tr>
</tbody>
</table>

**Comments:** This is poor analgesia, and possible convulsions and metabolic acidosis may occur. This should not be used for survival surgical procedures.

### Propofol/Thiopentone

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: 10/30 mg/kg</td>
<td>Dose: 26/30-40 mg/kg</td>
</tr>
<tr>
<td>Route: IV</td>
<td>Route: IV</td>
</tr>
<tr>
<td>Duration: 5-10 min (Short Duration)</td>
<td>Duration: 5-10 min (Short Duration)</td>
</tr>
</tbody>
</table>

### Local Anesthetics

Local anesthetics are injectable type, and they block nerve impulses locally by binding voltage gated Na+ channel in the nerve cell membrane. The routes of administration include injecting directly into tissues, around nerve bundles, and topical (nose, eye, etc.).
Key considerations

- It can be used as adjunct analgesic to opioid/NSAID prior to the painful stimulus.
- It should not be used as a primary analgesic due to the short duration.
- Fast onset and relatively long duration of action can be achieved when used in combination of lidocaine (quick onset and short acting) and bupivacaine (slow onset and long acting).
- Usually, dilution used for rodents: 1-2% lidocaine to 0.5% and 0.5% bupivacaine to 0.25% for volumes feasibility to infuse at the incision site (1% solution is equal to 10 mg/mL).

Lidocaine

Dose: 4 mg/kg (0.4 mL/kg of a 1% solution)
Route: It can be used locally as infiltration.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rat Drug</th>
<th>Mouse Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Acetylpromazine 1-2.5 mg/Kg, SC</td>
<td>2-5 mg/Kg, SC</td>
</tr>
<tr>
<td>Drug</td>
<td>Diazepam 2.5 mg/kg IP</td>
<td>2.5-5 mg/Kg, IP</td>
</tr>
<tr>
<td>Comment/s</td>
<td>Used as an adjunct to ketamine and xylazine in major surgical procedures</td>
<td></td>
</tr>
</tbody>
</table>

Bupivacaine

Dose: 1-2 mg/kg (0.4-0.8 mL/kg of a 0.25% solution)
Route: Local infiltration
Comments: It should not be exceeded 6 mg/kg total dose. Onset is slower (10-15 min), and longer duration (4-8 h).

D. Tranquilizers

It may be used to reduce stress and provide ease to restrain. It also smooths the induction of anesthesia and recovery.

Species

Rat

Mouse

Acetylpromazine 1-2.5 mg/Kg, SC

2-5 mg/Kg, SC

Diazepam 2.5 mg/kg IP

2.5-5 mg/Kg, IP

Comment/s Used as an adjunct to ketamine and xylazine in major surgical procedures

1. Analgesia Procedure & Analgesics

In animals, assessment of pain is difficult. So, pain can be identified by observing indirect signs such as abnormal posturing, vocalization, decreased appetite, and self-mutilation. It is best provided pre-emptively or prior to any painful procedure, irrespective of observing clinical signs. Administration of analgesics by systemic or local route may also reduce anesthetic requirements. Use of analgesic protocols may be chosen if required for the specific research model. All surgical protocols require anesthesia and analgesia, unless specifically justified by the principal investigator and approved by the IAEC.

A. Signs of Pain in rats/mice may include (but are not limited to) the following:

i. Unwillingness to move

ii. Reduced activity

iii. Not responsive

iv. Abnormal Posture

v. Back arching

vi. Reduced Appetite

vii. Ungroomed hair coat

viii. Vocalization

ix. Piloerection

x. Self-mutilation

xi. Social isolation

B. Prevention and Management of Pain in Rodents

Recommendation for rodents: Analgesics for different procedures and expected pain levels.
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<table>
<thead>
<tr>
<th>Drug/Analgesic</th>
<th>Rat (route and dosage)</th>
<th>Mouse (route and Dosage)</th>
<th>Comments/use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine (narcotics)</td>
<td>0.01-0.05 mg/Kg SC/IP (8-12 h; post-oper.)</td>
<td>0.05-0.1mg/Kg SC/IP (12 h; post-oper.)</td>
<td>Frequently used in laboratory animals, Administered preoperatively (pre-emptive analgesia) and postoperatively. Effective against pain of mild to moderate.</td>
</tr>
<tr>
<td>Meloxicam or Ketoprofen or Flunixin</td>
<td>1-2 mg/Kg, SC (24 h; post-op) or 2.5-5 mg/Kg, SC (every 24 h for 3 days maximum) or 2.5 mg/kg, sc/im; 12 h; post-oper.</td>
<td>1-2 mg/Kg, SC (24h; post-op) or 5 mg/kg;SC (every 24 h for 3 days max) or 2.5 mg/kg, sc/im; 12 h; post-oper.</td>
<td>Preferred. Effective against pain of mild to moderate. or It can used pre-operatively as preemptive analgesia and postoperatively every 24 h for 3 days. or postoperative use</td>
</tr>
<tr>
<td>Bupivacaine (Local Anaes.)</td>
<td>1 mg/Kg 2 mg/kg max SC</td>
<td>2 mg/Kg max SC</td>
<td>It is a local anesthetic, which provides preoperative analgesia when injected SC at the surgical incision site.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity of pain</th>
<th>Drug Combination</th>
<th>Comment/use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Analgesic may not be indicated</td>
<td>Non-painful procedure: restraint</td>
</tr>
<tr>
<td>Mild to persistent</td>
<td>On Procedure Day- Single dose of any one of the three analgesics, additional based on pain evaluation</td>
<td>Vascular cut down, tail snipping</td>
</tr>
<tr>
<td>Moderate</td>
<td>Combination of any two of the three analgesics on the day of procedure, Additional dose for at least 1-2 days following the procedure</td>
<td>Craniotomy, punch biopsy, subcutaneous(implantation), oophorectomy</td>
</tr>
<tr>
<td>Severe</td>
<td>All three types (multi modal) of analgesics provide at least for 3-5 days.</td>
<td>Thoracotomy, laparotomy, orthopedic procedure.</td>
</tr>
</tbody>
</table>

**Other Considerations**

1. If anesthesia procedure involves ketamine + xylazine, then analgesic should be administered post operatively.
2. If the procedure involves isoflurane, then meloxicam or buprenorphine should be administered preoperatively.
3. In major surgeries, tranquilizer may be used as an adjunct to ketamine + xylazine inj.
4. When pre-emptive analgesia is used, consider reducing the dose of anesthetic (whether inhalant or injectable) to the low end of the recommended range to reduce respiratory depression.

**Observation**

A. General considerations necessary for anesthesia are as follows:
- Acclimation, fasting, premedication, eye protection, monitoring, heat support, fluid support, and recovery.
B. Anesthesia is generally performed for surgical procedures / non-surgical procedures, which are painful /non-painful but require immobilization.
Anesthetic agents include:
- General anesthetics- commonly recommended as per duration and protocol requirement:
  Inhalant- Isoflurane
  Injectables- a. ketamine + xylazine  b. Pentobarbital
C. Local anesthetics: Agent/s can be injected directly into tissues, injected into nerve bundles, and applied topically.

- Commonly Recommended: lidocaine/bupivacaine.

D. Tranquilizer is used as an adjunct to ketamine and xylazine in major surgical procedures to minimize the distress during induction and produce a smooth recovery from anesthesia.

E. Buprenorphine, meloxicam, and bupivacaine are used as analgesics in a single or combined form for invasive/surgical procedures. These agents can be used preoperatively or postoperatively as per the severity of pain and/or anesthetic agent/s used in different procedures. When pre-emptive analgesia is used, consider reducing the dose of anesthetic (whether inhalant or injectable) to the low end of the recommended range.

**Conclusion**

The choice of the anesthetics, tranquilizers, and/or analgesics in severity of the painful procedures in animal is considered based on the research aim, severity of the pain, and animal age factor. Single or combination form of anesthetic/s is/are recommended in painful procedure such as non-invasive but painful or invasive procedures for short-duration or long-duration induction of anesthesia. Tranquilizer as preanesthetic can be employed for the reduction of distress in animals and effective in smooth induction of anesthesia and recovery. For prevention and management of pain, analgesics are preferred pre-emptively or prior to any painful procedure rather than assessing clinical signs. Therefore, it can be concluded that the meticulous use of these agents and procedures induces no or minimal distress in animals and also fulfil the ethical requirements, which may result into better scientific outcome.

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**References**

2. Standard operating procedure for institutional Animal Ethics Committee, CPCSEA.