

STANDARD OPERATING PROCEDURE ANESTHESIA AND ANALGESIA IN RODENTS

General anesthesia is needed for surgery, for any procedures that could potentially cause more than minor discomfort, and for terminal blood withdrawals. Analgesics are required after any major surgery (e.g., exposure of a body cavity such as laparotomy, thoracotomy or cranial procedure, orthopedic procedures) unless justified to the IACUC. Analgesics are suggested for minor surgeries (e.g., skin incision, cannulation).

Investigators are strongly encouraged to consult with the institute veterinarians in the design of their animal research projects, including the selection of the most efficacious anesthetic and analgesic regimens for the animals and the model.

(CS) = “Controlled Substance”. These agents are scheduled as controlled substances by the Drug Enforcement Agency. Special licensing is required in order to possess and maintain controlled substance.

The requirements for scientists using control drugs:

- The drug is for animal use, for approved protocols only.
- The drug must be kept in a locked safe.
- The principal investigator is responsible for the use of the drug in his laboratory.
- A record of each usage must be maintained specifying the date, the amount and the purpose.

Injectable anesthesia

When using parenteral anesthetics it is important to consider accurate dosing with correct multidrug use ratios, storage conditions, and feasibility of immediate use following reconstitution.

It is critical to weigh each animal accurately prior to administration of a calculated dose of anesthesia to avoid either over- or under-dosing.

There is relatively large variability in rodents’ response to the effect of anesthesia. Therefore, the dosage needs to be adjusted to the strain, age, health status of the animal and the specific experiment.

Commonly used compounds of anesthesia in mice:

| Anesthetic compound | Dosage | Rout of Administration |
|--|---|------------------------|
| Pentobarbital (CS) | 40-85 mg/kg (diluted 1:9 in saline) | IP or IV |
| Ketamine (CS) /Xylazine | 100 mg/kg Ketamine 10 mg/kg Xylazine | IP or IM |
| Ketamine (CS) /Xylazine/ Acepromazine | 30 mg/kg Ketamine 5 mg/kg Xylazine 1 mg/kg Acepromazine | IP or IM |
| Ketamine (CS) /medetomidine | 75-100 mg/kg Ketamine 1 mg/kg medetomidine | IP |
| Ketamine (CS) /diazepam | 100 mg/kg Ketamine 5 mg/kg Diazepam | IP |
| Propofol | 20-30 mg/kg | IV |

Commonly used compounds of anesthesia in Rat

| Anesthetic compound | Dosage | Rout of Administration |
|---------------------------------|---|------------------------|
| Pentobarbital (CS) | 40-50 mg/kg | IP or IV |
| Ketamine (CS) /Xylazine | 75-90 mg/kg Ketamine 10 mg/kg Xylazine | IP or IM |
| Ketamine (CS) / Acepromazine | 75 mg/kg Ketamine 2.5 mg/kg Acepromazine | IP or IM |
| Ketamine/medetomidine | 75 mg/kg Ketamine 0.5 mg/kg medetomidine | IP |
| Ketamine/diazepam | 75 mg/kg Ketamine 5 mg/kg Diazepam | IP |
| Propofol | 10 mg/kg | IV |

Reversal agents

Reversal leads to early termination of anesthesia which may reduce mortality and allow rapid return of the rodents to the home cage environment. Reversal agents also reverse bradycardia and bradypnea, but do not eliminate the hypothermic effects, thus thermal support remains essential. If reversal agents are used, both the anesthetic and analgesic properties of the drug may be terminated, thus alternative sources of analgesia must be provided.

| Reversal agents | Species | Dose range | Route |
|-----------------------------|----------------|-------------------|--------------|
| Atipamezole (Antisedan®) | Rodents | 1 mg/kg | SC |
| Yohimbine | Rodents | 2.1 mg/kg | IP |
| Naloxone | Rodents | 0.1 mg/kg | IP |

Inhalant anesthesia

Inhalant anesthetics provide a safe, reliable, reversible, and reproducible means of rendering rodents unconscious in order to perform surgeries and other intricate or potentially painful procedures.

Inhalant anesthesia of small rodents is generally maintained utilizing face masks or nosecones. Endotracheal intubation has also become a more common practice in mice and rats and should be considered where appropriate.

The most commonly used inhalant anesthetic is isoflurane. This is the drug of choice, especially for long procedures.

While using inhalant anesthetics scavenging of environmental anesthetic vapors is essential to protect human health.

Isoflurane anesthetic Machine Operating Procedure

Induction:

- Weight the Fluvac filter, if it weight more than 1400gr, it should be replaced.
- Open the O₂ faucet at the table
- Turn on the Fluvac scavenger and the vacuum red valve on top of it
- Open the O₂ valve in the anesthetic machine (200 – 1000 L/h)
- Turn on the Isolurane (2.5%)
- Place a mouse in the induction chamber (box)
- Open the induction chamber red valve - parallel to the pipe
- When the animal is sedated, close the induction red valve and take out the mouse

Maintenance:

- Place the mouse in the anesthetic mask and turn on the maintenance (mask) red value - parallel to the pipe
- When animal is sedated adjust the Isoflurane dosage (1.5 – 1%)
- At the end of the procedure turn off the mask valve

At the end of work

- Close the O₂ and Isoflurane valves
- Turn off the Fluvac system
- Close the O₂ faucet at the table

Isoflurane filling

- For the Key-Fill vaporizers use the Key Fill adapter. Use the upper opening.
- Release the upper cap and insert the key fill adapter attached to the Isoflurane bottle. Tighten the cap and tilt the bottle and fill the port. Remove the key by releasing the upper cap and tighten it again.

Monitoring during surgery

The anesthetized animal should be draped in such a way to allow access to the mouth and nose to monitor oxygenation status. The animal should be maintained at an adequate anesthetic level where the breathing is regular (not shallow - too deep or rapid - too light) and the mucous membrane of the nose and mouth remain pink (not blue - not enough oxygen). The animal should not have a blink reflex when the inner corner of the eye is touched and should not withdraw its feet in response to a toe pinch (loss of pedal withdrawal reflex). The animal should be assessed every 5 minutes during surgery. Anesthetic level should be adjusted as necessary. Use of the surgical heating bed is suggested to maintain the animal's core temperature. Rodents are prone to hypothermia due to their large surface area to mass ratio therefore must be kept warm during surgery and while recovering from anesthesia. The first dose of analgesia should be administered 20 minutes before the beginning of the surgery or at the start of surgery as pre-emptive analgesia.

Monitoring during recovery

Following surgery, the animal should be placed in a clean cage on a paper towel or absorbent lab diaper to prevent aspiration of bedding. Worming the animals until recovered is recommended. The animal must be monitored at least every 5 minutes until it is fully conscious and then returned to its regular room. Place some food on the floor of the cage and make sure water is accessible.

ANALGESIA IN RODENTS

Monitor the animal each day following surgery for at least 4 days. Record body weight and assess amount of eating and drinking. Be aware that rodents may mask signs of pain. Painful procedures are defined by the USDA as “any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures”. Signs of pain or discomfort are decrease in food and/or water consumption, decrease in weight, abnormal posture, Guarding, Mutilation, reluctance to move, Restlessness, grinding of teeth, and ruffled or unkempt fur, Abnormal behavior to handling. Licking, biting or scratching the painful area, writhe, twitching, back arch, belly press.

Systemic and/or local analgesics may also reduce the anesthetic requirements, and have a pre-emptive effect on pain perception which persists into the recovery period.

Analgesia should be administered pre-operative, after the procedure the analgesic regime should be adjusted according to animal’s response.

Non-steroidal anti-inflammatory drugs (NSAID)

NSAIDs such as aspirin, ibuprofen (Advil™, Motrin™), carprofen (Rimadyl™), and meloxicam (Metacam™) are useful for mild to moderate pain or discomfort. These drugs have Synergistic effect with Opioids therefor their combination is recommended. They can be dosed orally (through drinking water) or SQ injection and should be used for no longer than 3 to 5 days post-operatively. They are metabolized rapidly and have very few adverse side effects.

Dosages of commonly used NSAIDs:

| NSAID drug | Injection dosage (SQ) | Oral dosage Mice | Oral Dosage Rats |
|------------|--|---|--|
| Carprofen | 5 mg/kg SID | 27 µg/ml* (5 mg/kg/day) | 50 µg/ml* (5 mg/kg/ml) |
| Ibuprofen | N/A | 30 mg/kg 4.7 ml Children’s Motrin in 500 ml water | 15 mg/kg 2.35 ml Children’s Motrin in 500 ml water |
| Ketoprofen | 5 mg/kg SID | N/A | N/A |
| Meloxicam | 5.0 mg/kg SID Mice 2.0 mg/kg SID Rats | 1.7 µg/ml (0.3 mg/kg/day) | 10.89 µg/ml (1.0 mg/kg/day) |

*light sensitive in drinking water

Opioid analgesics

Opioids are used for moderate to severe pain. These are controlled substances and accurate records must be kept of their use. Full μ agonists like morphine are good for severe pain, but it should be administered every 4 hours.

Buprenorphine has long duration of activity and therefore can be administered only twice a day.

| Opioid | Species | Dose range | Route |
|--------------------|---------|----------------------|--------------|
| Buprenorphine (CS) | Rodents | 0.05-0.1 mg/kg | SC BID |
| Oxycod syrup (CS) | Rodents | 3 ml in 500 ml water | Oral |
| Morphine (CS) | Rodents | 2-5 mg/kg | SC q 4 hours |

Local anesthetics

Local anesthetics such as lidocaine, bupivacaine and others can be used topically at the surgical site to provide effective analgesia immediately post-op. They may be used to reduce the perception of pain at the surgical site as local or regional anesthetics. In conjunction with other agents, their use may allow reduced levels of general anesthetics, which may speed recovery and minimize mortality. When carefully used, direct injection of a local anesthetic can be a useful adjunct to anesthesia.

| Local anesthetics | Species | Dose range | Route |
|---|---------|-----------------------------|---------|
| Bupivacaine | Rodents | Local infiltration | SC |
| Lidocaine / Morphine (CS) | Mice | 0.85 mM / 1.7 mM in DMSO | Local |
| Lidocaine / prilocaine cream (EMLA Cream®) | Rodents | Local application | Topical |