NIDA-IRP ANIMAL PROGRAM

Anesthesia Guidelines for Rodents

Most recent ACUC approval: August 2018

Revised 9/1/2012 to add Telazol-xylazine

Introduction

This document provides guidance on the selection and use of appropriate anesthetics that meet standards of contemporary veterinary care, humane requirements, and the requirements of the research study. Use of anesthetics for non-human primates or other species should be discussed with the veterinarian in the development of the Animal Study Proposal (ASP).

I. Pre- Anesthesia Considerations

A. Fasting not needed

Although pre-anesthesia fasting is important in non-rodent species it is not necessary nor is it recommended for the type of rodent surgical procedures performed at NIDA.

B. Ventilation and Airways

Investigators should be aware of possible airway complications due to excess salivation/bronchial secretions and should monitor color of mucous membranes (or eye color in albino rodents). Membranes which are pink indicate good tissue oxygenation and circulation as opposed to grey and pale. Measures that can be taken to prevent poor air exchange include positioning and prophylactic anticholinergic treatment. Animals should be positioned with head/neck extended, prone, and head level/down when possible. The surgeon should have a means of clearing secretions with a syringe or swab if needed. Supplemental oxygen can also be provided.

Pre-anesthetic treatment with anticholinergic drugs such as atropine (0.04mg/kg SC) or glycopyrolate (0.02mg/kg SC) is very effective in preventing excessive salivation and bronchial secretions which can compromise air exchange. The use of an anticholinergic is recommended and especially when animals will be endotracheally intubated or anesthetized for lengthy procedures > 1 hour.

C. Hypothermia

Natural heat generating physiologic responses are blunted during anesthesia and rodents can rapidly lose body heat to the environment due to their large surface area/volume. The animals should be kept dry, insulated, and/or warmed to prevent excessive loss of body heat during anesthesia. Methods to conserve body heat should be implemented as soon as the animal is anesthetized. The animal should be insulated from contact with countertops and metal surfaces

using padded materials.
Wetting the animal during preparation of the surgical site should be minimized as much as possible. Use supplement heat sources intra-operatively and post operatively when possible. Water recirculating heating pads are recommended. Electric heating pads can cause thermal burns, therefore if used should not be in direct contact with the animal. Body temperature can be monitored by rectal thermometer or simply by holding the rodent in your hand (body temperature can be judged roughly through a gloved hand). Hypothermia also delays recovery from anesthesia.

D. Eye care
Apply ophthalmic ointment to the eyes to prevent drying of the corneas especially when ketamine is used. Drying is observed as post-procedural corneal opaqueness.

E. Ears
Instill local anesthetic (e.g. lidocaine or bupivacaine) in the ear canals prior to placement of sharp pointed ear bars.

II. Injectable Anesthetic Drugs

- Injectable anesthetics are easy to administer and typically given by intraperitoneal (IP) injection. The effect and duration of anesthesia can be variable because the injectable anesthetics are administered by a bolus injection rather than continuously infused. Long term anesthesia with injectable drugs is also a challenge due to the small size of rodents, and their rapid metabolism of drugs.

- There is less control of anesthesia with injectable anesthetic drugs. Once drugs are injected they cannot be removed and there are limited reversal agents available. Normal ventilation and oxygen exchange is compromised during anesthesia because most anesthetics depress respiration unless positive pressure ventilation is being used. The blood oxygen saturation is dramatically reduced in spontaneously breathing animals; rodents will become progressively acidotic; and drug metabolizing capacity by the liver and kidneys is diminished. The appropriate dosing for supplemental anesthesia can therefore be unpredictable. Supplemental oxygen provided via face mask will greatly improve blood oxygen saturation and reduce acidosis. It is recommended that supplemental dosing not be repeated more than twice. Supplementation with isoflurane gas anesthesia (used with a precision vaporizer) works well if additional anesthesia is needed and is safer than repeating drug injections.

- Rescue from an overdose of an injectable anesthetic has a low success rate. Resuscitation of acute respiratory arrest is possible following an acute initial overdose such as might occur with methohexital or

pentobarbital. The animal must be ventilated until the anesthetic has had time to be metabolized. Slow and steady positive pressure ventilation can be applied using a snug fitting face mask with oxygen or an abdominal folding technique to artificially ventilate the animal. Reversal agents for xylazine (atipamezole 1.0 mg/kg IP, or yohimbine 0.2 mg/kg IP or IV) can help if given immediately in the case of acute overdosage with xylazine. If respiratory arrest occurs following supplemental dosing or during prolonged procedure, resuscitation is unlikely to be successful due to a compromised physiological status.

A. Ketamine and Xylazine (mice and rats)

The combination of ketamine and xylazine is popular for anesthesia of many species including rodents. Ketamine is a dissociative anesthetic and xylazine is a potent sedative and analgesic. The drugs may be combined together and administered as a single intraperitoneal (IP) injection.

- General dosage ranges used:
  Mice: Ketamine 100-200 mg/kg and Xylazine 5-20 mg/kg
  Rats: Ketamine 60-100 mg/kg and Xylazine 5-20 mg/kg

- Supplemental dosing:
  Use up to one half of the original dose of ketamine and xylazine.

  The duration of xylazine's effect is much longer than ketamine, and the margin of safety for xylazine is much less than ketamine. If the original dose was effective then repeat only the ketamine using up to one-half of the original dosage for supplemental doses.

- Length of action (initial dose):
  Surgical level of anesthesia is 30-60 minutes (more variable in mice) and sedation of 1-2 hours.

- Other considerations:
  - For mice and small rats ketamine/xylazine should be diluted (with saline) to ensure accurate measurement of volumes (as in the formulations below).
  - Leave the animal undisturbed for several minutes minutes following injection to allow the anesthetic to reach its full effect. It is also helpful if the animal is calm prior to the injection.
  - The container should be dated when the solution is made and not be used after 7 days.
    Ketamine is a Schedule III controlled substance. Therefore, ketamine must be ordered through the NIDA Pharmacy and secured (locked when not in use). The usage of ketamine must be recorded as per NIDA Controlled Substances Policy.

Example # 1 formulation:

Drug	Volume ml	Mouse dose	Rat dose
		100 mg/kg ketamine + 10 mg/kg xylazine	80 mg/kg ketamine + 8 mg/kg xylazine
Ketamine (100 mg/ml)	1 ml	0.1ml per 10 gm of body weight	0.08 ml per 10 gm body weight
Xylazine (20 mg/ml)	0.5 ml		
Saline 0.9%	8.5 ml		
Total	10.0ml		

Example #2 formulation:

Drug	Volume ml	Mouse dose	Rat dose
		83 mg/kg ketamine + 17 mg/kg xylazine	83 mg/kg ketamine + 17 mg/kg xylazine
Ketamine (100 mg/ml)	1 ml	0.1 ml per 20 gm body weight	0.1 ml per 20 gm body weight
Xylazine (20 mg/ml)	1 ml		
Saline 0.9%	4 ml		
Total	6 ml		

B. Ketamine and Xylazine and Acepromazine (mice and rats)

Acepromazine, a phenothiazine tranquilizer that can be added to the above combination, can provide a smoother induction (especially for mice) and longer duration of anesthesia.

Example #3 formulation:

Drug	Volume ml	Mouse dose	Rat dose
		100 mg/kg ketamine + 10 mg/kg xylazine + 3 mg/kg acepromazine	80 mg/kg ketamine + 8 mg/kg xylazine + 2.4 mg/kg acepromazine
Ketamine (100mg/ml)	1 ml	0.1ml per 10 gm body weight	0.08 ml per 10 gm body weight
Xylazine (20mg/ml)	0.5 ml		
Acepromazine (10mg/ml)	0.3 ml		
Saline 0.9%	8.2 ml		
Total	10.0 ml		

C. Telazol (tiletamine and zolazepam) and Xylazine (rats only)

Telazol is a commercially available pharmaceutical grade combination of two drugs, tiletamine and zolazepam, originally marketed for use in cats. Tiletamine is chemically related to ketamine, but is longer lasting and more potent. Zolazepam is a diazepinone tranquilizer which improves muscle relaxation, CNS depression, and emergence from anesthesia. The addition of xylazine increases anesthesia time and adds a significant analgesic component.
If injectable anesthetics are to be used, then this is an excellent choice for long-term anesthesia. Anesthesia of up to 2 hours can be achieved with a single dose.

- Suggested dosage for rats:
  Telazol 40 mg/kg and xylazine 5 mg/kg administered IP

- Supplemental dosing:
  Suggest one-third of original dose (not published)

- Length of action (single dose):
  90-130 minutes

Dilutions should be used for accurate measurement of drug volumes. Telazol must be reconstituted with saline or sterile water and has a manufacturer's recommended expiration of 14 days after reconstitution under refrigeration. There is loss of potency if frozen.

D. Pentobarbital (mice and rats)

Pentobarbital had been a popular rodent anesthetic for many years. The margin of safety between surgical anesthesia and death is narrow. Animals are very prone to hypothermia. The optimum dose will vary with the strain, sex, diet, and other factors. Pentobarbital has become increasing difficult to obtain.

- Dosage:
  Mice 50-90 mg/kg administered IP
  Rats 40-60 mg/kg administered IP
- Supplemental dosing:
  10-15 mg/kg
- Length of action (initial dose):
  20-60 minutes
- Pentobarbital is a Class II controlled substance. Therefore, pentobarbital must be ordered through the NIDA Pharmacy and secured (locked when not in use). Usage must be recorded as per NIDA Controlled Substances Policy.

E. Methohexital (mice and rats)

Methohexital (Brevital) is a short acting barbiturate that is commonly used to verify intravenous catheter patency which is evidenced by immediate sedation.

- Dosage: 5 mg/kg administered via catheter
- Length of action: Sedation for 3-5 minutes

F. Other injectable drug combinations:

Consult with the veterinarian when considering any alternate injectable combinations. Numerous other anesthetic regimens have been used in rodents. These include:

- ketamine + diazepam
- ketamine + medetomidine
- Telazol (tiletamine/zolazepam)
- Telazol (tiletamine/zolazepam) + butorphanol
- fentanyl + droperidol + diazepam
- fentanyl + fluanisone + diazepam
- propofol

Although some of these drug regimens have been used successfully none of have gained wide acceptance for rodent anesthesia.
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III. Injectable Anesthetic Drugs- Non-Pharmaceutical Grade

- The following drugs have been used for rodent anesthesia. These drugs are not commercially available as pharmaceutical grade drugs and are no longer widely used in contemporary veterinary medicine due to the availability of more acceptable anesthetics. The drugs listed below are not recommended but may be permitted with scientific justification.

- The NIH Guide on Animal Care and Use and the NIH/NIDA has guidelines and policies on the use non-pharmaceutical grade drugs. http://oacu.od.nih.gov/ARAC/documents/Pharmaceutical_Compounds.pdf Contact the NIDA/IRP Pharmacy for guidance or assistance with compounding these drugs. Pharmacy will compound them by following the normal pharmacy standard.

- The use of chloral hydrate, equithesin, avertin, urethane, and alpha-chloralose must be scientifically justified in the Animal Study Proposal.

A. Chloral hydrate (rats)

Chloral hydrate has been a popular hypnotic anesthetic agent used in rats. The concentration used must not exceed 5%. The use of chloral hydrate is associated with chemical peritonitis which sometimes causes adynamic ileus (loss of GI motility with consequent fluid sequestration and constipation) leading to bloating and death within 1 week following injection. Until recent years this drug was commercially available as a pharmaceutical grade drug with the trade name Chloral Hydrate; however, it has become increasing difficult to obtain.

Dosage: 400mg/kg IP (4% concentration)

Supplemental dosing: Up to one-third of the original dose

Length of action (initial dose): 1-2 hours of surgical anesthesia

B. Equithesin (rats)

Equithesin is a drug combination that contains chloral hydrate, pentobarbital, magnesium sulfate and alcohol. The combination produces a long lasting anesthesia of 1-2 hours.

It is also associated with adynamic ileus as described for chloral hydrate. There seems to be no advantage to using this compound over chloral hydrate alone. This compound can be formulated by the NIDA pharmacy using standard formulation below.

Dosage: 3-4 ml/kg IP

Supplemental dosing: Up to 1 ml/kg

Length of action (initial dose): 1-2 hours of surgical anesthesia

Standard formula:

Ingredient	250 mls	50 mls	100 mls
Chloral Hydrate (44.4 mg/mls)	11.1g	2.22g	4.44g
Na Pentobarbital (9.72 mg/mls)	2.43g	0.486g	0.972g
$MgSO_4$	5.31g	1.062g	2.124g
Propylene Glycol	111 mls	22.2 mls	44.4 mls
EtOH (Absolute)	30 mls	6 mls	12 mls
Distilled H_2O	qs to 250 mls	50 mls	100 mls

- Place all weighed dry powders into a dry, volumetric flask according to the volume; 250 mls, 50 mls, or 100 mls.
- Add EtOH first.
- Add Propylene Glycol.
- When it is finally in solution, bring up to required volume (250mls, 50 mls, or 100 mls) with Distilled H_2O.

C. Avertin (mice)

Avertin (2,2,2-tribromoethanol) produces an anesthesia in mice lasting 15-30 minutes. This anesthetic should only be used one time in a given animal as a second anesthetic procedure may result in death. The use of avertin has been associated with development of fibrous peritoneal adhesions. Proper storage at 4 degrees Celsius under dark conditions is crucial to avoid decomposition and subsequent mortality. Dosage adjustments may need to be made depending on the particular strain of mice being used. This compound can be formulated by the NIDA pharmacy.

Dosage: 0.2 ml of 1.2% avertin per 10 grams of body weight IP
Other dosage: 0.015-0.017 ml of 2.5% avertin per 1 gram of body weight IP

Supplemental dosing: No information

Length of action: 15-30 minutes of surgical anesthesia

D. Urethane (rats)

Urethane produces a long-lasting anesthesia of 6-10 hours with minimal cardiovascular and respiratory depression. **Urethane (ethyl carbamate) is a known carcinogen**, therefore strict precautions must be taken (e.g. gloves, face masks, mixing under fume hood) to protect personnel. The use of rethane is limited to non-survival procedures only.

Dosage: 0.5-1.5 gm/kg IP, most commonly used dose is 1.0 mg/kg

Supplemental dosing: 0.5 gm/kg

Length of action (initial dose): 6-10 hours of surgical anesthesia

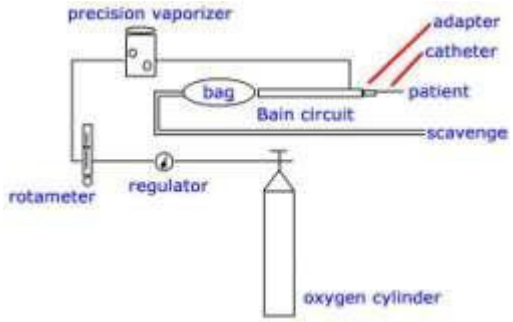
Use with alpha-chloralose:
Urethane has been combined with alpha-chloralose using IP doses of 250-400mg/kg urethane plus 35-40 mg/kg alpha-chloralose for minimizing physiologic changes (administer urethane 20-30 min prior to alpha-chloralose)

IV. Inhalation Anesthesia

Inhalation gas anesthesia with isoflurane is a very popular method for anesthesia of many animal species including rodents.
Advantages:

- Large margin of safety, depth of anesthesia is easily controlled.
- Can be used for any length of procedure and repeated with less stress to the animal.
- Recovery from anesthesia is fast
- Easy to learn to use

Isoflurane requires the use of specialized equipment including a precision vaporizer, oxygen source, flowmeter, delivery system, and method for scavenging waste gases. The equipment is relatively expensive, though the isoflurane solution is inexpensive.



Oxygen is the carrier gas for the liquid isoflurane. The isoflurane in oxygen is administered via a chamber for induction and via a face mask/nose cone for maintenance. The face mask/nose cone should be sized to cover the face and secured to stay in place. Scavenging becomes important as masks and nose cones are not tight fitting, isoflurane leaks into the immediate area and might be breathed by the surgeon. Isoflurane odor might not be readily detected from dilution but still exposes operator to chemical.
Administration via endotracheal intubation can be used but is not practical for most procedures with rodents.

Typical Isoflurane dosages used:

Delivery method	**O2 flow rate***	**% concentration isoflurane**
Small Chamber	1-2 liters/minute	3-5 %
Face mask	0.5-1 liters/minute	2-3 %

* The % concentration is not affected by the flow rate. Higher flow rates will produce an excess of waste gas to be scavenged. A flow rate to low may produce less accurate
% delivery. Larger chambers may require higher flow rates to fill appropriately.

Open drop jar method:
Isoflurane can be used without a precision vaporizer. This method typically uses a

glass dessicator jar in which several milliliters of isoflurane are placed on cotton wadding in the bottom. The rat is placed in the jar but protected from direct contact with any unabsorbed liquid isoflurane. A raised floor can be used to prevent contact. The anesthetic effect is very rapid (seconds); this is because isoflurane at atmospheric

pressure rapidly reaches high concentrations up to 30%. This is rapidly fatal if the animal is not removed quickly. The open drop jar method is best used for euthanasia procedures.
This method should be used over a downdraft table for personnel protection.

V. Analgesic Use with Anesthesia

The use of analgesics pre-operatively or intra-operatively will provide preemptive analgesia which improves patient stability and reduces post-operative pain. Local anesthetics are especially useful in this respect because they block nociceptive pain signaling to the CNS. See NIDA Analgesia Guidelines for information on analgesics.

Neuromuscular blocking agents (e.g. pancuronium) do not provide anesthesia or analgesia effects. The proposed use these agents should be discussed with the veterinarian in the development of the Animal Study Proposal (ASP).

VI. Monitoring Anesthesia

- Observe albino eye color and mouth membranes to evaluate adequate oxygenation (pink=good; grey/or pale = poor)
- Monitor regularity of breathing pattern, rate and depth of breathing (slow/shallow = excessive anesthetic; irregular/deep = inadequate anesthesia). Count breaths for 10 seconds x 6 for breaths/minute.
- Monitor pain reflexes – Adequate = no withdrawal of foot to gentle pinch
- Access body temperature by rectal thermometer or by hand, maintain warming procedures
- Place animal in a clean, bedded cage to recover and continue to provide warmth and monitor every 5 minutes until able to self-right itself from a lateral (on side) recumbent position.
- Administer analgesics
- Begin post-operative record keeping/documentation