West Virginia University
Institutional Animal Care and Use Committee

WVU IACUC Approved Guidelines: Anesthesia and Analgesia in Rats

Purpose

This document has been created by OLAR veterinary staff to serve as a **guideline** regarding delivering tranquilization/sedation, anesthesia and analgesia of laboratory rats, for use when planning or reviewing animal use protocols. Inclusion of a drug in this document **does not** equate authorization for use without IACUC review and approval. Any medication administered to animals *must* be included in the approved animal use protocol.

This document is not intended to be an exhaustive compendium of drugs or combination of drugs that can be used in rats for this purpose. Rather, the following guidelines are general recommendations and consequently do not include reference to specific research-associated drug protocols. If you have special needs, or questions about the use of anesthetics or analgesics for your particular situation, please contact the OLAR veterinary staff at 293-3737 or vetservices@hsc.wvu.edu.

More specific information on pre- and post-operative care for rodents undergoing survival surgical procedures may be found on the IACUC website at: http://oric.research.wvu.edu/animal/guidlines

Definitions

Anesthesia: A pharmacologically induced and reversible lack of awareness and/or sensory perception. *(Anesthetics are analgesic, but analgesics do not necessarily anesthetize.)*

Analgesia: A relief or absence of pain sensation without loss of consciousness

Anesthetic: An agent that causes loss of sensation with or without loss of consciousness

Analgesic: An agent used to lessen or eliminate pain

Tranquilizer: An agent that reduces tension/anxiety, an anti-anxiety agent

Sedative: An agent or drug having a soothing, calming, or tranquilizing effect

Scope

These guidelines are intended for use by WVU research investigators and staff, veterinary staff, and IACUC members when writing, reviewing or designing animal use protocols involving painful and/or anesthetic procedures in rats.

Special Considerations

Remember that body fat, or lack thereof, age, sex, overall health, and strain can all impact a rat's response to anesthetic agents. Thus, all of these factors should be considered when contemplating an anesthetic regimen.

Preanesthetic fasting is not usually necessary in rats. However, if fasting is employed it should be limited to *no more than 2-3 hours* prior to the procedure, due to the high metabolic rate of small rodents. Water should *never* be restricted during the pre-anesthetic period.

Because rats have a greater body surface area to body mass ratio than larger animals and humans, thermal support is critical to their survival and successful anesthetic recovery. Hypothermia during anesthesia results in prolonged recovery, additional stress to the animal, and increased risk of anesthetic death. Supplemental heat should be provided to rats undergoing anesthesia. Re-circulating warm water blankets and other isothermal heat sources such as Safe and Warm®, Snuggle Safe®, etc, are recommended for use with rodents. Electric heating pads and heat lamps are not generally recommended because uneven heating may present danger to anesthetized animals, with the tendency to cause thermal injury, and increased potential for ocular damage and hyperthermia. If a heating pad or other heated surface is used, there should be some insulating material between the rat and the heated surface. Regardless of heat source, animals' temperatures should be monitored closely during anesthetic recovery.

Anesthesia Administration

Parenteral anesthesia may be administered to rats via **intraperitoneal** (IP), **subcutaneous** (SQ), **intravenous** (IV), or **intramuscular** (IM) injection. Intravenous injections are most typically made into a lateral tail vein. Vessel dilation can be increased by warming the tail using warm (not hot) water or heat lamp exposure for 1-2 minutes prior to injection. Acceptable locations for IM injections in rats are the caudaomedial thigh muscles (semimembranosus or semitendonosus) or the muscles just lateral to the backbone (epaxial muscles). IM injections should be reserved for non-recovery procedures because IM injection of irritating substances (e.g. ketamine) may lead to muscle necrosis, lameness, self-mutilation of the limb, or cutaneous ulceration. Documented training for proper restraint and injection techniques is strongly recommended for persons performing these procedures, and particularly for those without prior experience. Laboratory personnel who wish to obtain training or practice to increase proficiency with approved parenteral administration techniques may contact OLAR veterinary services (3-3737) or the IACUC training associate (3-9368) to schedule a training or practice session at no charge.

Inhalation anesthesia may be delivered by facemask, endotracheal tube or by chamber induction. **Flow-through anesthesia chambers** and **facemasks** require a gas anesthesia machine with an oxygen source and a precision vaporizer. Use of a precision vaporizer is preferred for inhalant most anesthetics. A non-rebreathing system should always be used with rats. Anesthesia chambers and facemasks for rats are commercially available, or can be devised in-house. Facemasks should be used for anesthetic events lasting more than 5 minutes. **"Drop jar" chambers** can be made of covered glass or plexiglass containers containing premeasured amounts of an anesthetic-soaked absorbent material in the bottom. It is important that chambers provide physical separation of the rat from the anesthetic-soaked substrate. Use of a mesh grid or histopathology cassette can facilitate this. This method is generally adequate for very short-term procedures, such as subcutaneous tumor implantation. **Endotracheal intubation** is technically difficult in the rat and requires some expertise, and demonstration of appropriate technique by experienced personnel is recommended prior to use. An intravenous catheter (size 14-20) with the stylet filed down may be suitable for rat intubation. Proper positioning is important and greatly facilitates intubation. An appropriately fabricated laryngoscope or light source is typically used in order to visualize the pharyngeal and laryngeal structures before attempting intubation. Contact OLAR to arrange training if there is a specific need for this method. *Any time inhalant anesthesia is used, it is important to do so with an anesthetic system equipped with a gas scavenging system or in a fume hood to minimize occupational exposure to exiting gases.*

Monitoring and Recovery

Irrespective of the anesthetic used, each rat must be monitored to avoid excess cardiac and respiratory depression or, at the other extreme, insufficient anesthesia which is characterized by poor muscle relaxation, and movement in response to either a noxious stimulus (firm toe pinch). Other signs of insufficient anesthetic depth include response to surgical stimulation, vocalization, or a sudden and significant increase in heart rate during an anesthetic procedure.

Parameters that should be monitored for rats during anesthesia include:

1. **Anesthetic depth**: assessed by an inability to remain upright, loss of purposeful voluntary movement, loss of blink reflex, muscle relaxation, *and* loss of response to reflex stimulation (toe pinch with firm pressure)
2. **Respiratory rate, depth and pattern**: assessed by observing chest wall and abdominal movements (normal respiratory rate for an awake rat at rest is 70–110/min, a slow rate drop of 50% with a regular even pattern is acceptable during anesthesia).
3. **Heart/pule rate**: can be assessed by thoracic auscultation or palpation of the chest wall (normal heart rate for an awake rat at rest is 260–500/min).
4. **Body temperature**: can be assessed rectally, using a regular digital thermometer or temperature probe (normal body temperature for an awake rat at rest is 96.6°F – 99.5°F or 35.9°C – 37.5°C). Because rats have a greater surface area to body mass ratio than larger animals, thermal support is critical to successful surgery and recovery.
5. **Mucous membranes**: mucous membranes or eye papillary color (if albino) should be reddish pink (*not* grey, muddy, purple, blue or white). Mucous membranes should be moist and shiny, not tacky or dry.

An ophthalmic ointment, such as Paralube®, Lacrilube® or equivalent, must be applied to the eyes of any animal anesthetized with gas anesthetics >10 minutes or receiving injectable anesthetics. For procedures lasting more than 45 minutes, ophthalmic ointment should be re-applied every 45 minutes or more frequently if indicated, until the animal is recovered and mobile.

Placing a warm water re-circulating blanket and drape between the rat and the table, or administering warmed fluids (SQ and/or IP) during or after the surgery (not to exceed 5 mL/kg/hr; or 12 mL/rat/12 hr) can minimize heat loss. Warm fluids such as normal saline or lactated Ringer's solution are also important for correcting volume deficits following surgical procedures.

Rats should be recovered from anesthetic events either in a clean cage without cage mates or bedding. Recovering rats should remain under relatively constant (every 5-10 minutes) observation until they are deemed to be stable and recovered. Recovery in rodents is defined as complete and unassisted self-righting (e.g. not just the front end) from dorsal recumbency without losing consciousness thereafter. Rats can be returned to cages with bedding and cage mates when fully recovered from anesthesia.

Nutritional support is critical during post-procedure recovery periods. Placing moistened rodent chow on the cage floor is recommended to encourage animals to eat following anesthetic events. Oral glucose solution can be helpful in pups undergoing anesthesia, especially with hypothermia. Contact OLAR for additional information.

Animals may not be returned to animal housing rooms following an anesthetic procedure until they are alert and fully ambulatory, with a body temperature of *at least* 97°F (36°C).

General Anesthetics

Isoflurane is generally the inhalant anesthetic agent of choice (when study parameters allow) for both short and lengthy procedures, due to its rapid and reliable recovery and personnel safety profile. When using a precision vaporizer to deliver an inhalant anesthetic, the machine must be compatible with the specific agent being used (isoflurane vs sevoflurane). *NOTE: Older halothane vaporizers can be recalibrated to use with isoflurane, and enflurane vaporizers can be recalibrated to use with sevoflurane.*

The injectable anesthetic combination of choice for most common procedures is ketamine (40–90 mg/kg IP) with xylazine (5–10 mg/kg IP) OR dexmedetomidine (0.25–0.5 mg/kg) to produce 40–90 minutes of anesthesia. NOTE: When using this combination, if additional anesthetic is needed, only re-dose 1/3 to 1/2 the original calculated dose of *ketamine*. Xylazine or dexmedetomidine should *not* be re-dosed due to their profound alpha-2 cardiovascular effects.

Table 1: Inhalant Anesthetics Used in Rats

Drug	Dosage	Comments
Isoflurane (Forane®, Aerane®) **Recommended**	4-5% for induction 1-2% for maintenance	Maintenance requires use of a calibrated vaporizer
Sevoflurane	To effect (shorter induction)	Maintenance requires use of a calibrated vaporizer

Table 2: Injectable Anesthetics, Tranquilizers and Sedatives Used in Rats

Drug	Dosage & Route	Duration of Anesthesia	Comments
Barbiturates			
Methohexital 1% soln (Brevital®)	7-15 mg/kg IV	5-10 min	Respiratory depression / poor analgesia at low doses / insufficient for surgery / sedation only
Pentobarbital (Nembutal®)	30-50 mg/kg IP Give diluted in saline (< 10mg/ml)	80-90 min	Severe respiratory depression / poor analgesia in rats Anesthesia / immobilization
Thiobutobarbital (Inactin®)	80-110 mg/kg IP	60-160 min	This drug is indiosyncratic in its effect, depending on strain and use. Use with caution
Dissociatives			
Ketamine (Ketoset®) Pre-med only **Not recommended**	45-100 mg/kg IM or IP	Unproven	Poor muscle relaxation / only mild cutaneous analgesia

Ketamine + acetylpromazine	30-75 mg/kg + 2.5-3 mg/kg IM or IP	20-30 min	For sedation/ immobilization only
Ketamine + dexmedetomidine (Dexdomitor®)	60-80 mg/kg + 0.1-0.25 mg/kg IP	20-30 min	Surgical anesthesia Anesthetic depth varies ♀ more sensitive than ♂ **Dexmedetomidine should *not* be re-dosed**
Ketamine + diazepam (Valium®)	40-80 mg/kg + 5-10 mg/kg IP	20-30 min	Sedation/immobilization
Ketamine + midazolam (Versed®)	60-80 mg/kg + 5 mg/kg IP	20-30 min	Sedation/immobilization May see extended recovery times (up to 120 min)
Ketamine + xylazine (Rompun®) **Recommended**	50-100 mg/kg + 5-10 mg/kg IP or IM	45-80 min	Longer duration than ketamine/xylazine alone Surgical anesthesia **Xylazine should *not* be re-dosed**
Ketamine + xylazine + acepromazine	40-50 mg/kg + 2.5 mg/kg + 0.75 mg/kg IM or IP	Unproven	Surgical anesthesia **Xylazine should *not* be re-dosed**
Tiletamine/zolezepam (Telazol®)	20-40 mg/kg IP or 20 mg/kg IM	25-45 min	Sedation/immobilization (corneal, pedal, and swallowing reflexes remain intact)
Tiletamine/zolazepam (Telazol®) **+ xylazine**	20-40 mg/kg + 5-10 mg/kg IP	130-180 min	Good analgesia but may cause marked cardiovascular depression **Xylazine should *not* be re-dosed**
Telazol® **+ butorphanol**	20-40 mg/kg + 1.25-5 mg/kg IP	50-120 min	Good analgesia but transient hypotension, bradycardia, and dose dependent respiratory depression

Other			
Alpha-chloralose (5% w/v concentration) **NOT recommended** for survival surgery in rats	55-65 mg/kg IP	Unproven	Poor analgesia. Only light sedation/ immobilization. Metabolic acidosis and/or convulsions possible. **Non-recovery use only**
Alpha-chloralose + urethane **NOT recommended** for survival surgery in rats	50-60 mg/kg IP + 500-800 mg/kg IP (administer urethane 20-30 min. *before* α-chloralose)	Extended	For **non-recovery use only**
Alphaxalone/ Alphadolone (Saffan®)	10-15 mg/kg IV	5 min	Brief, deep anesthesia
Dexmedetomidine (DexDomitor®)	0.03-0.25 mg/kg SC or IP (30-250 µg/kg)	Approx. 1 hr.	Light to heavy sedation / immobilization Mild analgesia **Dexmedetomidine should *not* be re-dosed****
Diazepam (Valium®)	2.5-5.0 mg/kg IP or IM 2.0 mg/kg IV	Unproven	Light sedation / immobilization Recommended as **pre-med** only Cause muscle necrosis when given IM
Fentanyl + dexmedetomidine (Dexdomitor®)	0.3 mg/kg + 0.1-0.2 mg/kg IP (300 µg/kg + 100-200 µg/kg)	60-70 min	Surgical anesthesia
Midazolam (Versed®)	2.0-5.0 mg/kg IM or IP or 2.0 mg/kg IV	Unproven	Light sedation / immobilization Recommended as a **pre-med** only
Propofol (Diprivan®)	7.5-10 mg/kg IV (induction) 44-55 mg/kg/hr CRI (maintenance)	5-10 min Extended	Respiratory depression may occur; Compromised patients may experience cardiovascular depression

Tribromoethanol (Avertin®)* **Not recommended** for use in rats	300 mg/kg IP	Unproven	Must be stored in the dark at 4°C
Urethane **NOT recommended** for survival surgery in rats	1000 mg/kg IP	Extended (up to 24 hr)	Carcinogenic, mutagenic / For **non-recovery use only** / progressive acidosis, hyperosmolality of body fluids, and osmotic toxicity of mesenteric vasculature
Xylazine	1-5 mg/kg IP (doses up to 10 mg/kg may be appropriate in some instances)	Approx. 1 hr	Light to heavy sedation / immobilization Mild analgesia **Xylazine should *not* be re-dosed****

SC = subcutaneous IP = Intraperitoneal IV = Intravenous

***Tribromoethanol (Avertin®)** should not be used routinely without scientific justification and IACUC approval. Tribromoethanol is an irritant, especially at high doses and/or concentrations, or with repeated use. It can result in peritonitis, and the risk of fatal peritonitis increases with each subsequent use in the same animal. It degrades in the presence of heat or light to produce toxic byproducts, resulting in nephrotoxicity and hepatotoxicity. The effects of tribromoethanol are unpredictable in rats <16 days and in animals with altered carbohydrate metabolism (e.g. obesity and diabetes models). **Cautions for use:** Because Avertin® is not commercially available as a pharmaceutical grade drug, it must be made in the laboratory from the reagents tribromoethanol and tertiary amyl alcohol. It must be carefully prepared under aseptic conditions with a working dilution of 1.25% prepared fresh. Sterile filter the prepared solution with a 0.2-micron filter. Store in a brown glass or foil-covered container at 4°C for no longer than 4 months. Do not use if the solution becomes discolored or has a precipitate. Check the pH before each use and use only when pH >5. It is not to be used twice in the same animal that will survive. Therefore, second-time use should *only* be for a terminal/acute procedure.

****Xylazine** and **Dexmedetomidine** may only be re-dosed if surgical anesthesia is required beyond the limit of the alpha-2 agonist used (1 hr), when administered without other agents. However, if longer duration anesthesia is needed, consultation with an OLAR veterinarian for an alternative anesthetic regimen is strongly recommended.

Table 3: Antagonists to Anesthetics used in Rodents

Drug	Dosage & Route	Anesthetic	Comments
Atimpamezole (Antisedan®)	Equal volume as dexmedetomidine (or xylazine) being reversed IP, SC, or Half IP, half SC	Any regimen using xylazine or dex-medetomidine	Specific alpha-2 adrenergic receptor antagonist. Reverses xylazine or dexmedetomidine
Yohimbine (Yobine®)	2.1 mg/kg IP, SC, or Half IP,	Any regimen using xylazine	Alpha-2 adrenergic receptor antagonist.

	half SC		
Flumaznil	0.1-10 mg/kg	Reversal of midazolam or diazepam	Benzodiazepine reversal agent Re-sedation may occur
Naloxone	0.01-0.1 mg/kg IV, IM, or IP	Reversal of opioids	Pure opioid (e.g. fentanyl) antagonist Reverses analgesia as well as respiratory depression. Re-sedation may occur
Naltrexone	Give 2 times the dose (2 mg per mg opioid given) for opioid to be reversed Split SC and IM or IV		Pure opioid (e.g. fentanyl) antagonist Reverses analgesia as well as respiratory depression. Effects typically last longer than Naloxone.

SC = subcutaneous IP = Intraperitoneal IV = Intravenous

Local Anesthetics

The two most commonly used local anesthetics in veterinary patients are lidocaine (Xylocaine® or Novocaine®) and bupivacaine (Marcaine® or Sensocaine®). Lidocaine has a rapid onset (1–2 min) and a short duration of action (1½ –2 hr). Bupivacaine has a slower onset (5–10 min) and a much longer duration of action (4–12 hr, site dependent).

Maximum safe doses for most veterinary species are:

Lidocaine: 4 mg/kg (0.4 ml/kg of 1%, or 10 mg/ml solution)

Bupivacaine: 1–2 mg/kg (0.4–0.7 ml/kg of 0.25%, or 2.5 mg/ml solution)

These doses can be diluted in sterile saline to provide a larger injection volume. Intravenous administration of lidocaine or bupivacaine can result in cardiovascular effects including hypotension and dysrhythmias, as well as central nervous system depression and seizures. To avoid these adverse consequences, the maximum safe dose should never be exceeded. Always aspirate prior to injection to insure that IV injection is avoided.

PLEASE NOTE: local anesthetics are available in a variety of concentrations with our without epinephrine. Epinephrine causes vasoconstriction and prolongs the action of the local anesthetic. Adrenaline should *not* be used in animals with suspected cardiac compromise.

Neonatal Rat Anesthesia

A neonatal rat is defined as a pup < 5 days of age. There are a variety of anesthetic techniques currently described in the literature for use in neonatal rodents including injectable, inhalant, and physical methods.

Neonatal rodents demonstrate an increased sensitivity to most **injectable** anesthetic agents and these have been associated with high anesthetic mortality in neonates. **Inhalant** anesthetics are considered to be safe and effective in neonatal rodents, but they may have a longer induction time than adult rodents, and this should be anticipated when using these anesthetics. **Hypothermia** is the primary physical method utilized in neonatal anesthesia and is believed to provide anesthesia 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 (e. g. ice or alcohol in dry ice) should be avoided. It is unknown if hypothermia-induced anesthesia is distressful to neonatal rodents.

NOTE: it is important to make sure the neonate is fully recovered (warmed) from anesthesia prior to placing back in the mother's cage in order to reduce the likelihood of parental rejection.

Table 4: Neonatal anesthesia methods

Inhalant Anesthetics (Neonatal)			
Stage of Anesthesia	**Route**	**Oxygen (L/min)**	**Isoflurane (%)**
Induction	Mask or Chamber	0.5–1.0	4–5
Maintenance	Mask	0.5–1.0	1–3.5
Injectable Anesthetics (Neonatal)			
Rat Age	**Drug**	**Dose & Route**	**Comments**
< 7 days	Ketamine + Xylazine	50–120 mg/kg + 5–10 mg/kg **IP**	25 g needle 1 mL syringe max. vol. 2 mL
> 7 days	Ketamine + Xylazine	50–150 mg/kg + 5–10 mg/kg **SC**	25 g needle 1 mL syringe max. vol. 3 mL
Physical Anesthesia Method (Neonatal)			
Rat Age	**Technique**	**Procedure**	
< 5 days	Hypothermia	Place neonate(s) either 1) on a latex covered bed of crushed ice; 2) on a paper towel-lined test tube in crushed ice/ice water; Animals have reached their proper plane of anesthesia when they no longer respond to a firm toe pinch Once the proper anesthetic plane is reached, remove the neonates from the ice bath and place them on a chilled cold pack or bed of ice Optimally, fiber optic light sources should be used during neonatal hypothermia-anesthetic procedures, as incandescent bulbs will warm the surgical field. Following anesthesia, the animals should be warmed *slowly* (rapid warming can cause tissue damage) on a circulating warm water blanket (40°C) or in an incubator (33°C) Animals can be returned to the dam once they are fully awake and able to ambulate normally.	

Analgesia

Unrelieved pain can have profound negative physiologic consequences and, subsequently, alter research results. Rats show a variety of responses to pain, some of which may be fairly subtle and easily overlooked on cage side exam. The **IACUC policy on Pain and Distress** (http://oric.research.wvu.edu/animal/guidelines) provides further guidance and should be consulted regarding methods for detecting and alleviating pain or distress in rats.

Table 5: Systemic Analgesics Used in Rats

Drug	Dose & Route	Duration
Buprenorphine (Buprenex®)*****	0.01–0.05 mg/kg SC or IP	8–12 hrs
Butorphanol (Torbutrol®)***#**	0.5–2.0 mg/kg SC or IP	2–4 hrs
Carprofen (Rimadyl®)	5 mg/kg SC or 1.5 mg/kg PO	24 hrs 12 hrs
Flunixin (Banamine®)	1.1–2.5 mg/kg SC or IM	12–24 hrs
Ketoprofen	1-5 mg/kg SC or IM or 5 mg/kg PO	24 hrs
Meloxicam (Metacam®)	1–2 mg/kg PO or SC	24 hrs
Morphine*§	2–10 mg/kg SC	2–4 hrs
Oxymorphone*	0.15-0.5 mg/kg SC or 0.15-0.25 mg/kg IM	6–12 hrs

SC = Subcutaneous IP = Intraperitoneal IV = Intravenous PO = By mouth

* In addition to being an analgesic, this drug also has sedative properties; If this drug is administered as an animal is recovering from anesthesia, the animal *must* be observed carefully for cumulative sedative effects of the anesthetics and sedating analgesics.

The analgesic properties of this drug dissipate in domestic animals well before the sedative effects; however, in Kappa opioid receptor dominated species (e. g. pigeons) it may be a drug of choice.

§ This drug has a broad range of recommended doses reflecting unique individual sensitivities to its effects. It is recommended that the animal be given the lowest dose in the range and be observed for signs of pain or discomfort. Additional analgesic may be administered if necessary at the next scheduled dosing time.

References

This document was adapted from "ULAM Mouse Anesthesia and Analgesia Guidelines" referenced below.

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