

WVU IACUC GUIDELINES:

Veterinary Recommendations for Anesthesia and Analgesia

Purpose

This document serves as a guide for the anesthesia and analgesia in laboratory and agricultural animals. All surgical procedures, anesthetics, analgesics, antibiotics or other medications used on animals must be described in the animal use protocol, be approved by the IACUC, and performed by adequately trained personnel listed on the protocol. According to the 8th edition of the Guide for the Care and Use of Laboratory Animals (NRC), "Guidelines for the selection and proper use of analgesic and anesthetic drugs should be developed and periodically reviewed and updated as standards and techniques are refined."

Guidelines

- 1) Appropriate anesthetic and analgesic agents must be used to eliminate or reduce potential pain or distress. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
- 2) Withholding anesthesia/analgesics or under-treating pain or distress must be justified and approved in the IACUC protocol.
- 3) A veterinarian can suggest or prescribe an anesthesia/analgesic regimen which is not approved in the IACUC protocol. The drugs will be administered under veterinary oversight and this should be reflected in the medical records. If a permanent change to the anesthesia or analgesia regimen becomes necessary, an amendment must be submitted.

Definitions

- 1) **Systemic anesthesia:** Temporarily induces loss of sensation with loss of consciousness. Only provides pain relief due to or during loss of consciousness. Can be administered via inhalation, injection, or immersion (aquatic).
- 2) **Local anesthesia:** Temporarily induces loss of sensation to a specific part of the body. May provide pain relief.
- 3) **Analgesia:** Provides pain relief without loss of consciousness. Some drug classes may produce sedative effects.
- 4) **Sedation:** Central depression causing stupor where the animal is unaware of its surroundings but still responsive. Sedation may or may not provide analgesia depending on drug used.
- 5) **Surgical plane of anesthesia:** Stage of anesthesia in which the animal is at an appropriate anesthetic depth and surgical procedures can commence.
- 6) **Multimodal analgesia:** Use of different categories of analgesics/anesthetics in combination to address different sources of pain perception/stimulation.
- 7) **Preemptive analgesia:** analgesia administered shortly before or immediately after an animal is anesthetized, but prior to initiation of a painful stimulus. Benefits include preventing "wind-up phenomenon", ensure pain is managed at point of anesthesia recovery, lower amount of anesthetic agents required, improve cardiovascular stability during surgery.

Anesthetics

1) Inhalant Anesthetics

- a. The inhalant anesthetics include gases such as isoflurane and sevoflurane.
- b. An anesthetic machine set-up is required for surgical procedures or experiments that require prolonged anesthetic exposure.
- c. For short, non-surgical procedures, it may be possible to administer inhalant anesthesia (isoflurane only) via a drop jar. For this procedure a cotton ball or gauze soaked with the anesthetic is placed in a jar with the animal. This procedure must be performed under a chemical fume hood or ducted biosafety cabinet, the animal CANNOT contact anesthetic, and can only be used for minor procedures (e.g., genotyping, injections, tumor implantation (trocar), drug pellet or microchip implantation). Animals will recover quickly after removal from jar.
- d. Use of a scavenger system is required to prevent personnel exposure to the waste anesthetics.
- e. **Advantages:** safe and reliable, predictable and rapid control of anesthetic depth, not controlled substances
- f. **Disadvantages:** induction must be closely monitored, personnel training, special equipment required, potential risk to staff (if not appropriately scavenged)

2) Injectable anesthetics

- a. In rodents, injectable general anesthetics include ketamine/xylazine combination and pentobarbital, which are typically administered via intraperitoneal (IP) injection.
- b. Local anesthetics are often delivered subcutaneously along the incision site. They could also be used in nerve blocks or epidural administration. Local anesthetics are not adequate as the only analgesic for any surgical procedure unless scientifically justified in the protocol.
- c. **Advantages:** They can be used without expensive supporting equipment such as the anesthesia machines required with the use of inhalants, they are easily transported, and are relatively inexpensive.
- d. **Disadvantages:** Prolonged recovery times. The animal will have to metabolize the drug in order to completely recover from anesthesia. In addition, once the agent is injected, the anesthetic depth cannot be adjusted throughout the procedure except to achieve a deeper anesthetic plane by giving additional drug if the animal demonstrates signs of arousal. Animals which are sick or compromised may have a difficult time with these anesthetics due to changes in their ability to metabolize the drugs. Also, many of the commonly used injectable anesthetics are controlled substances which will require the laboratory comply with all rules regarding controlled substances and obtain a DEA license. OLAR does not provide controlled substances to laboratories for experimental procedures.

3) Monitoring and Recovery

- a. Animals should be monitored continuously, and parameters recorded at least every 15 minutes while under anesthesia. Animals must be fully awake (ambulatory, able to maintain body temperature, able to obtain food/water) before returning to their housing room. Parameters that should be monitored for rodents during anesthesia include:
 - i. **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)
 - ii. **Respiratory rate, depth and pattern:** assessed by observing chest wall and abdominal movements

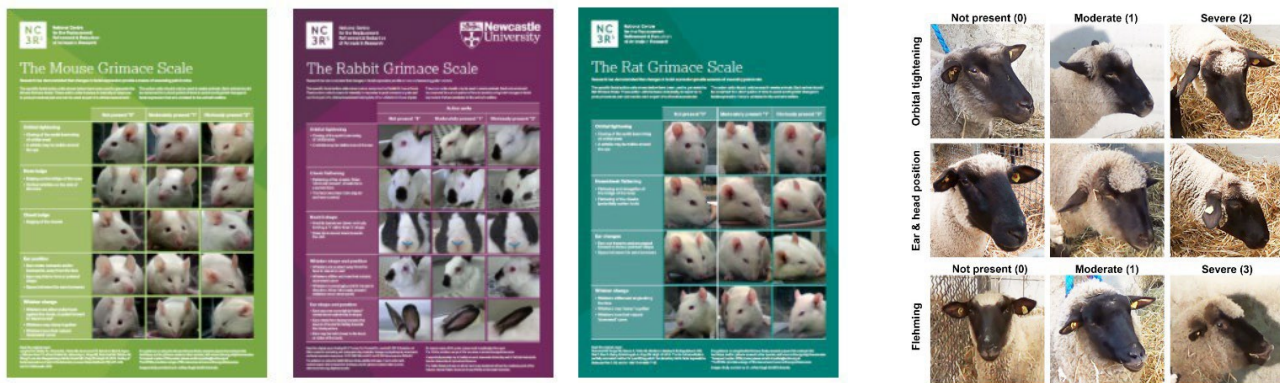
Analgesia

- 1) Assessing pain is difficult in animals. Some animals are species of prey and are adapted to hide signs of pain and distress. Clinical signs associated with pain are species specific,
- 2) Signs of pain/distress:
 - Hunched, scruffy, porphyrin staining (rats/mice)
 - Bruxism (ruminants)
 - Changes in activity, including less active or inactive, hyperactive or pacing, abnormal postures such as back arching
 - Belly pressing, wound guarding, or writhing
 - Decreased appetite (weight loss)
 - Isolation from cage/pen mates
 - Exaggerated or decreased response to handling
 - Vocalization
 - Changes in species specific behavior such as nest building (mice) can indicate pain or distress
 - Recently, there has been focus on assessing facial expression or grimace scale in order to assess pain (see Grimace Scale below). It is important to pay close attention to the animal's appearance and behavior post-surgery in order to observe subtle changes that may indicate the need for additional pain management.
- 3) Analgesia should be administered to **ALL** surgical animals unless otherwise justified in the protocol. It is recommended that the initial dose of analgesics is administered prior to the surgical procedure (i.e., pre-emptive analgesia). If there is concern regarding an animal's clinical condition post-surgery and additional analgesia is necessary, contact OLAR veterinary staff for further guidance on treating the animal.
- 4) Whenever possible multimodal analgesia is recommended. This involves providing a more 'balanced analgesia' through multiple methods or modalities. Local anesthetics at the incision site are often used in conjunction with a stronger opioid or NSAID analgesic.

Grimace Scale

Grimace scales for mouse, rabbit and rat can be found on the National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) website:

<https://www.nc3rs.org.uk/grimacescales>. You can also find examples of a sheep grimace scale online (see article link in References section).



Analgesia Recommendations based on procedure/model type:

Minor Surgery: Does not expose a body cavity or causes little or no physical impairment.

Major Surgery: Penetrates and exposes a body cavity, produces substantial impairment of physical or physiological function or involves extensive tissue dissection or transection.

Type of Pain	Severity	Examples	Analgesia	Duration
Surgical	Mild	Punch Biopsy, vascular cut down, simple skin incision	Pre-emptive dose	Local +/- NSAID
Surgical	Moderate	Head cap, craniotomy, subcutaneous procedures, castration, muscle incision	Pre-emptive analgesia + 24 hours post-procedure	Local + NSAID or Opioid
Surgical	Severe	Thoracotomy, Laparotomy, Orthopedic	Pre-emptive + 48 hours post-procedure	Local + Opioid; NSAID PRN
Visceral	Variable	Cancer, Inflammatory bowel syndrome, pancreatitis, peritonitis	Long-term management based on established scoring system	Opioids or NSAID based on model

Non-rodent species:

- 1) A protocol planning meeting with a veterinarian is required for all large animal surgical or invasive procedures which require anesthesia.
- 2) The veterinarian will provide recommendations on an appropriate anesthetic/analgesic protocol.
- 3) Species specific signs of pain will be covered during the protocol planning meeting with veterinary staff.

Mice and Rats Formulary

Table 1: Inhalant Anesthetic

Drug		Dosage
Isoflurane	Mice and Rats	4-5% for induction
		1-3% for maintenance

Table 2: Local Anesthetics

Agent		Dose	Duration
Lidocaine (1-2%) AND Bupivacaine (0.5% Marcaine) * RECOMMENDED	Mice and Rats	SQ incision site; do not exceed 0.5 mg/kg Lidocaine and 1.5 mg/kg Bupivacaine	Recommend mixing in same syringe; provides quick onset short duration PLUS later onset long duration (see below)
Lidocaine (1-2%) * used ALONE	Mice and Rats	SQ incision site; do not exceed 7 mg/kg	5-10 min onset, 0.5-1 hr duration
Bupivacaine (0.5% Marcaine) * used ALONE	Mice and Rats	SQ incision site; do not exceed 2 mg/kg	15-30 min onset, 4-8 hr duration
Liposomal Bupivacaine	Rats	1 mg/kg SQ incision site	Up to 4 days

Table 3: Injectable Anesthetics and Sedatives

Drug		Dosage & Route	Duration	Comments
Survival Surgeries				
Ketamine* / xylazine** Recommended	Mice	90-120 K/ 10 X mg/kg IP	30-45 min	Surgical anesthesia
	Rats	40-80 K/ 5-10 X mg/kg IP		
Ketamine* / xylazine** / acepromazine	Mice	100 K/ 2.5 X/ 2.5 A mg/kg IP	40-50 min	Surgical anesthesia
	Rats	40 K/ 8 X/ 4 A mg/kg IP		
Tribromoethanol (Avertin)	Mice	125-250 mg/kg IP	15-45 min	Single use only, adverse effects likely with repeat dosing. Product is non-pharmaceutical grade and use will require protocol justification.
	Rats	300 mg/kg IP		
Pentobarbital (Nembutal®)*	Mice	40-80 mg/kg IP	80-90 min	Sedation/immobilization, severe respiratory depression Dilute in pharmaceutical grade saline (<10mg/ml)
	Rats	30-40 mg/kg IP		

*DEA controlled substance

**Xylazine should NOT be re-dosed

Table 4: Anesthetic Antagonists (Reversal Agents)

Drug		Dosage & Route	Comments
Atipamezole	Mice and Rats	1 mg/kg SC	Reverses xylazine and medetomidines
		1-2.5 mg/kg IP or equal volume of dexmedetomidine	
Naloxone	Mice and Rats	0.01-0.1 mg/kg SC, IP	Reverses opioids
Yohimbine	Mice and Rats	0.5-1 mg/kg IP	Reverses xylazine and medetomidines

Table 5: Analgesics

Drug		Dose & Route	Duration
Opioids			
Buprenorphine*	Mice	0.05–0.1 mg/kg SC	4-8 hrs
	Rats	0.01-0.1 mg/kg SC	8-12 hrs
Buprenorphine SR-ER* (Wedgewood-compounded/requires script from veterinarian) (Available in multiple Concentrations)	Mice	0.6- 1.0 mg/kg SC	up to 72 hrs
	Rats	1.0 - 1.2 mg/kg SC	
Buprenorphine XR* (Ethiq®- FDA indexed in rodents, recommended) (1.3 mg/ml concentration)	Mice**	3.25 mg/kg SC	up to 72 hrs
	Rats***	0.65 mg/kg SC	up to 72 hrs
Non-steroidal Anti-Inflammatory			
Carprofen	Mice and Rats	5 mg/kg SC	24 hrs
Carprofen (bioserv®)	Mice and Rats	Dose based on manufacturer instructions PO	24 hrs

Meloxicam	Mice	1-2 mg/kg SC, PO	12 hrs
	Rats		12-24 hrs
Meloxicam SR	Mice	2-4 mg/kg SC	24 hrs[†]
	Rats		72 hrs

*DEA controlled substance

**Manufacturer does not recommend administering Ethiqva with NSAIDs concomitantly.

***Rats should be maintained on paper or soft bedding for 72 hours after administration

[†]**Mice metabolize meloxicam at a rate 20X higher than rats. Therefore, mice should be treated daily with meloxicam SR, as needed.**

Table 6: Neonatal Anesthesia Methods

Physical Anesthesia Method (Neonatal)	
Technique	Procedure
≤ 6 days	Place neonate(s) on a covered bed of crushed ice or on a paper towel-lined test tube in crushed ice/ice water. Neonates should not come in direct contact with ice.
Hypothermia	Animal should be warmed <i>slowly</i> on a 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 crawl.

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