

Laboratory Animal Resources Center		
Title: Guidelines for Evaluating and Treating Surgical Pain in Mice & Rats		
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Author: Marcel Perret-Gentil	LARC Director (Initials)	

Objectives

1. To establish guidelines for analgesic care of rodents and to facilitate the efficacy and safety of pain management in the perioperative setting. These guidelines are written in accordance with the Public Health and Safety *Guide for the Care and Use of Laboratory Animals (The Guide)*, and current/established veterinary practices.
2. To serve as a reference for UTSA investigators, IACUC and LARC personnel.
3. The present guidelines and associated categorizations of surgical pain are to be distinguished from the “Animal Use Pain and Suffering” categorization defined by the USDA and IACUC. Use the present guidelines to determine a suitable postoperative pain control plan for your experimental subjects. When describing the Animal Use category for your IACUC protocol, please refer to the USDA categories B-E.

Caveat

1. This guideline covers a wide range of species from rodents to larger species. For more rodent-specific recommendations refer to *Recommended Surgical Analgesic Protocols for Mice and Rats* (<http://vpr.utsa.edu/larc/training.php>) and rodent specific information at the end of this guideline.
2. All procedures performed on animals at UTSA must receive prior IACUC approval, including those described in this guideline.
3. Investigators should keep the following principles in minds:
 - a. The variability of individuals’ responses to surgery and analgesic therapies based on age, strain, experimental conditions, surgeon’s experience, etc.
 - b. The nature of clientele (investigative) – there will always be new surgical procedures not specifically addressed by these guidelines.
4. To overcome these obstacles, ongoing interaction between investigators and LARC veterinary staff is recommended.

Rationale for Assigning Pain Classifications to Surgical Procedures

The amount of pain produced by a surgical procedure is proportional to the *type of tissue* involved (Table 1), the *type of tissue damage* (pain receptors are stimulated by excessive pressure, stretch, heat or cold, and irritant chemicals including blood and hydrogen ions, which are released during ischemia and inflammation), and the *extent* of the tissue damage. An estimate of the degree of pain expected to be experienced during or after a procedure can also be based on the incidence and severity of pain-related signs observed in animals previously subjected to similar procedures, and on the degree of pain known to be experienced by humans undergoing similar procedures. Surgeries will be divided into two categories (*minor* and *major*) accordingly. The *minor* category will be subdivided (**A** & **B**) depending on duration of postoperative analgesia required.

Analgesia for Surgeries Producing Minor Pain

Minimum postoperative analgesic duration for minor pain:

Type A: 6-12 hrs or until signs of pain subside

Type B: 24 hrs or until signs of pain subside

Minor pain is generally associated with minor surgeries. According to the *Guide for the Care and Use of Laboratory Animals* (“*The Guide*”) “Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time.” For analgesia purposes, this list includes the following examples:

- Wound suturing (**A**)
- Peripheral vessel cannulation (**A**)
- Placement of peripheral vascular access ports (**B**)
- Placement of epidural catheters (**A**)
- Preparing a chronic gastric fistula (rat pups) (**A**)
- Placing small (non-disfiguring) subcutaneous implants (**A**)
- Craniotomy with minimal cranial or soft tissue manipulation (**A**)
- Skin incision of a non-noxious volume of a non-noxious substance e.g. into abdomen or viscera (**A**)
- Non-extensive skin biopsies (**A**)
- Stereotaxis – use intraaural topical bupivacaine drops (**A**)
- Extraabdominal orchiectomy (**A**)
- Thyroidectomy (**B**)
- Laparoscopy (*The Guide*) (**B**)
- Gonadectomies in rodents (**B**)
- Embryo transfers (**B**)
- Peripheral nerve transection (**B**)

- Non-extensive mouth and teeth surgeries (**A/B**)
- Carotid artery ligation (**B**)
- Intraventricular (brain) catheterizations (**B**)

Examples of suitable analgesic protocols for minor pain (Type A or B) (for specific doses and dose intervals, see tables below):

1. Apply topical local anesthetic (bupivacaine every 6 hrs) OR
2. Infiltrate wound with local anesthetic (bupivacaine every 6 hrs) OR
3. Systemic analgesic agent (buprenorphine; ketoprofen; carprofen or buprenorphine + NSAID) OR
4. Include analgesic agent in anesthetic plan e.g. ketamine, dexmedetomidine, xylazine. Check that its duration of action persists for an adequate time into the postoperative period.

Analgesia for Surgeries Producing Major Pain

Minimum postoperative analgesic duration for major pain: 48–72 hrs or until pain subsides.

Major pain is generally associated with major surgeries. According to *The Guide*, “as a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection.” For analgesia purposes, this list includes the following examples:

- Laparotomy (adrenalectomy, cesarean section, nephrectomy, catheterization of major abdominal or thoracic blood, duct or lymph vessels, hepatectomy, procedures that produce ischemia of abdominal organs, splenectomy, intraabdominal reproductive organ surgery, gall bladder surgery, urinary bladder surgery, bowel surgery, prostatectomy)
- Thoracotomy
- Thymectomy
- Craniotomy with extensive cranial or soft tissue manipulation
- Hypohysectomy (parapharyngeal and intraaural method)
- Pinealectomy
- Joint replacement
- Surgery to repair a fracture or bone defect
- Limb amputation
- Laminectomy with or without spinal cord injury
- Intraocular or corneal surgeries/manipulations
- Extensive ear surgeries
- Skin grafting
- Extensive mouth and teeth surgeries

Examples of suitable analgesic protocols for major pain (for specific rodent doses and dose intervals, see tables below and *Recommended Surgical Analgesic Protocols for Mice and Rats* (<http://vpr.utsa.edu/larc/training.php>):

1. Opioids, given by injection or oral route
2. NSAIDs, given by injection or oral route
3. Repeated infiltration/infusion of local anesthetic/opioid e.g., via subcutaneous, intrapleural or epidural catheter

Signs and Severity of Pain Associated with Surgical Procedures

Tissue or Site	Possible Signs of Pain	Severity of Pain	Duration of Pain
Skin	Rubbing; licking; biting; scratching	Punctures/incisions: <i>mild</i>	Prolonged
		Burns/inflammation/scarification: <i>moderate to severe</i>	
Muscle	Reluctance to move, lameness, rapid respirations	<i>Mild to severe</i> , depending on site and extent	Determined by extend of surgery
Viscera	Reluctance to move; abnormal posture, biting or kicking abdomen; rolling, writhing, guarding	Mild to severe, depending on organ and type of injury e.g. distention, obstruction, ischemia and inflammation cause greatest pain.	Determined by extend of surgery
Bones, joints	Reluctance to move, lameness, stiffness, guarding, licking, biting, self-mutilation	Bones (esp. femur and humerus): <i>moderate to severe</i>	Intermittent
		Joints: <i>mild to severe</i> . Greatest with inflammation	
Spine	Cervical: reluctance to move, especially head, standing with head down, stiff gait	Cervical cord: <i>moderate to severe</i>	Continual
	Thoracic/lumbar: few signs	Thoracic/lumbar cord: usually <i>mild to moderate</i>	Determined by extend of surgery
Nervous system	Acute injury: none Post-injury neuropathy: licking, biting, self-mutilation	Acute: <i>none</i> Post injury neuropathy: nonpainful abnormal sensations: <i>mild to moderate</i> pain.	Short
Thorax	Reluctance to move; stiff gait; rapid or shallow respirations	<i>Moderate to severe</i>	Continual
Rectal area	Rubbing; licking; biting; abnormal excretory behavior	<i>Moderate to severe</i>	Intermittent to continual
Abdomen	Arched back, hunched posture, guarding; anorexia	<i>Moderate to severe</i> after extensive surgery.	Short
Eye	Rubbing; pawing or scratching at eye; blepharospasm; lids closed,	Intraocular/corneal injury: <i>moderate to severe</i>	Intermittent to continual

	porphyria (red staining) around eye		
Ear	Rubbing; pawing or scratching at ear; head shaking; head tilt	<i>Moderate to severe</i>	Intermittent to continual
Mouth and teeth	Anorexia, abnormal chewing, excessive salivation, rubbing, scratching	<i>Mild to severe</i>	Intermittent to continual

Adapted from Recognition and Alleviation of Pain and Distress in Laboratory Animals. Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences. National Research Council. National Academy Press, Washington, DC. 1992.

Physiological and Biochemical Changes Associated with Pain*

Physiological changes	Biochemical changes	
	Increased plasma conc.	Decreased plasma conc.
Cardiovascular Vasoconstriction (elevated blood pressure) Increased heart rate Increased stroke volume Increased cardiac output	Epinephrine, Norepinephrine Cortisol, corticosterone Glucose Glucagon Sodium Endorphins, enkephalins	Phosphorus, Magnesium, Testosterone, Insulin
Respiratory Rapid, shallow respiration Decreased tidal volume Hypoxemia Hypercapnia	Lipotropin Substance P Amino Acids Lipids Ketones	
Peripheral blood count Lymphopenia Eosinophilia Neutrophilia	Renin, Angiotensin 2, aldosterone, vasopressin, ADH	

* Adapted from information in ACLAM textbook series *Anesthesia and Analgesia in Laboratory Animals*. Edited by Kohn, Wixson, White and Benson.

Advantages of Using Analgesics

- Humane care of research animals
- Improved food and water intake
- Improved respiration
- Less stress resulting in better immune function and fewer infections

- Improved “self-maintenance” behavior e.g. grooming
- Greater mobility, so less muscle atrophy, pressure sores

Adverse Effects of Analgesic Agents

Although generally beneficial, analgesic agents may produce side effects in individuals. Often, these can be corrected by adjusting the dose of drugs. Alternative methods of analgesia may be required in some cases. Consultation with the university veterinary staff is advised if difficulties arise.

- Sedation – less food, water intake (opiates, xylazine)
- Suppression of breathing reflexes (opiates, xylazine), especially if in combination with other analgesic or anesthetic agents. For example pentobarbital anesthesia and buprenorphine should on the same subject should be used with caution, as the combined respiratory depressive effects of these two agents could be severe
- Constipation, urinary retention (opiates)
- Gastric irritability, kidney toxicity (NSAID's)
- Abnormal behaviors (opiates)
- Excitement, increased locomotor activity (opiates, local anesthetics)
- Cardiac arrest (local anesthetics, all if excessive)

Commonly Used Systemic Analgesics in Mice and Rats

	Mouse	Rat	Class
Buprenorphine*	0.05-0.1 mg/kg SC q8-12h	0.01-0.05 mg/kg SC q8-12h	Opioid
Oxymorphone	0.2-0.5 mg/kg SC q4h	0.2-0.5 mg/kg SC q4h	Opioid
Morphine	1-2.5 mg/kg SC q2-6h	1-2.5 mg/kg SC q2-6h	Opioid
Ketoprofen	2-5 mg/kg SC q24h	2-5 mg/kg SC q24h	NSAID
Carprofen	2-5 mg/kg SC q12-24h	2-5 mg/kg SC q12-24h	NSAID
Meloxicam	1-2 mg/kg SC, PO q12h	1-2 mg/kg SC, PO q12h	NSAID
Flunixin	2.5 mg/kg SC q12h	1.1-2.5 mg/kg SC q12h	

*Buprenorphine is the only opioid with long duration effect in rodents.

NSAID: Non-steroidal anti-inflammatory drug.

NSAID dosing caution:

1. Ensure that animals are adequately hydrated (skin pinch test, or serum Total Protein test) before administering an NSAID to avoid renal damage.
2. NSAIDs must be used with caution beyond 3 days as it may have deleterious effect on the gastrointestinal mucosa. This may be especially true when using ketoprofen and flunixin.

Opioid dosing caution:

1. Opioid agents enhance sedative and respiratory depressive effects of anesthetics.
2. For rodents anesthetized without respiratory support (intubation, ventilation and oxygen supplementation), you may wish consider opioid administration until the end of surgery.
3. In this case, an NSAID may be the preemptive analgesic of choice
4. If ventilatory support can be provided and an opioid is used as a preemptive analgesic agent, expect to reduce the dose of the injectable or gas anesthetic agent by 30-50%.
5. Some opioids lower body temperature and supplemental heat may be necessary. However, supplemental heat should be provided during anesthesia and recovery regardless of the anesthetic protocol used.

Oral dosing caution:

1. Animals should be acclimated to oral medications before surgery. When added to the drinking water, rodents will initially refuse to drink until they become adjusted to the flavor, which could be disastrous postoperatively.
2. When used in drinking water, analgesics should generally be administered 5-7 days prior to the anticipated pain insult.
3. When other forms of oral dosing is given, e.g., tablets or pellets supplements, they should be administered in a palatable formulation (such as Bacon Softies from Bio-Serv, <http://bio-serv.com/>). The supplement should be started at least 2-3 days prior to surgery and the researcher should verify that the animals are consuming the supplement.
4. Consideration to the use of analgesics in drinking water or in supplements must take into account that postoperatively, animals may decrease fluid and food intake and may therefore not receive the intended analgesic dose.

Preemptive Analgesia Concepts

Preemptive analgesia is the prevention of pain before the actual pain insult occurs. As an adjunct to general anesthesia, a local anesthetic may be used to desensitize a body area before making an incision. This reduces the pain of the surgical wound postoperatively and during the healing process. Preemptive analgesia is also accomplished by administering a systemic analgesic before the pain develops (e.g. before the surgical incision is made). Generally, analgesics are more effective when administered prior to surgery.

Much of the post surgical pain is the result of the sensations produced in the skin and body wall of the incision area. Anesthesia of the local nerves prior to the incision will greatly reduce post-op pain and lead to improved recovery.

When the skin and tissues are incised, local sensory nerves become excited and transmit impulses to the brain that are interpreted as pain. During general anesthesia, the animal is unconscious and is unable to perceive the neural stimulations from the incision site and so is unaware of painful sensations. However, when the anesthetic has worn off, the brain will process these neural excitatory impulses, which continue postoperatively for days until the incision is healed. The result is that the surgical wound is painful and sensitive to touch and movement.

A local anesthetic infiltration prior surgery will block or diminish the sensory neuroexcitation caused by the incision and trauma to tissues. When the animal wakes up, it will have a relative reduction in sensory stimuli from the incision area, and pain of the surgical wound will be decreased both initially and throughout the period of wound repair.

An effective, inexpensive and easy to administer local analgesic cocktail of lidocaine and bupivacaine may be used. The incision site and underlying tissues may be infiltrated with lidocaine 1-2% /bupivacaine 0.25-0.5% preoperatively (preferred) or intraoperatively. Lidocaine provides almost immediate pain control for 20-40 minutes while bupivacaine provides longer pain management for up to 4-6 hours. Since bupivacaine tends to sting upon injection, we recommend injecting it after the animal is anesthetized. Generally, major surgery requires systemic analgesics, as the lidocaine/bupivacaine infusion only provides pain management to the incision site. Lidocaine and bupivacaine doses should not exceed 10 and 6 mg/kg respectively. Higher doses may lead to cardiac arrhythmias.

Neither drug (lidocaine or bupivacaine) requires DEA registration.

	Onset	Duration	Do not exceed (toxic dose)
Lidocaine	1-3 minutes	20-40 minutes	10 mg/kg
Bupivacaine	~20 minutes	4-6 hours	6 mg/kg

Adjuvants: Adding epinephrine (1:50:000 to 1:200,000) to plain solutions of local anesthetics just before administration shortens the onset time and prolongs the duration of action. A 1:200,000 dilution is obtained by adding 0.1 ml of 1:1000 epinephrine (with a tuberculin syringe) to 20 ml of local anesthetic. Epinephrine should not be used for peripheral nerve blocks in areas with poor collateral circulation e.g., digits, tails. Use caution if patient has cardiac problems.

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