NIDA-IRP ACUC

# Analgesia Guidelines

Most recent ACUC Approval: August 2018

## Introduction

The NIDA ACUC is responsible to ensure that all animals within the intramural program are used humanely and that usage complies with NIH guidelines and federal regulations and policies. This NIDA guideline supplements the NIH-ARAC Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation in assisting Principal Investigators (PI) to anticipate the pain and distress potential of proposed animal procedures, to evaluate animals for the presence of pain and distress, and to minimize significant pain and distress. Pain and distress can develop consequent of any experimental procedure (i.e., post-procedural pain) including drug treatment, experimentally applied stressors, and surgery (i.e., post-surgical pain).

The PI is obligated to ensure that research staff comply with the Animal Care and Use Committee (ACUC)-approved Animal Study Proposal (ASP) and execute the ASP's plans for anesthesia and analgesia activities. Failure to adhere to the ASP, failure to monitor animals post-procedurally as necessary to ensure well-being, failure to maintain appropriate animal-related records, and implementation of significant change to approved protocols without prior ACUC approval, are examples of non-compliance which require NIH to report non-compliance to the Office of Laboratory Animal Welfare (OLAW).

## Guidelines

### I. Animal Care and Use Committee- Animal Study Proposal (ASP)

The PI will provide sufficient detail in the ASP for the ACUC to evaluate the degree of anticipated or potential pain or discomfort, to evaluate the necessity and adequacy of anesthesia and analgesia usage, and to evaluate the necessity to perform procedures which can result in pain or distress.

A. Two approaches are used for determining the need for analgesics: one uses anthropomorphic criteria, based on knowledge of the degree of pain experienced by humans after comparable procedures, and the other uses behavioral criteria, based on knowledge of normal behavior of the species, and preferably the individual animal, to determine whether analgesics are needed.

1) The ACUC will evaluate the potential of pain or distress in the proposed study following the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training statement that unless the contrary is known or established, investigators should assume that procedures which cause pain or distress in humans probably cause pain or distress in animals.

2) The use of behavioral criteria relies on post-procedural monitoring by research staff for signs of pain or distress to determine the need for analgesics.

**B. ASP Sections F and G.**

The PI will provide sufficient detail in the ASP for the ACUC to evaluate the adequacy of plans for a) post-procedural monitoring, b) treatment to address pain and discomfort and c) documentation of observations, analgesic use, and treatments.

1) In ASP Section "F. Description of Experimental Design and Animal Procedure", the PI should describe anticipated effects from experimental (e.g. pharmacological, behavioral, etc) manipulation of animals and describe the alternative humane endpoints.

2) In ASP section "G. Survival Surgery", the PI should describe the post- operative care procedures and address minimization of pain and distress.

**C. ASP Section H.**

In ASP section "H. Pain or Distress Category", the PI will describe how it was determined that alternatives to procedures that may produce more than momentary or transient pain or distress and are not available, and the PI will tabulate the number of animals which will be subject to the following pain categories.
Category C: **Minimal, transient, or no pain or distress**
Category D: **Pain or distress relieved by appropriate measures**
Category E: **Unrelieved pain or distress**

Guidance and examples of procedures and associated pain categories can be found Guidelines for Assigning Pain and Distress Categories in Research Animals.

Category E: With ACUC approval, the PI may withhold medical treatment to prevent or relieve potential or expected post-procedural/post-surgical pain or distress. Pain category E activities typically include studies on pain itself for which analgesic drugs are withheld. The PI must provide a justification and provide scientific reasons for assertions such

as:

a) The use of anesthetic, analgesic or tranquilizing drugs would interfere with the research objectives, and,

b) Harm/'benefit analysis; the benefit of scientific finding justifies harm inflicted to the animals using the proposed model/procedures.

Since ASPs are available to the public through the Freedom of Information Act (FOIA), please use layman's terms when writing the justification for withholding measures to mitigate pain and distress. For studies involving rats and mice the justification should be provided in Section F. For studies with USDA regulated animals such as Non-human primates the justification must be provided in the ASP Attachment 2. "Explanation for Column E listing (Attachment Two)" for inclusion in the NIH Annual Report to the United States Department of Agriculture (USDA).

## II. Procedures for Minimizing Pain and Distress

### A) Maintenance of Body Temperature

Preventing heat loss during the procedure is most desirable and is accomplished using materials to insulate the animal from any cold surfaces. Rodents will lose heat quickly due to their large surface area/ body weight ratio. Recovery from anesthesia may be prolonged if animals are hypothermic especially when injectable anesthetics are used. The use of supplemental heat sources are recommended during and following procedures such as water re-circulating heating pads. Heating lamps have the potential to produce too much heat and therefore must be carefully monitored as animals can gain heat quickly and overheat.

Animals should be recovered in a clean, dry, comfortable area. Animals should not be returned to the facility until they have sufficiently recovered from anesthesia and remain able to right themselves from lateral recumbency.

### B) Supportive Fluids

Post-operative recovery is aided by the administration of warmed physiologic fluids (such as sterile saline or lactated ringers solution) to compensate for water loss and reduced water consumption post operatively. This is especially important with lengthy procedures or procedures in which there is any blood loss. Fluids can be warmed in a hot water bath. Suggested fluid volumes for each species:

| **Route** | **Mouse 30gm** | **Rat 300gm** | **Sq monkey 600-900gm** | **Rhesus monkey** |
|---|---|---|---|---|
| Subcutaneous | 1-2 ml | 5-10 ml | 20-30 ml | 30 ml/kg |
| Intraperitoneal* | 0.6 ml (2.4) | 3.0 ml (6.0) | | |
| Intravenous (1 minute)* | 0.15 ml | 1.5 ml | 2.0 ml | |
| Oral gavage* | 0.3 ml (1.5) | 3.0 ml (12) | 8.0 ml (12) | |

* Based on dosages from *J. Appl. Toxicol.* ***21****, 15-23 (2001)*, published maximum ml in parenthesis ( ).

## C) Use of Analgesics

Analgesics should be given as early as possible; the use of more than one class of analgesic agent will be more effective; and the doses and duration of action should match the expected pain and distress from surgery. The timing of analgesic intervention has a significant bearing on the intensity of post- operative pain. (Pain Management in Animals- Flecknell)
Analgesics given preemptively prevent CNS sensitization, reducing post-operative pain intensity and decreasing post-operative analgesic requirements. for periods much longer than the normal duration of action. (Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research-NRC).
The use of appropriate analgesics should hasten return recovery to normal food/water consumption and behavior.

There are three classes of analgesics commonly used in rodents:

- Local anesthetics
- NSAIDS (non-steroidal anti-inflammatory drugs)
- Opiates.

**Local anesthetics** are most effective when administered by subcutaneous infiltration prior to making the incision. Local anesthetics block the action potential of the nerve fibers therefore preventing the transmission of nocioceptive input to the CNS. Use of local anesthetic prevents post-operative "wind-up" pain (hyperalgesia) resulting from chronic nerve stimulation while under general anesthesia alone.
Caution: Local anesthetics can be cardiotoxic in high doses. Bupivacaine and Lidocaine should be diluted especially when used in mice.

**NSAIDS** are best given immediately post-operatively. The use of some NSAIDS pre-operatively can potentially precipitate acute renal failure. NSAIDS should not be used for prolonged treatment.

**Opiates** given well in advance pre-operatively may augment anesthesia or may be given post operatively following recovery. Opioids can depress respiration so should not be given while the animal is still under anesthesia.

The following is an abbreviated formulary of recommended analgesia drugs and regimens for rodents. (PIs should consult with the attending veterinarian during ASP development to devise anesthesia and analgesia plans for NHPs.)

**1) Common Analgesia Regimens (Rodents)**

SC= subcutaneous, PO= orally, IM=intramuscular

| Drug Name | Suggested Dose (mg/kg) | Route | Frequency or Schedule | Supplied Concent |
|---|---|---|---|---|
| **Local anesthetics** | | | | |
| Bupivacaine 0 .25% or 0.5% (Marcaine) | Apply directly or dilute to 0.25% for injection, do not exceed 8 mg/kg total dose. Note: 1% = 10 mg/ml | SC | Infiltrate under the skin prior to skin incision or apply to incision | 2.5 mg/ml and 5 mg/ml |
| | | Instill in ear canal | Prior to insertion of ear bars | |
| Lidocaine 1% or 2% | Dilute to 0.5% for injection, do not exceed 7 mg/kg total dose | Same as above | Same as above | 10 mg/ml and 20 mg/ml |
| **NSAIDs** | | | | |
| Meloxicam | 1 mg/kg | SC | Once daily | 5 mg/ml |
| Carprofen | 5 mg/kg | SC PO | Once daily | 50 mg/ml 25 mg/tablet |
| Ketoprofen | 2.5-5 mg/kg | SC | Once daily | 100 mg/ml |
| Ibuprofen (Advil) | 10-30 mg/kg/day | PO | Self-administered | 20 mg/ml suspension |
| Acetaminophen (Tylenol pediatric elixir)** | ~ 200 mg/kg/day or 450mg/100ml drinking water (70 ml suspension per | PO | Self-administered through drinking water | 32 mg/ml suspension |
| **Opiates** | | | | |
| Buprenorphine (Buprenex) *see caution below | 0.05-0.10 mg/kg mice 0.01-0.05 mg/kg rats | SC | Every 8-12 hrs | 0.3 mg/ml |

* Caution: Buprenorphine can cause respiratory depression and death if given prior to recovery from anesthesia

** Literature regarding efficacy of acetaminophen in drinking water is not conclusive; consult with veterinarian for alternative analgesia if treatment appears to be not providing adequate analgesia.

**2) Treatment Plans for Analgesia**

Surgical procedures are the most common type of procedure requiring analgesia.. Major surgery is defined as that which penetrates and exposes a body cavity or produces substantial impairment of physical or physiological function. Major surgeries are more likely to produce post-operative pain than minor surgeries.
The following are the most common surgical procedures performed at NIDA with recommendations for analgesia.

**A. Intravenous catheter implantation with subcutaneous routing of the catheter** is the most common minor rodent surgical procedure and generally produces minimal post-operative pain.

i. Potential therapeutic approaches include use of local anesthetics and/or NSAIDs.

ii. Local anesthetics such as lidocaine or longer acting bupivacaine (Marcaine) are administered by subcutaneous infiltration prior to the initial incision to preempt pain signaling to the CNS and reducing post- operative pain sensitization. They may also be applied directly to the open incision (cut surfaces) at the time of surgery and/or prior to closure.

iii. NSAIDs can be administered by several routes. A regimen which provides 12-24 hours of analgesia activity is commonly sufficient.

**B. Intracranial (craniotomy) surgery** is the most common major rodent surgical procedure. Craniotomies involve cutting the scalp skin, displacing or removing the overlying muscle and periosteum, drilling the calvarium, and infusion of drugs or implanting guide cannulae or microelectrodes and inserting bone screws to secure a dental acrylic "cap" to hold cannulae, electrodes, or hardware to exteriorize intravenous catheter or to attach a spring arm. These types of manipulations are likely to produce pain in humans, but the lack of behavioral changes in rodents after intracranial surgery suggests they do not experience more than minimal pain.

i. Local anesthetic infiltration of the surgical site or judicious topical application to open tissue, as described above, is acceptable as a post-operative analgesic treatment for simple routine intracranial surgeries. Human brain surgery is frequently conducted with only local anesthetics with no significant pain to the human patient since there are no pain receptors inside the brain, only in the dura and other

membranes lining both sides of calvarium.

ii. If the intracranial procedure involves extensive periosteal tissue dissection or large head mounts, consideration should be given to providing additional analgesia such as an NSAID (a single dose or 24 hours orally) or single dose of opiate (buprenorphine).

iii. Commonly, intracranial procedures require placement of ear bars for stereotaxis. The tips of the ear bars may be blunt or taper to a narrow point. Tapered ("sharp") ear bars frequently produce trauma to the ear canal and tympanic membrane; if tapered ear bars are used 1-2 drops of bupivacaine or lidocaine must be instilled into each ear canal prior to insertion of the ear bars to reduce trauma associated pain.

**C. Other major surgeries.** For more invasive intracranial procedures, e.g. stroke model surgery, or procedures involving opening the abdominal cavity, additional post-operative analgesia should be provided. The use of a combination of a local anesthetic along with the use of NSAIDs or opiate should be provided and for a longer duration of 48 hours post-surgery.

i. Local anesthetics plus NSAIDS or opiates**:** The NSAIDs should be administered after major survival surgery by the dose and route in the tables above.

ii. If not contraindicated by the scientific goals of the study, buprenorphine may be administered up to every 8-12 hours for the first 24 hours followed by an NSAID.

**D. Recognizing Pain and Distress in Rodents**

Research personnel should be able to identify these health and behavioral changes that can relate to post-procedural pain and distress in rodents:

**1) Mild, moderate, and sometimes profound pain might be expressed by:**

i. Unusual postures during ambulation or at rest, e.g., hunched up, nose touching ground, head pressing against the cage.
ii. Matted or dull hair coat because they have stopped grooming
iii. Altered temperament e.g. increased aggression, biting.
iv. Vocalization upon handling or applying pressure to the affected area.
v. Tensing or tightening of the abdominal muscles when touched or held

**2)** Redness and swelling around a surgical site.More profound or significant pain might be expressed by:

i. Shallow and rapid breathing, abdominal breathing, open- mouth breathing.
ii. Grinding teeth.
iii. Bulging, pale eyes; red-stained eyes and nose (in rats)
iv. Reduced consumption of food or water or refusal to eat or drink with consequent reduction in fecal output and weight loss.
v. Dehydration, skin tenting, sunken eyes.
vi. Muscular tremors (a sign of deep pain), muscle rigidity, lack of muscle tone.
vii. Restlessness, self-imposed isolation or hiding.
viii. Self-mutilation, breaking skin on limbs.

## III. Assessment, Monitoring, and Recordkeeping.

### A. Assessment

The animals should be observed at least once daily for the following.

i. Level of activity (mobility, nose in the air, rearing).
ii. Normal ambulation and posture
iii. Signs indicating pain listed above
iv. Body weight relative to pre-procedure weight
v. Hydration status by skin turgor (looseness of skin)
vi. Presence of fresh feces in bedding
vii. Appearance of the surgical site (redness, swelling, self- trauma, intactness)

### B. Monitoring

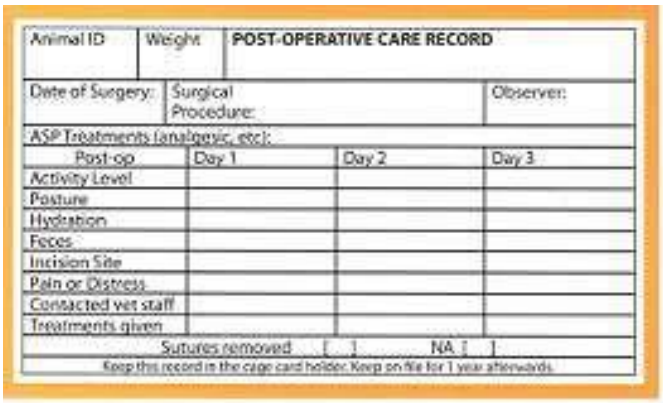
The PI will ensure that their research personnel can recognize health and behavioral changes that can relate to post-procedural pain and distress in rodents. Research personnel will perform regular, daily health and behavioral assessments related to signs of post-procedural pain and distress for the first 3 consecutive days, including weekends or holidays. .

Post-surgical monitoring will also include attention to the surgical incision site for dehiscence, post-surgical infections, and timely removal of skin sutures or wound clips (7-14 days). Contact the veterinary staff if animals are not recovering as expected.

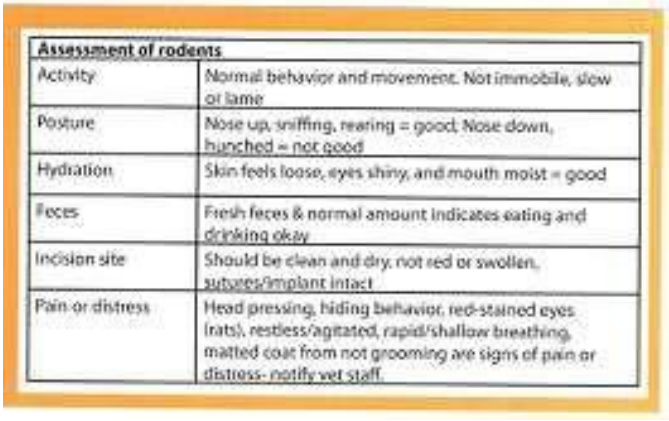
### C. Recordkeeping (Rodents)

The Post-Operative Care cage cards will be used for documenting 3 days of post-operative care and completion of ASP prescribed

treatment plan (e.g. analgesics) The PI's research staff is responsible for completing the cards, using one card for each animal. The post-operative cage card(s) should be kept on the cage card holder with the rodent(s) until the end of the study.If pain or distress or illness is observed the veterinary staff should be notified.



| Animal ID | Weight | POST-OPERATIVE CARE RECORD | |
|---|---|---|---|
| Date of Surgery: | Surgical Procedure: | | Observer: |
| ASP Treatments (analgesic, etc): | | | |
| Post-op | Day 1 | Day 2 | Day 3 |
| Activity Level | | | |
| Posture | | | |
| Hydration | | | |
| Feces | | | |
| Incision Site | | | |
| Pain or Distress | | | |
| Contacted vet staff | | | |
| Treatments given | | | |
| Sutures removed [ ] NA [ ] | | | |
| Keep this record in the cage card holder. Keep on file for 1 year afterwards. | | | |



| Assessment of rodents | |
|---|---|
| Activity | Normal behavior and movement. Not immobile, slow or lame |
| Posture | Nose up, sniffing, rearing = good; Nose down, hunched = not good |
| Hydration | Skin feels loose, eyes shiny, and mouth moist = good |
| Feces | Fresh feces & normal amount indicates eating and drinking okay |
| Incision site | Should be clean and dry, not red or swollen, sutures/implant intact |
| Pain or distress | Head pressing, hiding behavior, red-stained eyes (rats), restless/agitated, rapid/shallow breathing, matted coat from not grooming are signs of pain or distress- notify vet staff. |

Post procedural care must follow what is written in the ASP. Any need or deviations or omissions should be discussed with the veterinary staff.

Lack documentation implies failure to provide adequate medical care or to follow ASP prescribed treatment plan and ACUC may need to investigate and report to OLAW.

## IV. Veterinary Assistance

The NIDA ACUC, following the requirements of the Animal Welfare Act, charges the attending veterinarian with the responsibility to provide research personnel with working guidelines and advocates the use of professional judgment concerning the choice and use of analgesic and anesthetic drugs. Contact the Animal Program Director (APD) for additional guidance regarding specific indications for use of these guideline's or alternate analgesics.

A. Contact the facility veterinarian or veterinary technician anytime there is a question about whether an animal is experiencing pain.

B. The veterinarian may prescribe anesthetic and analgesic regimens for clinical care of specific animals. Whenever possible, the facility veterinarian will notify investigators prior to the administration of clinically indicated analgesics whenever possible since analgesic treatment could confound the experimental usefulness of the animal leaving euthanasia as a reasonable alternate choice.

C. If the APD determines that ASP plans requires change to meet actual requirements, the PI is required to modify the ASP to assimilate any significant changes in anesthetic and analgesic monitoring and treatment regimens for ACUC review and approval.