CHAPTER 10

PAIN, DISTRESS, ANALGESIA AND ANESTHESIA

I. How and by Whom are Levels of Pain and Distress Assessed and Categorized

The levels of pain and distress are initially categorized by the investigators as they complete the protocol form. The IACUC then determines how appropriate their selections are during protocol review. Criteria for assessing pain in different species differ since species vary in their response to pain. It is essential that the personnel caring for and using animals be familiar with species-specific behavioral, physiologic and biochemical indicators of well-being. In general, unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals.

II. IACUC Guidelines for Avoiding Unnecessary Pain or Distress

The IACUC has established guidelines on humane endpoints to ensure that unnecessary pain or distress does not occur. Study staff must know the IACUC approved endpoints for their respective studies. Endpoints are posted in all ARC animal rooms.

III. Training and Experience of Personnel Performing Anesthesia

Individuals performing anesthesia should be trained by both the investigator and the veterinarian. In addition to in-person training, a Canvas course is assigned to each study staff member who will perform surgery. The Canvas course contains videos and quizzes that must be completed as well as additional resources.

IV. General Considerations of Anesthesia

A. What is Anesthesia?

Anesthesia is a state of unconsciousness induced in an animal. The three components are analgesia (or pain relief), amnesia, and immobilization

B. Pre-anesthetic Considerations

1. Animals should be allowed a week to acclimate to a new environment after transportation prior to anesthesia if possible.
2. Assess the animal prior to anesthesia to form a baseline for comparison in the postoperative period.
3. It is not necessary to withhold food and water from rodents since vomiting does not occur in these species.

C. Monitoring Anesthesia

1. Response to Pain
    a) Pedal withdrawal reflex (toe pinch): using fingernails, pinch web of skin between animal's toes. If the limb is withdrawn, the muscles twitch or the animal cries out the depth of anesthesia is insufficient.
    b) Tail pinch in small rodents: use as a substitute for pedal reflex.

2. Alteration in Eye Reflexes
    a) Palpebral reflexes: touching the eyelids causes blinking. The animal is light if it is blinking. Difficult to assess in small rodents
    b) Corneal reflex: touching the cornea of the eye with a tuft of cotton causes a blink. The animal is too deep if it has lost this reflex.

3. Alterations in Respiratory functions
    a) Rate, depth and pattern can be monitored by watching the chest wall.

4. Alterations in the Cardio-Vascular System
    a) Rate, rhythm and quality of peripheral pulse.
    b) Stethoscope for heart sounds and rate.

5. Alterations in Blood Pressure
    a) Quality of pulse: use femoral artery.
    b) Capillary refill time: blanch gums with digital pressure.

6. Alterations in Temperature
    a) Rectal temperature with clinical thermometer.

7. Muscle tone
    a) Decreases as depth of anesthesia decreases, unless using cataleptic drugs; Test by pulling on the lower jaw or a limb. Rigid tone indicates inadequate depth of anesthesia.

D. Anesthetic Problems and Emergencies

1. Respiratory System

Most anesthetics cause direct depression of the respiratory center in the brain and reduce ventilation. When an animal is in lateral recumbancy the lung that is down is being compressed by the rest of the body. In dorsal recumbancy the animal may have depression of the diaphragm by abdominal viscera. The airway can be compromised by regurgitated food or tracheal or pharyngeal secretions due to normal reflexes being lost during anesthesia.

a. <u>Signs of impending failure</u>

- Rate: in rodents a decrease to less than 40 % of pre-anesthetic rate indicates impending failure. Increased rate may indicate lightening of level. Blue mucous membranes indicate a lack of oxygen.

b. Correction:
   - Extend head and neck; open mouth and pull tongue forward.
   - Ventilate with 100% oxygen if possible or assist ventilation by manual compression of thorax- thumb and forefinger in rodents.

c. Prevention
   - Pre-medication with atropine or glycopyrrolate (anticholinergics) may reduce respiratory tract secretions in some animals.
   - Intubate the trachea when possible to prevent aspiration pneumonia and allow you to assist respiration.

2. Cardiovascular System

Many anesthetics have direct effect on the heart or vasculature, decreasing cardiac output and blood pressure.

a. Signs of impending failure
   - Decreased capillary refill time; blanching of mucous membranes; limbs cool to touch.

b. Correction
   - Ensure unobstructed airway
   - Ventilate with 100 % oxygen or with a face mask or with intermittent compression of chest (gentle and rapid compression between thumb and forefinger for rodents)
   - For complete arrest: In rodents- easier to compress all areas of thorax simultaneously and combine cardiac massage and assisted ventilation.

c. Prevention
   - Place an intravenous catheter whenever possible to provide access for fluids.
   - Supplemental fluids, IV, IP or SC; Rate of 5-10 ml/kg/hour; Monitor for over-hydration or under-hydration.

3. Thermoregulation

Anesthesia frequently causes hypothermia because of inhalation of cold gasses, exposure of body cavities to room air and loss of normal thermoregulatory mechanisms and behaviors.

a. Prevention
   - Monitor body temperature.
   - Prevent heat loss by insulating cold surfaces.
   - Supplement heat with a thermal blanket.

V. Analgesia

To comply with PHS policies on minimizing pain and distress in laboratory animals, analgesics are required for all vertebrates, including rodents that are subjected to a painful procedure. Analgesia should be provided to animals if they undergo a procedure that is likely to cause pain or distress, unless specific exception is granted by the IACUC based on scientific justification. A painful procedure is considered to be any procedure that, in the absence of evidence to the contrary, would cause pain in a human. Pain in rodents is usually manifested as decreased activity, grooming or food and water consumption, guarding behavior (e.g. limping or a hunched posture) or increased aggression. The analgesic should be selected based on the level of pain expected. Ophthalmic, orthopedic and visceral procedures will result in higher levels of post-operative pain than will skin incisions, catheterizations, blood collection or subcutaneous implants.

Analgesics should be administered pre and postoperatively. If administered pre-operatively they can reduce the dosage of anesthetic needed by 10 –15%. It is not appropriate to wait until the signs of pain or distress are demonstrated before administering analgesics since monitoring for clinical signs of pain may be an unreliable method in rodents post anesthetic administration due to normal changes produced by the anesthetics themselves.

A. Recognition of pain

1. Activity: reduced level or unusual restlessness; altered gait; altered posture
2. Appearance: lack of grooming; unusual posture
3. Temperament: aggressive or apathetic
4. Vocalizations: pitch of cry abnormal
5. Feeding: food and water intake are reduced; weight loss
6. Alterations in physiological variables: change in pattern and rate of respiration; cardiovascular system may be altered; severe pain may cause shock.
7. A “1” or “2” on a grimace scale (posted in appropriate species rooms)

VI. Anesthetic/Analgesic Agents

Federal Regulations require that guidelines and consultation be provided to research personnel on the type and amount of anesthetics, analgesics and tranquilizers used for each species of animal. Anesthetic agents and doses can be found on UWM’s Animal Care Program website.

A. Inhalational anesthetics

Isoflurane is the first choice of anesthetic used for animal restraint or surgical procedures in laboratory animal species. Isoflurane is delivered via a nose cone and inhaled in rodents or provided through an intra-tracheal tube in larger species. The concentration of drug can be administered to effect by adjusting the percent of displacement of oxygen with a precision vaporizer and compressed oxygen.

Anesthetic chambers can be used for high vapor pressure anesthetics, however there is great risk of overdose.

Advantages: Rapid induction and recovery. A precision vaporizer provides the ability to precisely titrate the level of anesthesia during a procedure. Liquid Isoflurane is not a DEA controlled drug and is non-explosive and non-flammable.

Disadvantages: Upfront cost associated with a precision vaporizer; requires either passive or active scavenging of waste and exhaled anesthetic gas; occupational health exposure to anesthetic gas should be limited; prolonged analgesic effect is not achieved after the animal is awake; depressed respiratory rate and decreased blood pressure.

Additional notes: The duration of anesthesia can be easily adjusted for a variety of procedures ranging from 30 seconds up to many hours. Concurrent use of analgesics such as opioids or NSAIDs is encouraged as Isoflurane has no analgesic properties once the animal is awake from the procedures.

B. Injectable Anesthetics

Injectables are given to effect. Doses should be used as guidelines. Effects may vary among individuals. Drugs that have exceeded their expiration date may not be used even for terminal procedures. These agents can be given by the intra-venous, intra-muscular, intra-peritoneal or subcutaneous routes. The agents are in general metabolized in the liver and excreted in the kidneys. Inhalation agents are therefore safer to use in debilitated animals.

List of injectable anesthetics and analgesics:

1. Dissociative Agents

a. Ketamine (Ketoset®) , Tiletamine

Ketamine is the most commonly used injectable anesthetic in a variety of species. In most cases, Ketamine is used in combination with other injectable agents such as agonists or benzodiazepines to reduce or eliminate many of the less desirable side effects if used alone. In rodents, ketamine combined with Xylazine or Xylazine plus Acepromazine are the preferred anesthetics when gas anesthesia cannot be used.

Advantages: ketamine has a wide margin of safety in most species; residual analgesic effect following anesthetic recovery, most commonly used drug in combination for injectable anesthesia in rodents.

Disadvantages: ketamine alone does not provide muscle relaxation and muscle spasms may be observed; DEA license required for use as Ketamine is

a Schedule 111 controlled substance; surgical anesthesia may be limited depending on the species; prolonged recovery as compared to gas anesthetics. The degree of analgesia produced is variable and severe respiratory depression is produced in small rodents. IP and IM injections are painful as the drug is acidic.

Additional notes: Produces a state of cataleptic sedation with apparent lack of awareness of surroundings. The corneal blink reflex is lost in some species and eye ointment should be applied to prevent drying of the cornea. Laryngeal and pharyngeal reflexes are maintained and salivary secretions are increased. Induction time for IM injections is 3 to 5 minutes and peak effect lasts about 20 minutes. IP induction times are longer and recovery may be prolonged. IV induction is rapid and provides about 10 minutes of anesthesia.

Ketamine combinations:
Ketamine plus Xylazine: Both drugs can be mixed in a single syringe prior to administration. This is the most common injectable anesthetic used in rodent species.
Ketamine plus Xylazine plus Acepromazine: All three drugs can be mixed in one syringe. In rodents, the addition of Acepromazine to the cocktail increases the depth of anesthesia and prolongs the duration of anesthesia as well as recovery time.
Ketamine plus Diazepam: Both drugs can be mixed in a single syringe prior to administration. In rodents, this combination only provides light anesthesia so it may only be appropriate for chemical restraint.

b. Alpha-2 Agonists: Medetomidine (Domitor®), Dexmedetomidine (Dexdomitor®), Xylazine (Rompun®)

Alpha-2 agonists are used for their sedative and analgesic properties in a variety of species. Used as the sole agent, they do not produce an adequate level of anesthesia for even minor surgical procedures. However, in combination with Ketamine, they become much more useful and effective as anesthetics for surgical procedures.

Advantages: Produces analgesia of short duration; can be combined with Ketamine to produce adequate surgical anesthesia in many species; effects can be reversed with a subcutaneous alpha- 2 antagonist injection; not a DEA controlled drug; not irritating when administered IM or IP.

Disadvantages: Cardiovascular depression (decreased heart rate, cardiac output, and hypotension; transient hyperglycemia following administration which may have research significance

Additional notes: When re-dosing an injectable anesthetic combination of Ketamine and an alpha-2 agonist, it is recommended to only re-dose Ketamine

as the duration of action of the alpha-2 agonist is much longer than the duration of the effect of Ketamine.

c. Alpha-2 Antagonists: Atipamezole (Antisedan®), Yohimbine

Alpha-2 antagonists are used a reversal agents for alpha-2 agonists. Administration at the end of a procedure where the anesthetic combination included Xylazine or Medetomidine, an alpha-2 antagonist will aid in reducing anesthesia time and prompting anesthetic recovery.

Advantages: Can reduce duration of sedation and anesthesia caused by alpha-2 agonist.

Disadvantages: Reverses any analgesic benefit of alpha-2 agonist; can cause muscle tremors, increased respiratory rate and hyperemic mucous membranes.

d. Benzodiazepines: Diazepam (Valium®), Midazolam, Zolazepam:

This class of drug can provide marked sedation in a variety of species; however, there is no analgesic effect. These drugs are primarily used as sedative, pre-anesthetics and the induction of anesthesia but are never used alone to provide or maintain anesthesia.

e. Barbiturates: Sodium Pentobarbital(Nembutal®), Methohexital, Thiopental

Barbiturates are irritating to tissues because of their acidic properties. They redistribute rapidly to all body tissues so obese animals may require more drug to anesthetize. Metabolism is slow so recovery times are slow. They are controlled substances and as such require appropriate licensing to obtain and appropriate record keeping. Barbiturates function as $GABA_a$ agonist and are considered to be good anesthetic agents but provide unreliable sedation at low dosages and inadequate analgesic effect at any dose.

Advantages: Rapid anesthetic onset; provides a prolonged duration of surgical anesthesia;

Disadvantages: Prolonged recovery time, inadequate analgesic properties; extremely expensive; narrow margin of safety; produces respiratory depression higher dosages; DEA License required

f. Opioids: Buprenorphine (Buprenex®), Oxymorphone, Fentanyl, Morphine, Butorphanol

*Advantages***:** Provide potent analgesia; concurrent administration can lower the dose of inhalant or barbiturate general anesthetic for surgery; mechanism

mediated by receptor binding in the brain and spinal cord; reversible with Naloxone.

Disadvantages: DEA Controlled drugs; relatively short duration or action; repeated use may result in tolerance development.

g. Non-steriodal Anti-Inflammatory Drugs (NSAIDs): Carprofen (Rimadyl®), Meloxicam (Metacam®) Flunixin meglumine (Banamine®), Ketoprofen (Ketofen®), Ibuprofen (Advil®)

Advantages: New drugs (Carprofen®, Meloxicam®) include a long duration of analgesic activity; not a DEA controlled substance; there are multi-route administration methods for several NSAIDs; relative safety when administered at prescribed dosages.

Disadvantages: Contraindicated for inflammation models, infectious disease, or coagulation research due to anti-inflammatory properties; COX-1 side effects such as gastrointestinal complications, prolonged coagulation times, and changes in kidney function with non-COX-2 selective forms.

Note: Analgesic combinations that include NSAIDs plus opioids would be considered an ideal combination for the control and prevention of discomfort due to the demonstrated harmony and difference in mechanism of action. Oral dosing of analgesics following anesthesia results in questionable consumption of the drug due to decreased water consumption following anesthesia, as demonstrated in rodents.

h. Local anesthetics: Lidocaine, Bupivacaine (Marcaine®), Rjpivacaine (Naropin®), Proparacaine (Alcaine® Ophthalmic)

Local anesthetic routes of administration include topical to mucus membranes or injected directly into tissues and around nerve bundles. Use as the primary analgesic is discouraged due to the short duration of effect (hours).

Advantages: Pre-operative and intra-operative administration can provide a good adjunct in pain relief to general anesthesia and systemic analgesics administered after a procedure. Drugs of this class are not controlled substances.

Disadvantages: Avoid administering by intramuscular and intravenous injections as both routes reach systemic circulation very rapidly. Dilution of stock concentration is encouraged to provide more accurate dose administration.

Additional notes: Lidocaine is a fast acting, short duration local anesthetics. Bupivacaine is a slow onset, long acting local anesthetic.

VII. Additional Anesthesia/Analgesia Information by Species

A. **Rat** Anesthesia and Analgesia

1. Normal Parameters
    a. Lifespan: 2-3 years (maximum 4)
    b. Average weight: 250-400 gm
    c. Heart Rate: 250-500 beats per minute
    d. Respiratory Rate: 65-110/minute
    e. Temperature: 99.2 degrees F average
    f. Blood volume 6-7 % of body weight
    g. Ear pinch, toe pinch, corneal reflexes

B. **Mouse** Anesthesia and Analgesia

1. Normal Parameters
    a. Lifespan: 1-2 years (maximum 3)
    b. Average weight: 30 gms
    c. Respiratory Rate: 60-220/minute
    d. Temperature: 98.8 degrees F average
    e. Blood volume: 5.5% of body weight
    f. Surgical anesthesia- toe pinch, corneal and tail pinch reflex

C. **Amphibian** Anesthesia and Analgesia

Amphibians in the early stages of their life cycle (e.g., tadpoles) are entirely aquatic and have gills for respiration. Most amphibians lose their gills during metamorphosis and develop lungs. Most importantly, amphibian skin acts as a semipermeable membrane that allows for respiration (cutaneous respiration) and absorption of substances through the skin.

Amphibians may be anesthetized by immersion in an anesthetic solution, placement in an anesthetic gas induction chamber or by anesthetic preparations applied to the skin.

Amphibians can remain out of water for long periods of time if they are kept moist.

Fasting for 12-24 hours prior to anesthesia is recommended to decrease the incidence of regurgitation which will foul the water of the anesthetic or recovery container.

Amphibians go through an excitement phase during anesthetic induction. It is important to induce anesthesia in a container that will prevent injury due to the animal jumping or falling out.

Pulmonary respiration will cease during anesthesia in amphibians and cannot be used to monitor anesthetic depth. Cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia.

Heart rate may be monitored during anesthesia by direct observation (ventral midline, caudal to the shoulders). Normal values for heart rates have not been published.

D**. Fish** anesthesia and analgesia

When a fish reaches the level of anesthesia sufficient to perform surgery, there is a total loss of equilibrium and muscle tone, decreased respiratory rate and no response to stimuli. A firm squeeze at the base of the tail may be used to determine response to stimuli.

Respiratory rate may be evaluated by observing movement of the operculum (rigid flap that covers the gills) as it opens and closes. Gill color should be dark pink to light red. If respirations become extremely slow or stop, the fish may be placed in anesthetic-free recovery water until respirations resume.

Water temperature should be maintained at the species normal optimum during both anesthesia and recovery.