



Guidance on Anesthesia in Research and Instruction Animals

Route of Drug Administration

- Injectable agents
 - Intraperitoneal (IP) or subcutaneous (SC) are the most common routes of administration in rodent species.
 - IP injection is a convenient route of administration for most properly restrained rodents.
 - SC injections require minimal restraint and are associated with few injection site complications.
 - Intramuscular (IM) injections are appropriate for many drugs in large animal species but are discouraged in most rodent species due to their relatively small muscle mass. IM injections can result in localized inflammation, tissue necrosis, and/or neuropathies followed by self-trauma due to the relative size of the needle and injection volume.
 - Intravenous (IV) injection of anesthetic agents is possible but technically challenging in small species. Often administration of anesthetics by the IV route is reserved to those studies which require vascular cannulation for reasons other than anesthesia alone.
- Inhalant anesthesia (Gas anesthesia)
 - Rodents
 - Induction of gas anesthesia for mice and rats is done in an induction chamber attached to a precision vaporizer anesthetic machine with proper scavenging system.
 - Animals should be monitored closely, and once unconscious, moved out of the chamber for maintenance on a tightly fit nose cone attached to the machine.
 - The anesthetic range should be from 0.5-5.0%, using medical grade oxygen, and must be maintained at a rate that will keep the animal at a surgical plane of anesthesia for the duration of the procedure. The range may also vary depending on the health status of the animal, and the use of other drugs, such as injectable anesthetics, and analgesics.
 - Larger species
 - Induction of gas anesthesia for larger animal species often requires pre-anesthetic sedation and administration of other supportive medications such as anticholinergic medications to reduce salivation and gastric activity.
 - Once sedated, animals should be fitted with a tight-fitting endotracheal tube and connected to a precision vaporizer anesthetic machine that allows accurate, real-time management of anesthetic depth.

- **Local anesthesia**

Local anesthetics block passage of sensory nerve impulses responsible for the generation and conduction of pain perception. When used superficially, they block sensory nerve endings and pain associated with local, superficial, painful procedures. When used to infiltrate nerve trunks, the effect is regional blockage of pain perception and propagation.

 - Using local anesthesia at the site of skin incisions is an expectation.
 - Lidocaine (xylocaine) is a local anesthetic with a rapid onset (1-3 minutes) and a short duration of action (20-40 minutes). Bupivacaine (Marcaine) is a local anesthetic with a slower onset (~20 minutes) and a relatively long duration of action (4-6 hours). Because of bupivacaine's long duration of effect, it is often used in rodent species both for its local anesthetic effect and as a local analgesic. A solution combining lidocaine and bupivacaine provides the benefits of both. Local anesthetics supplement but do not replace a systemic analgesic.
 - Ideally, local anesthetics are injected using a small gauge needle (25 - 30 gauge). The needle is injected up to the hub, following the line of the surgical site. The drug is slowly administered as the needle is pulled out. They may also be applied by "splash" block, where the solution is put in the open surgical site.

Anesthetic Dose Calculation and Dilution

- The importance of accurately weighing every animal in order to accurately calculate individualized doses of anesthetics cannot be over-emphasized
 - Example: The difference in accurate dosing between a 20 gram and a 30 gram mouse can equate to complications ranging from inadequate anesthesia to anesthetic overdose.
 - When dosing multiple animals with slightly different weights, having a chart prepared in advance listing weights in small increments and associated calculated doses and injection volumes for each agent to be administered can help facilitate dosing accuracy and safety for the animal.
 - Diluting doses
 - Many drugs used in rodents are not formulated for easy use in very small animals. Many drugs have to be diluted in order to administer accurate doses in rodent species. When calculating dilutions, it is important to consider the total volume that will be administered to the animal. Volumes less than 0.1 ml (100µl) are often too small for dosing accurately and volumes greater than 0.5 ml (500µl) may be excessive for the size of the animal and/or route of administration. Further, diluting drugs will also dilute the preservatives in injectable medications, thereby shortening the safe shelf life of the diluted drug.

Fasting and Water Restriction

- Most large animals used in research must be fasted and have water restriction for a number of hours (consult a URAR veterinarian) prior to administration of anesthesia to prevent regurgitation or vomiting and aspiration of gastric contents.

- Rodents and rabbits, however, generally do not regurgitate or vomit, and do not need to be fasted, or have water restriction preoperatively, unless there is scientific justification.

Physiologic Support

- While anesthetized, from induction to recovery, animals must be provided appropriate supportive care.
 - The sophistication of the support will vary with species, and the type and duration of the procedure, but typically will minimally include use of ophthalmic (ocular) lubricant on the eyes, thermal support for maintenance of normal body temperature, and, in larger animal species, administration of IV fluids.
- Supplemental heat and temperature monitoring
 - Supplemental/external heat sources must be provided for all but the most rapid anesthetic events (i.e., ≤ 5 minutes). Supplemental heat should be provided throughout anesthesia, from induction, and through recovery from anesthesia.
 - Supplemental heat sources to counter hypothermia include slide warmers, chemical or microwaveable heat packs, warm water recirculating pads, heat lamps and forced air heaters such as Bair Huggers.
 - All of these have the potential to cause thermal burns.
 - All thermal sources (exception: warm water recirculating pads) must have a barrier thick enough to diffuse the heat, such as a small towel, placed between the animal and the pad.
 - Electric heating pads are discouraged because of their increased risk for thermal burns compared to the other sources. If used, electric heating pads must be stored flat, to avoid damage from repeated folding/rolling of the wires.
 - Another means of supportive care is the administration of sterile fluids subcutaneously. Warmed (not hot) saline or lactated Ringer's solution, 38.5-40° C, can be given at 2ml/100g during or immediately after surgery.
 - Whenever possible, body temperatures should be monitored. Temperature monitoring during anesthesia is important as decreases in body temperature (hypothermia) are commonly encountered when animals are anesthetized, especially small animals like rodents. Hypothermia enhances anesthetic effect such as respiratory and cardiovascular depression which can adversely affect (e.g., delay) recovery and survival from anesthesia.
 - Rectal thermometers and probes allow for accurate measurement of body temperature. Close temperature monitoring is important to minimize risk of hypothermia or overheating of the unconscious animal.

Monitoring anesthesia

- Monitoring an animal's physiologic and vital signs is necessary to assess both the level (depth) of anesthesia, and the animal's general condition.

- Toe pinch
 - Toe pinch is used to assess perception of pain in an anesthetized animal. If the toe or distal paw has pressure applied using a digital pinching action, withdrawal of the limb or overt movement of the animal signals response to stimulus and pain.
 - This test should be used during anesthesia induction to determine when an animal has achieved an adequate level of anesthesia to begin a painful procedure.
 - This test is also used intermittently during anesthesia to assess continued depth of anesthesia and lack of response to painful stimuli. If after having lost the toe pinch response, the animal begins to regain it, or shows other signs of movement, this is an indication that additional anesthetic doses may need to be administered.
- Heart rate
 - Heart rate is a primary factor for effective monitoring of large animal species, but is difficult to assess accurately in most rodent species due to their rapid heart rates.
 - If electrocardiography or pulse oximetry is being used, measurement of heart rate may be possible, and trends can be observed over the anesthetic interval.
 - Increases in heart rate may indicate pain perception and the need for additional anesthesia.
 - Conversely, if heart rate decreases, this may be an indication that the animal is reaching a deeper plane of anesthesia and additional anesthetic is not needed or that too much has been administered.
- Respiratory rate and character
 - Respiratory rate and character are primary factors for monitoring large animal species. Respiratory character may be monitored in rodents, but respiratory rate may be difficult to accurately measure in many rodent species.
 - Trends in respiratory rate and character can be judged over time to help assess anesthetic depth.
 - Increases in respiratory rate and shallow breaths may indicate that an animal is responding to manipulations being performed and may need additional anesthetic.
 - Alternately, if respiratory rate decreases or the animal demonstrates exaggerated respiratory effort, this may be an indication that the animal is reaching a deeper plane of anesthesia and additional anesthetic is not needed.
- 'Large' animals (USDA covered, larger than a rodent)
 - Physiological monitoring should include measurement of heart rate, respiratory rate, and blood pressure (systolic, diastolic, MAP) once every five minutes. Additionally, core body temperature, and anesthetic depth (pupillary dilation/position, presence/absence of jaw tone) should be assessed at a minimum of once every fifteen minutes.
- Rodents
 - Physiological monitoring should include a quantification of respiratory rate (acquired by counting excursions of the chest wall during respiration) once every five minutes at minimum. Qualitative indicators of physiologic status (skin color, muscle tone) should be assessed as necessary depending on an animal's status.
- Documentation of these parameters during monitoring should occur at least every 15 minutes.
 - The frequency of data collection should increase if the animal shows signs of physiologic decompensation.

- Monitoring and documentation of these parameters must continue until the animal has recovered from anesthesia.

Recovery from General Anesthesia

- Animals recovering from an anesthetic event must be watched continually by laboratory personnel until the animal has demonstrated physiologic stability. Recovering animals must not, under any circumstance, be permitted to recover immediately following anesthesia without constant oversight from qualified personnel.
 - Signs of this status will vary by species but generally describe a fully-conscious state, capable of supporting body weight and moving smoothly/independently (without staggering, or assistance from staff) throughout the housing environment.
 - Food and water should be removed from the enclosure until the animal has reached this stable physiologic plane. An exception is a rodent cage's food hopper, water bottle, or lixit.
 - Physiologic monitoring should continue (along with recording of aforementioned parameters) until the animal is fully recovered.
- Animals should be stimulated frequently until they have fully recovered (vigorous rubbing or positional adjustments). Failure to stimulate anesthetized animals until they regain consciousness may lead to gradual decreases in respiration, resulting in gradual oxygen deficiency, which could ultimately lead to cardiac failure and death.
- Animals which are pair or group housed should receive particular attention during the post-anesthesia/sedation recovery phase as individuals may recover at varying rates. Animals recovering sooner than others may harm those still in the recovery stage.
- For URAR housed animals, enclosures housing animals which have been anesthetized must be marked with the URAR provided Post-Procedure cards.

Documentation

- Each administration of anesthesia and recovery from anesthesia must be documented, either in the animal's individual Clinical Health Record, or, for rodents and non-mammals, on a group record.
 - The IACUC website provides general templates for Anesthesia/Surgery and Post-Procedural Monitoring, which may be used.
- The hard copies of these records must be kept with the animal in its housing room until the animal is fully recovered from anesthesia and behaving normally, no monitoring or physiological support is needed, and food and water, if removed, have been returned to the enclosure.