Small Animal Handling, Care, and Anesthesia

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3.1 Animal Care: Introduction

The use of animals in any type of biomedical research brings with it implicit ethical considerations that should be thoroughly reviewed prior to performing the research. At most institutions, these ethical reviews will be conducted by an institutional animal care and use committee (IACUC) or an animal care committee (ACC) after a research group has accepted the need to use animals and defined their use. This review is mostly based on accepted national standards such as the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources et al. 1996) in the United States or the guidelines and policies defined by the Canadian Council on Animal Care in Canada (Olfert et al. 1993). These guidelines, standards, and policies attempt to ensure improvement in animal welfare: reduction in animal numbers, animal pain, and stress, while at the same time not compromising the results of animal-based research.

In the book, The Principles of Humane Experimental Technique (Russell and Burch 1959), William Russell and Rex Burch defined their “3Rs” concept—replacement, reduction, and refinement—in relation to the use of animals in research. In this fundamental publication, they were ahead of their time, defining accepted practices in the design and implementation of animal-based research today.

“Replacement” refers to using a nonanimal model or excised animal tissue to replace the use of live animals, eliminating stress and pain for the animal. It may also refer to using a “less sentient” species such as replacing the use of a vertebrate animal with an invertebrate. The use of cell cultures and animal and human tissues would also be a “replacement.” In small animal imaging, there may be limited opportunities for “replacement” available in a study as most imaging equipment is specialized for small rodents. The use of models and postmortem carcasses or tissues may be considered a viable replacement in some studies.
“Reduction” refers to minimizing the number of animals used in a study by appropriate experimental design and improved data analysis. This can be further enhanced by using genetically homogeneous animals and controlling for other research variables. Small animal imaging often allows for substantial “reduction” as living animals are often followed longitudinally, allowing for fewer animals within a study, and for noninvasive research. Imaging-based research also allows each animal to serve as its own control, thereby increasing statistical power.

“Refinement” is concerned with improvements in an experiment that reduces stress, distress, and pain in the animal. Refinement can also apply to improving an animal’s environment allowing for decreased stress and normal species-specific behaviors. Examples would include providing appropriate analgesia and postoperative care to an animal, establishing and adhering to early experimental endpoints, or group housing social animals allowing for positive conspecific behaviors such as grooming. Imaging is inherently nonpainful and may allow for the use of animals without clinical disease. Unlike human imaging, animals often require anesthetics during imaging procedures and this may be done repeatedly. Repeated recovery anesthesia should be considered a negative aspect of imaging and attempts to limit the frequency and duration of anesthetic sessions should be sought.

As mice, and secondly rats, are the mostly widely used rodent species in biomedical research worldwide, this chapter deals with only these two species.

### 3.1.1 Housing

Rodents can be housed and maintained in many types of environments within the laboratory setting. In conventional housing, rodents are often housed in open-topped cages or cages with a filter lid. They are often handled in open air at the room or procedure room level. Negative aspects of conventional housing systems are that they may allow for human exposure to allergens and rodent disease transmission within the colony. Alternatively, modified barrier or barrier housing may have rodents contained within individually ventilated cages or filtered cages and animals are only handled within a biological safety cabinet or other equipment that protects them from pathogens that may be transferred by contact or via room air. Depending on the type of containment used in housing, the requirements for moving animals to imaging equipment may differ among research facilities.

The standard housing environments for rodents have inherent stressors but it is most important to ensure proper groupings of rodents as this may have research and welfare implications. It is typical to keep rodents in groups of three to five animals per cage. Not only is this useful to maximize holding space but it has a strong welfare implication. Rodents are social species (Smith and Hargaden 2001), and it has been shown that even male rodents will work to be in contact with another male rodent (van den Broek et al. 1993; Van Loo et al. 2001). However, caution should be taken when group housing certain strains of male mice as they are known to fight which can result in injury or even death. Studies should be designed to allow group housing when possible, and stable groups and group sizes should be maintained throughout a study so as to decrease potential variability.

Enrichment or environmental enrichment is the addition of objects or alterations in an animal’s environment that allows an animal to display species-specific behaviors. For example, group housing rodents would be considered an environmental enrichment as it allows for conspecific grooming. Other standard enrichments in rodent environments may include nesting, group housing, hiding spaces/devices, climbing devices, foraging devices, or food treats. Enrichment, while often not standardized or well defined, does have the potential to produce positive or negative effects on many types of research (Bayne 2005). Little is known about the effects of standard enrichments such as nesting on imaging outcomes. However, for example, nesting has a significant impact on body temperature (Gaskill et al. 2012), which may be critical...
for physiological homeostasis and survival post-anesthesia/imaging. Enrichment should always be a study component and should be evaluated prior to its use.

3.1.2 Handling

3.1.2.1 Basics: Mouse and Rat

In addition to general handling required for husbandry, animal handling is a standard practice for preparing animals for imaging, monitoring animals post-imaging, and providing treatments. It is generally an accepted practice to handle mice by first gently grasping them by the base of the tail and then lifting them and supporting their body weight in the same or other hand (Figure 3.1a). In rats, it is general practice to avoid handling them by the tail, as there is potential for damage. Instead, “scooping” a rat into an open hand is preferred (Figure 3.1b).

Handling can induce unwanted stress or distress that has the potential to complicate research. Certain types of handling may mitigate some of this stress. It appears that handling mice by the tail induces aversion to the handling process and induces anxiety, whereas using tunnels to handle mice or using an open hand improves these unwanted conditions (Hurst and West 2010) (Figure 3.1c). Rodents may not habituate to repeated handling, so all handling should be thought of as a potential stressor (Balcombe et al. 2004; Longordo et al. 2011).

3.1.3 Administration of Substances

In all cases, it is preferable to get specific training on handling and injections long before a research project is to take place. At most institutions, training is available from veterinary staff or facility staff. As consistency and technique are critical to both animal welfare and animal research, training should continue until an individual is proficient at all the techniques required for a study.

![Figure 3.1](image-url)
3.1.3.1 Subcutaneous Routes of Administration

The subcutaneous route is frequently used for the administration of substances and fluids to prevent dehydration that can ensue following anesthesia and/or surgery, but rarely used for the administration of anesthetics. The rate of absorption will depend on the formulation of the substance. In general, the smallest needle should be used taking into account the viscosity of the substance. The recommended volumes are 10 mL/kg (mouse) and 5 mL/kg (rat) (Diehl et al. 2001). This would be approximately a 0.25 mL injection for a 25 g mouse. However, for subcutaneous fluid administration, up to 1 mL is normally given for a mouse. Normally the scapular region is the preferred site for subcutaneous injections; however, due to rodent’s loose skin, these injections can be given along the back and along the abdomen. A “tent” of skin should be created by lifting the skin in the area, and the needle should be inserted at an angle into the tented area. The entire needle should be placed into the subcutaneous space to ensure that the injected substance does not leak out of the puncture site. Before injecting, aspiration should occur to ensure that the needle has not penetrated a blood vessel or exited the skin, in which case air will be aspirated.

3.1.3.2 Intramuscular Routes of Administration

In almost all instances, intramuscular injections are not recommended for rodents as their muscle bellies are quite small, leading to pain and potential muscle and nerve damage on injection. Intramuscular injections should only be given when no other alternatives exist, and well-trained personnel should perform these injections.

Intraperitoneal and intravenous injections are the most common routes for injecting substances in rodents, and they are discussed in detail in the following sections.

3.1.4 Transportation

In most instances, animals will need to be moved or relocated to a facility that houses imaging equipment. Transport, even from the simplest move of animals from one room to another, can alter basic physiological functions, such as elevated heart rate and blood pressure. Consideration of potential stress induced by transport between facilities, between rooms within a facility, and between holding areas and imaging areas should be given. Short-distance transport may result in stress due to changes in the environment including diurnal light/dark cycles, temperature, and relative humidity (Tuli et al. 1995). Rats transported between holding room and testing room showed a significant elevation in body temperature that lasted for 2 h (Dallmann et al. 2006). Long distance transport also causes alterations in physiological function including behavioral changes, corticosterone levels, food and water consumption, and body weight (van Ruiven et al. 1998). Extreme temperature variations during air shipment have been documented and this should be expected to cause changes in the animals. Other factors to consider in movement of animals are the change in the microenvironment (cage type, bedding type, animal density, temperature, diet, etc.) and/or the change in personnel that can cause a stress response.

While it may be difficult to quantify the stress imposed by transporting animals to and from imaging equipment, it is still critical to establish standards of acclimatization to attempt to mitigate these mostly unforeseen effects. In general, a minimum period of 2–3 days of acclimatization is recommended when transporting animals between facilities (Conour et al. 2006).

3.1.5 Disease Transmission Concerns

One of the most important considerations in small animal imaging is that of disease transmission or biosecurity at the level of multiuser equipment. As imaging equipment is expensive and often scarce within an institution, it is likely that many different research groups will use the equipment. If mice from one facility that allows certain opportunistic or pathogenic organisms in their rodent colonies are scanned the day before mice from a barrier facility that excludes all known pathogenic organisms, what are the risks? There are very few publications on this topic yet it is likely the most difficult-to-manage issue in small animal imaging. Methods and standard operating procedures to exclude pathogens from imaging equipment and imaging facilities should be well established.
Regardless of the movement of animals within an imaging facility or suite, it is best practice to
disinfect equipment either between groups of same health status animals or between individual ani-
mals. In most cases, equipment such as the imaging bed, the induction chamber, and the anesthetic
circuits can be disinfected with an appropriate solution. In some instances, equipment that cannot
be appropriately disinfected can be covered with a disposable material to help reduce contamina-
tion. Note that certain pathogens of rodents are highly resistant to disinfection and so there are
inherent risks to sharing equipment between animals of a different health status.

Some strategies that are commonly used to maintain rodent facility health status is to move
rodents to imaging equipment and then not allow those rodents to return to their facility of origin.
In some cases, rodents are held and euthanized within the imaging facility. Another option would
be to provide a short-term holding facility that accepts rodents after imaging and allows them to be
transported back and forth to the equipment as needed, without returning to and possibly contami-
nating the facility of origin. Another strategy is to have the biosecurity level at the individual animal,
where each animal is in a mobile self-contained container that can be used for imaging.

In cases where animals are held post-imaging in groups that arrived from different sources or facili-
ties, sentinel testing, or health monitoring should be considered. Sentinel rodents are used to monitor
the pathogen status of a group or colony of rodents. Sentinels are often exposed to the air that the colony
is exposed to, and to the dirty bedding of colony rodents. In this way, sentinels are exposed to whatever
pathogens may be present in the colony. At the end of a designated monitoring period, the sentinels are
sampled and tested for a specific list of infectious agents. Another option would be to sample directly
from the animals that are used for research purposes. There are commercially available diagnostic tests,
including serological and polymerase chain reaction based tests, for determining the status of either
sentinel or research animals. Monitoring strategies should be developed in conjunction with experi-
enced laboratory animal veterinarians who are familiar with the risks of rodent pathogens to research.

3.2 ANIMAL PREPARATION AND SUPPORTIVE
CARE FOR ANESTHESIA

3.2.1 Fasting
In imaging studies, there is sometimes a need to fast rodents; however, it is not typical to fast rodents
for anesthesia or manipulations as in other larger species of animals. Vomition during anesthetic
induction and anesthesia is not a concern in rodents due to the limiting ridge of the forestomach
(Luciano and Reale 1992). It is still possible to have regurgitation though, so appropriate animal
positioning without weight on the abdomen is important. Fasting can be an effective way to ensure
uniformity in PET imaging especially in fluorodeoxyglucose (FDG) imaging to decrease blood glu-
cose levels (Hildebrandt et al. 2008). However, fasting in rodents should be done with care as rodents
have high metabolic rates causing quicker physiologic alterations compared to larger animals. Food
should not be withheld from small rodents for more than 2–3 h as hypoglycemia and dehydration
may occur, as these species typically will not drink water if they do not eat (Harkness et al. 2010).
Regardless, water should be available at all times during the fasting period.

Fasting may have significant research effects. When food was withheld from the beginning of
the dark period, even short periods of food deprivation will affect variations in metabolism. A 3 h
fasting period during the dark phase caused a substantial decrease in liver weight and glycogen con-
tent in rats (Palou et al. 1981). Fasting rats resulted in depleted glycogen stores and a significantly
lower liver attenuation observed in computed tomography (CT) images compared to normal rats
(Leander et al. 2000). The fact that rodents reingest feces as part of their normal behavior also means
that the ability to actually fast these animals is limited.

3.2.2 INJECTIONS OF ANESTHETICS AND CONTRAST AGENTS
Many guidelines are available on the administration of substances to animals regarding volumes
and routes of administration. Properties of substances to be injected including pH, tonicity, sterility,
viscosity, and rate of absorption are all important to consider. In imaging, contrast agents are usually given intravenously, although in very small animals, such as a 20 g mouse, this can be difficult. An alternative route in rodents is to give contrast agents via the intraperitoneal route that is most similar to the uptake rates of intravenous administration. However, substances given by the intraperitoneal route are possibly first absorbed into the portal circulation, resulting in potential biotransformation of the substance before it reaches circulation (Nebendahl 2000).

Intravenous injections, while more complicated in rodents than larger species, have the advantage of rapid absorption and allow the injection of some substances that are too irritating to be given by other routes. Intravenous access can be obtained in both mice and rats. In rats, the use of 24-gauge over-the-needle catheter in the lateral tail vein can be accomplished relatively easily (Figure 3.2a). In mice, there are no commercially available catheters for tail vein catheterization but catheters can be self-made with a small-gauge needle and polyethylene tubing. Alternatively, injections can be given directly with a needle but there is increased chance of injecting the substance outside of the vessel (Figure 3.2b). In mice, a 25- to 27-gauge needle is typically used, and in rats, a 25-gauge needle is standard. In both species, the typical maximum volume for bolus administration would be 5 mL/kg (Diehl et al. 2001). For rapid IV administration, a substance must be compatible with blood and not be too viscous. If large volumes are given, the substance should be warmed to body temperature. For injections in a conscious animal, many commercially made restrainers are available, and in some cases, these restrainers have built-in heating systems to ensure vasodilation.

While over-the-needle catheters may be useful for a single imaging session, longer-term catheterization of many vessels can be achieved surgically. Frequently used vessels for chronic catheterization are the internal or external jugular veins, the femoral vein, or the carotid artery. This allows for multiple injections or blood samples to be performed over time. Several commercial companies can provide catheterized rats or mice for studies. If catheters are not properly maintained, clotting or infection may occur. It may be necessary to house rodents with external catheters separately.

Intraperitoneal injections are common in rodents but may cause complications including injecting into the intestinal tract, laceration of organs/vessels, and peritonitis caused by irritating substances or bacteria. These injections are generally done without anesthesia and proper restraint and injection techniques are important to avoid complications. Mice and rats can be properly restrained and injected by one person, but in the case of rats, it is preferable to have two people for an IP injection, with one person responsible for restraint. In mice, the scruff hold works best for these injections, and in rats, the animal can be restrained with a “v” hold, and the hind legs restrained by one person and the injection by another person (Figure 3.3a). If only one person is available to hold and inject the rat, the authors preference is to gently towel wrap the rat and cradle it in dorsoventral hold
in the forearm while retracting the hind legs (Figure 3.3b). The abdomen can be thought of as four quadrants separated by midline and a perpendicular line through the umbilical area (Figure 3.3c). Injections should be given into one of the lower quadrants of the abdomen to avoid damage to internal organs such as the liver or the spleen. To avoid accidental injection into the cecum, a large organ of the abdominal cavity, injections in mice should be given in the lower left quadrant, and in rats, the lower right quadrant (Suckow 2001). Typically, the animal is held in a slight head-down position and injections are given with a quick motion at a 20°–30° angle. Only the tip of the needle needs to enter the peritoneal space. Aspiration should occur before injection to ensure that no blood, urine, or other fluid is aspirated. Failure of intraperitoneal injections is common and injections by this route should be justified prior to the study (Gaines Das and North 2007).

3.2.3 Supportive Care

Basic supportive care, regardless of the rodent species, is critical for ensuring that physiological parameters are maintained during and after anesthesia. Supportive care also has implications for imaging. The five essential components of supportive care are discussed next.

3.2.3.1 Heat

Rodents are prone to hypothermia due to their large surface-area-to-body-mass ratio and their rapid metabolism (Balaban and Hampshire 2001). Anesthesia lasting longer than 10–15 min may result in hypothermia in rodents. Therefore, monitoring and maintaining body temperature is imperative. The normal body temperature of a mouse is 37.4°C and that of a rat is 38°C (Flecknell 1996). With select types of imaging equipment, heating devices are built in (Figure 3.4). In some cases, a heated imaging bed is provided with a rectal probe that provides feedback to the bed and adjusts the temperature appropriately. In the case of optical imaging equipment, the temperature within
the chamber can be regulated. If a heating device is not part of the imaging equipment, a commercial heating device should be obtained as well as a way to continuously monitor body temperature. Circulating warm water blankets, warm air devices, or thermal pads with rectal temperature probes are recommended. Electrical heating pads, microwavable heating pads, water bags or bottles, and lamps should be used with extreme caution as overheating and thermal burns are common. In addition to heating devices, small rodents can be covered or wrapped in a lightweight drape or bubble wrap, taking care not to impede respiration. Heat is important in the anesthetic recovery period as well. Rodents should be maintained in environmental temperatures of 30°C–35°C (86°F–95°F) during recovery (Harkness et al. 2010).

3.2.3.2 Fluids
Preoperative or preanesthesia administration of fluids given subcutaneously may be beneficial for long-duration anesthesia or debilitated small rodents. Fluids may also be provided during the imaging session, especially if intravenous access has been obtained with a catheter. Lactated ringers solution or 0.9% sodium chloride can be given intravenously, subcutaneously, or intraperitoneally at a rate of 5–10 mL/kg/h (Harkness et al. 2010). Fluids should be warmed as cold fluids can cause hypothermia. Syringe drivers or syringe infusion pumps may be very useful at delivering these small volumes. Rodents will require 40–80 mL/kg/day of fluids in the postimaging period until it is certain that the animal is eating and drinking sufficiently.

3.2.3.3 Oxygen
While oxygen is typically delivered with inhalant anesthetics, it is a critical additional supportive care when using injectable anesthesia and may hasten recovery from anesthesia. Cyanosis and pulse oximeter readings of less than 70% are often observed in rodents given injectable anesthetics, especially ketamine and xylazine. In order to counteract hypoxemia, oxygen can be provided via facemask for the duration of the anesthesia. Corneal hypoxia resulting in damage to the cornea may be a consequence of low oxygen saturation (Harkness et al. 2010). Rodents that are debilitated or having subclinical disease may benefit from pre-oxygenation with 100% oxygen for 3–5 min prior to anesthetic induction.

3.2.3.4 Eye Lubrication
Due to their large size and somewhat exophthalmic eyes, rodents may not be able to close their eyelids completely during anesthesia. Certain injectable drugs may also cause further exophthalmia of the eyes. Sterile, nonmedicated ophthalmic ointments or drops must be used to minimize corneal drying and corneal damage. This should be placed in the eyes immediately after anesthetic induction.
Animals should be positioned to avoid eye contact with heated or other surfaces and inhalant anesthetics to avoid corneal damage and desiccation. Eye lubrication should be repeated as needed especially for long-term imaging.

### 3.2.3.5 Analgesia

While pain is not expected during imaging sessions, if rodents have undergone painful procedures such as surgery prior to imaging, appropriate analgesics should be used. Some analgesics, such as opioids, may be used in conjunction with anesthesia to lower the required amount of anesthetics needed.

### 3.3 ANESTHESIA

Unlike imaging in humans, small animal imaging almost always requires anesthesia in order to maintain the animal in one position without movement. Repeated anesthesia and recovery brings challenges to small animal imaging. It is also a variable that may have research implications when comparing results in nonanesthetized animals or correlating to human research.

#### 3.3.1 STRAIN, GENDER, CIRCADIAN RHYTHM EFFECTS

The first parameter to consider in mouse or rat anesthesia is rodent strain. There are a vast number of strains, particularly of mice, many of which have been genetically altered. Strain differences alone are known to influence the sleep time of anesthetics (Kohn et al. 1997). Genetically modified rodents may have unexpected variability in their response to anesthetics since the location of insertion and number of copies of genetic material will vary (Gaertner et al. 2008). Anesthetics doses cannot easily be extrapolated from one strain to another and mice often require much higher doses of anesthesia than rats. Pilot studies should be undertaken when changing to a new anesthetic regimen in research models (Flecknell 1993). Gender also has significant effects on pharmacokinetics, metabolism, and other physiological parameters (Curry 2001). This effect must be considered when anesthetizing rodents. Rodents are nocturnal and most of their activity including food consumption happens in the dark cycle. Because of this, the time of day plays an important role in anesthesia of rodents and it has been documented that the same doses of anesthetics produce different levels of anesthesia depending on the time of day that they are given (Challet et al. 2007). There is less concern for these variables when inhalant anesthetics are used as the depth of anesthesia can be easily altered.

#### 3.3.2 OPTIONS FOR ANESTHESIA: INHALANTS versus INJECTABLES

It is important to define the use of anesthetics in imaging studies. In almost all cases, the goal is immobilization and decreased stress (light anesthesia) compared to anesthetizing an animal for surgery that would also require loss of consciousness and analgesia (deep anesthesia). However, due to the size of rodents and difficulty with vascular access, there are very few anesthetics or combinations of anesthetics commonly used. Drugs given to induce and maintain general anesthesia are either inhalant anesthetics or injectable anesthetics. There are many online resources for determining the doses of anesthetics in rodent species and, therefore, doses will not be discussed here. In some cases, it may be beneficial to consider the use of injectable and inhalant anesthetics together, often allowing the dose of each anesthetic to be lowered. This may result in fewer unwanted side effects of each anesthetic.

#### 3.3.2.1 Inhalant anesthetics versus inhalants

Inhalant anesthetics are considered the gold standard for rodents as they are easy to administer, easy to remotely administer via a breathing system, safe for many ages and strains, and allow for control of the depth of anesthesia. Inhalant anesthetics include halothane, isoflurane, sevoflurane, and desflurane. Minimum alveolar concentration (MAC) is the alveolar concentration of an
anesthetic required to block the response to a specific stimulus in 50% of animals, so the lower the MAC value, the more potent the anesthetic. The most commonly used inhalant anesthetic in veterinary medicine and rodent research is isoflurane. The MAC for isoflurane in rats is 1.38% (Flecknell 1987). It is readily available and relatively inexpensive. Isoflurane is usually delivered at 3.5%–4.5% gas in oxygen to induce anesthesia, which is then maintained with a concentration of 1.5%–3% (Flecknell 1996). For longer-term anesthesia, Constantinides et al. (2011) showed isoflurane at 1.5% to provide the most stable heart rate and mean arterial pressure in mice anesthetized for 90 min. Isoflurane maintains better cardiac function than most injectable anesthetics but is a respiratory depressant.

Due to the respiratory depressant concerns with the use of inhalant anesthetics, it has become common to intubate rodents for prolonged imaging sessions. Intubation allows for positive pressure ventilation and gives a direct connection from the rodent to the anesthetic machine and a secure airway. Rodent intubation kits are available commercially and there are many choices of techniques available for rodent intubation. The most common method is to use intravenous catheters or laboratory tubing of appropriate size to intubate. Artificial ventilation can be done via commercially available rodent ventilators and may reduce variability in studies by decreasing hypoxemia and hypercapnia.

Induction of inhalant anesthetics is safely done in an induction chamber, with appropriate scavenging systems to prevent human exposure to waste gases. The induction chamber should allow for the visualization of the animal and should be sized as small as possible to accommodate the animal and to ensure a rapid induction phase of anesthesia (Figure 3.5).

Once induced, the animal should be quickly removed from the induction chamber and either intubated or attached to a nonrebreathing anesthetic circuit with a tight-fitting nose cone with minimal dead space. Nose cones are an ideal way to deliver inhalant anesthetics to rodents, as rodents are obligate nasal breathers; however, anesthetic-induced respiratory depression is common and rodents may require positive pressure ventilation. As rodents have a compliant pulmonary system, it is possible to ventilate a rodent with a nose cone (Gaertner et al. 2008). Appropriate scavenging should be used to ensure human safety, especially when a facemask is used. Because rodents have a relatively large abdomen, which could lead to thoracic compression, they should be positioned at a slight incline with the head slightly above the tail (Balaban and Hampshire 2001).

For inhalant anesthetics, an anesthetic machine consisting of a calibrated vaporizer, a flow meter, and a delivery circuit is necessary for proper administration. The carrier gas is normally oxygen, allowing for a built-in supportive care mechanism. Recovery from inhalant anesthetics is rapid, which for a nonpainful procedure such as imaging can be an advantage.
3.3.2.2 Injectable anesthetics versus injectables

Injectable anesthetics come with inherent risks in small animals, as once drugs are given by injection, the dosage cannot be reduced. Therefore, injectable drugs should be used at low doses and drugs with a wide safety margin should be used. It is also advisable to do a small pilot study on the particular species and strain to be anesthetized as there are significant strain effects on anesthesia. There are occasions where the use of injectable anesthetics are warranted such as when inhalant anesthetics may interfere with the research in question or when appropriate anesthetic equipment and scavenging are not available. Injectable anesthesia requires only a needle, syringe, and appropriate training to give the drug in the appropriate location (Flecknell 1993).

As close as possible to the timing of anesthetic induction, animals should be evaluated for health status and weighed to ensure that accurate dosing is used. Because of the small body size of rodents and the fact that many drugs are designed for larger species and must be diluted, it is easy to inadvertently overdose or underdose an individual rodent. In almost all instances, injectable drugs are given intraperitoneally. Obese mice may have an altered biodistribution of lipophilic agents and a high incidence of liver dysfunctions and are, therefore, at high anesthetic risk because of hypoventilation and hypoxia. However, cachectic mice present low plasma protein binding and might hide renal, hepatic, or cardiac deficiencies (Gargiulo et al. 2012).

Injectable anesthetics may be a single drug (e.g., pentobarbital) or a combination of drugs (e.g., ketamine/xylazine). In general, combinations of drugs are preferred allowing for lower doses of individual drugs and the potential to reduce side effects. The most common anesthetic drugs and combinations are outlined in the following sections including their advantages and disadvantages. In the author's experience, published doses of injectable anesthetics for rodents can be used at lower doses when a surgical plane of anesthesia is not being sought, such as for most imaging studies.

3.3.2.2.1 Alpha-2 Agonists (Xylazine or Dexmedetomidine)

Alpha-2 adrenergic agonists xylazine (Rompun®) and dexmedetomidine (Dexdomitor®) are commonly used with ketamine to provide surgical anesthesia in rodents. Used as sole agents, these drugs are muscle relaxants and sedatives often used for chemical restraint. In some species, dexmedetomidine appears to lead to greater anesthetic depth than xylazine, and it is more reliably antagonized by a reversal agent.

**Advantages:** Advantages of alpha-2 agonists are that they provide analgesia and can be combined with ketamine ± acepromazine to produce surgical anesthesia in some animals. These drugs are not controlled substances and they are reversible with intravenous or subcutaneous reversal agents.

**Disadvantages:** Disadvantages in most species include cardiovascular depression (decreased heart rate, decreased cardiac output, and hypotension) and, therefore, these drugs are not recommended for the studies of cardiac function. In addition, these drugs can cause more profound hypothermia and irreversible corneal opacities. They also act as diuretics making fluid replacement important. Oxygen supplementation is helpful to counteract the effects on the cardiovascular and respiratory systems. These anesthetics cause a transient hyperglycemia, which may have research implications.

3.3.2.2.2 Ketamine

Ketamine is a dissociative anesthetic used in a wide variety of rodent species. At low doses, ketamine provides chemical restraint with minimal analgesia. In most instances, ketamine is used in combination with other injectable agents, as it does not lead to muscle relaxation or a surgical plane of anesthesia when used alone. Incremental additional doses of ketamine can be given to extend the period of anesthesia but can cause severe respiratory depression (Flecknell 1987).

**Advantages:** Advantages of ketamine are its wide margin of safety in most species, its N-methyl-D-aspartic acid receptor blocking action providing special pain control, and its limited effects on the cardiovascular system. In combination with other drugs, it can provide a surgical plane of anesthesia for about 30 min when given intraperitoneal.
Disadvantages: Disadvantages of ketamine include a mild irritancy on injection due to low pH and insufficient anesthesia in some species and strains (especially mice) for some procedures. Ketamine is a controlled substance and requires a license for use.

3.3.2.2.3 Ketamine Combinations

3.3.2.2.3.1 Ketamine and Alpha-2 Agonists (Xylazine or Dexmedetomidine) Ketamine may be combined with the alpha-2 agonists, xylazine or dexmedetomidine, and is normally the preferred anesthetic combination when the equipment for inhalant anesthesia is not available (Gaertner et al. 2008).

Advantages: Advantages of ketamine/alpha-2 agonist combinations are that they may be combined for injection and that they may produce short-term surgical anesthesia with good analgesia in some animals. This combination can be partially reversed by reversing the alpha-2 agonist with reversal agent.

Disadvantages: Disadvantages of ketamine/alpha-2 agonist combinations are that rodents will not reliably reach a surgical plane of anesthesia in all cases in addition to the disadvantages of both drugs listed earlier. The reversal of the alpha-2 agonist results in the reversal of the analgesic component of the combination and should only be done when there is no need for pain control. If a ketamine/alpha-2 agonist combination is used for surgery longer than 20–30 min, animals will likely require additional anesthetic. It is likely to get a much longer duration of sedation. Redosing with a lower dose of ketamine rather than the combination is usually safer, as the cardiovascular depression of alpha-2 agonists is often longer lasting than the sedation or analgesia produced. However, repeated redosing with ketamine alone will not produce a surgical plane of anesthesia.

Adding acepromazine to the ketamine–alpha-2 agonist combination may result in deeper and/or longer plane of anesthesia in small rodents, especially rats, and possibly some strains of mice as well (Arras et al. 2001). These combinations should be mixed immediately prior to use since the drugs are incompatible and their efficacy, once mixed, will decrease over time.

3.3.2.2.4 Ketamine and Benzodiazepines (Midazolam or Diazepam) Benzodiazepines such as diazepam (valium) are used in combination with ketamine as anesthesia or as a premedication combination for inhalant anesthesia. Diazepam alone is a muscle relaxant and works on centers in the brain to cause a calming effect. In combination with ketamine, it counteracts the muscle rigidity of ketamine. This sedative combination will require an inhalant agent or other anesthetic to achieve surgical anesthesia.

Advantages: Advantages of ketamine/benzodiazepine combinations are that they may be combined in one syringe and can produce profound sedation without the negative aspects of ketamine/xylazine or dexmedetomidine.

Disadvantages: Disadvantages of ketamine/benzodiazepine combinations are that rodents will not reach surgical anesthesia. This combination, however, is preferred for imaging and other nonpainful procedures as it is safer than the ketamine/alpha-2 agonist combinations. Diazepam should be restricted to intravenous or intraperitoneal use.

3.3.3 Barbiturates

Although the other injectable anesthetics addressed have many advantages over barbiturate drugs, barbiturates are still frequently used for rodent anesthesia. They are most frequently used in terminal or acute studies, as recovery can be prolonged and unpleasant. Concurrent use of an analgesic (opioid or nonsteroidal anti-inflammatory drug) is encouraged as it may improve pain relief with barbiturate use and lower the required dose of barbiturate. The most commonly used barbiturate in
rodents is sodium pentobarbital given intraperitoneally. For long-term nonrecovery imaging, urethane is a commonly used barbiturate anesthetic in rodents. Urethane is likely to cause minimal respiratory and cardiovascular depression compared to other injectable anesthetics (Flecknell 1987). However, urethane is considered a mutagen and a carcinogen and its use should be well justified and precautions taken to protect personnel.

**Advantages:** Barbiturates may provide longer sleeping times than other commonly used anesthetics or anesthetic combinations. They are often preferred for physiological recordings and appear to provide more stable anesthesia in rats than in mice.

**Disadvantages:** Disadvantages of barbiturates include a narrow margin of safety, primarily associated with respiratory depression. Pain sensation is only decreased at surgical planes of unconsciousness and may even be heightened (hyperalgesia) at subanesthetic doses.

Barbiturates are controlled substances and require a license for use.

### 3.3.3.1 Equipment Requirements for Anesthesia

With the number of rodents used in research, there are many commercially available products specifically for mice and rat anesthesia. This includes rodent ventilators and kits for endotracheal intubation, heating devices, induction chambers, nose cones, anesthetic circuits, and a variety of monitoring equipment. It is necessary to ensure that appropriate monitoring equipment is available that will work with the species being used (e.g., a pulse oximeter that can register heart rates above 400 bpm for mice under isoflurane anesthesia).

While injectable anesthetic regimes for short-duration imaging may not require much equipment, where possible a heating device with a rectal feedback probe and a source of oxygen should be used.

### 3.3.3.2 Monitoring Anesthesia and Monitoring Equipment

Monitoring of rodents under anesthesia can be difficult due to their small size and their rapid heart and respiratory rates, which can exceed 350 beats/min and 90 respirations/min. Monitoring will also depend on the imaging modality, the type and length of anesthesia, and the access to the animal. The goal of monitoring is to recognize expected changes in physiological mechanisms and to adjust the anesthesia or supportive care to prevent short- or long-term adverse effects on the animals. Many types of monitoring devices for large animals have been adapted for use with rodents and are commercially available.

Anesthetic monitoring of small rodents, without the use of monitoring devices, typically includes testing of rear foot reflexes before any painful procedure is started, and continuous observation of respiratory pattern, mucous membrane color, and responsiveness to manipulations and rear foot reflexes throughout the procedure. This, however, may be difficult with many types of imaging equipment and often a surgical plane of anesthesia is not warranted. Some types of imaging machines have built in monitoring devices such as beds with heating devices and rectal probes that continuously record body temperature and ECG leads that record heart rate and function. Without these devices, other than the monitoring noted earlier, it is critical to have a reliable way to monitor body temperature for longer-term imaging. Any device purchased must work with the particular imaging modality (i.e., nonferrous metal for MRIs). As anesthetics are known to cause respiratory depression, and in some cases cardiovascular depression, monitoring of these vital signs should occur. Monitoring respirations and circulation in rodents can be difficult due to rodent heart rates exceeding 250 beats/min. To monitor these parameters, it is necessary to use specialized respiratory monitors and electrocardiograms specifically designed for rodents (Hildebrandt et al. 2008). Many commercially available monitoring devices such as pulse oximeters are available that can register heart rates over 400 beats/min. Because magnetic resonance imaging can produce artifacts in electrocardiograms, it is necessary to synchronize the heart and respiratory data with the imaging data, which is known as “gating.”
3.3.3.3 Anesthetic Recovery

Rodents should be removed from imaging equipment prior to recovery. It is beneficial to provide oxygen until the animal starts purposeful movement. A recovery cage should be ready prior to recovery; it should be prewarmed and ideally not contain bedding or other devices that could harm the animal during recovery. The author prefers a standard rodent cage lined with paper towel and supplied with prepared nesting material allowing the animal to seek shelter from light. The rodent should be continuously monitored until it is conscious and moves around the cage easily. The time for recovery is normally directly related to the time under anesthesia for inhalant anesthetics. Rodents receiving injectable anesthetics may take over 30 min to begin to recover. Heat is critical during recovery and the animal’s cage may be placed on a surface with supplemental heat (e.g., water circulating heating pad). Be cautious with supplemental heat sources; hyperthermia can be as detrimental as hypothermia. Provide replacement fluid therapy as needed.

Animals should not be returned to the animal room until they can stand and move about their cage. Once returned to the holding room, animals should be monitored sufficiently to ensure their complete recovery. Animals that are not fully awake may injure cage mates, so group housing of animals at recovery should be avoided.

3.3.3.4 Influence of Anesthesia on Imaging

Anesthetics themselves and their complications including hypothermia can have profound effects on imaging. It is critical to understand the potential physiological effects of a particular anesthetic to be used, to carefully consider the time period between imaging sessions and to standardize anesthetic techniques. In the case of specific types of anesthetics affecting specific imaging modalities, some general anesthetics are known to inhibit the luciferase enzyme reaction. Isoflurane, sevoflurane, desflurane all inhibited luciferase activity, which was thought to be due to their hemodynamic effects (Keyaerts et al. 2012). Other effects such as immunomodulatory effects have been documented in mice for up to 9 days following three 40 min weekly exposures to sevoflurane (Elena et al. 2003).

Hypothermia can negatively affect the quality of some imaging procedures such as PET imaging with FDG, increasing FDG uptake by interscapular brown fat, and meddling the visualization of nearby structures (Fueger et al. 2006). It was also shown that isoflurane impeded FDG uptake in mouse heart and brain (Toyama et al. 2004). Respiratory acidosis, a complication of anesthesia with increased pCO2 and reduced pH, has been shown to affect the uptake of tracers (Fuchs et al. 2012).

3.4 ANIMAL WELFARE CONCERNS

While imaging generally improves upon the 3Rs in research, including the longitudinal analysis of often fewer animals, the potential elimination for the need for surgery and serial sacrifice, and the ability to use earlier endpoints, there are still some concerns faced by ethics committees, veterinarians, and researchers. The decision to use live animal imaging should be well justified, as the possibility of postmortem imaging exists. In the case of postmortem imaging, it can likely provide morphological data and biochemical data without motion and without conventional gross pathology procedures.

3.4.1 Number and Duration of Anesthetic Sessions

The duration and number of imaging sessions and the intervals between them will depend on factors such as the duration of time it takes to acquire the images, an animal’s tolerance to anesthesia, and the half-life of the contrast agents. Anesthesia, depending on the type, can cause profound physiological changes and these need to be accounted for in imaging studies.

Knowledge of the negative physiological effects and pharmacological effects of repeated imaging and anesthesia, as well as the animal welfare implications, is still limited. Isoflurane used at six time points within a 24 h period for short-duration anesthesia caused an elevation in corticosterone, which was highest during the initial anesthesia with further elevation at the second anesthesia.
(Altholtz et al. 2006). Suggestions for limiting anesthetic sessions to 2–3 h in a 24 h period and when performing repeated anesthesia no more than five sessions in a 1–2 week period with no more than one anesthetic session per day have been made; however, further research in this area is needed (Workman et al. 2010).

### 3.4.2 Stress

Longitudinal imaging studies create numerous occasions for rodents to be stressed including repeated injections, repeated handling, transportation, experimental conditions including tumor burden, anesthesia effects including hypothermia, and fasting (Hildebrandt et al. 2008). These acute or chronic stressful conditions need to be understood and studies should be planned to reduce these where possible.

### 3.4.3 Imaging of Conscious Animals

Because of the effects of anesthesia on many physiological functions, some imaging research has incorporated imaging of conscious animals, mostly rats. Restraint devices have been developed including stereotaxic devices for MRI and PET imaging. These devices require training and conditioning of the animals for up to 2 weeks in order for them to remain visually calm during imaging (Hildebrandt et al. 2008). However, there is a major welfare and physiological concern as restraint stress is an established model for stress induction in rodents causing elevated heart rate and blood pressure, increased cortisol levels, and gastric and duodenal ulceration (Glavin et al. 1994). Some optical imaging systems are designed to image freely moving conscious animals negating the need to restrain them.

### 3.4.4 Repeated Injections/Radiation Exposure

Radiation exposure during single or serial imaging depends on the tracer dosages and the imaging parameters (e.g., micro-CT). In CT, the radiation dose experienced by an animal is related to the desired resolution of the scan, the number and duration of scans, and the energy of the X-ray used (Glavin et al. 1994). Small animal CT causes radiation doses ranging from 70 to 400 mGy. However, different scan protocols can be used to reduce this exposure level. A longitudinal imaging study in mice consisting of five FDG–PET and CT scans resulted in up to 1 Gy of exposure in a single animal. While 6.5–7 Gy is a lethal exposure dose for mice, these lower doses can show biological effects (Taschereau et al. 2006). Common radiotracers used in micro-PET are relatively short-lived but do leave the animal briefly radioactive. Whole body radiation doses of 6–90 cGy for mice and 1–27 cGy for rats are typical with common PET and SPECT radioisotopes (Funk et al. 2004). This whole body dose does not take into account the biodistribution of the radionuclide and, therefore, may underestimate the radiation risk to the animal (Hildebrandt et al. 2008). Care must be taken to ensure that animals are kept physically separate from other animals until background radiation levels are measured. It is important that all persons working with these animals are trained appropriately to handle radioactive material.

### 3.4.5 Experimental Conditions

Imaging modalities and longitudinal studies will benefit research related to aging, and the progression of diseases such as cancer, heart disease, and neurological conditions. However, the consequences of an aged rodent population with inherent spontaneous diseases should be considered as well as the lifetime degree of stressors to the animal.

Typically, study endpoints should be defined such as tumor volume or the progression of disease. With imaging, it should be possible to refine these endpoints as more detailed information about disease state/stage can often be obtained.

### 3.5 Future of Small Animal Imaging

It is clear that rodents as models of human disease will continue to be the most widely used animals in research in the near future. As imaging equipment adapted for these small animals becomes more available and accessible, it appears that imaging as an adjunct to biomedical research will increase.
The intent of this chapter is to introduce the reader to the animal care and use aspects of rodent-based research and imaging. They are many resources, many online, in which to find details relating to the topics discussed and further information should be sought prior to imaging-based studies. In addition, a laboratory animal veterinarian is a great resource in helping to plan studies using best practices.

REFERENCES


Chapter 3. Small Animal Handling, Care, and Anesthesia


