Tools and tips for mouse imaging and spectroscopy:
Anesthesia and physiological monitoring

Brenda A. Klaunberg, M.S., V.M.D.

NIH Mouse Imaging Facility
National Institutes of Health
10 Center Drive, Room B1D-69
Bethesda, MD 20892-1060 USA
klaunbeb@ninds.nih.gov
301-594-3898 (phone)


Introduction
Veterinarians and scientists face many challenges while utilizing animal models for research. One major goal of experimental design is the reduction of animal numbers needed to perform a useful study. Serial in vivo imaging techniques not only reduce animal study numbers and husbandry expenses, but also provide data during the course of disease or treatment in individual animals. For most imaging procedures, it is necessary to anesthetize the animal to alleviate stress and discomfort, and prevent image artifacts from movement.

Anesthesia of small animals can be challenging under any circumstances, but anesthesia during in vivo imaging presents a unique set of obstacles to the investigator (Nicholson 2008, Hildebrandt 2008). Some problems are relatively easy to overcome, while others require specialized equipment and creative improvisation. This chapter will point out some of the important challenges to maintaining physiological homeostasis in the laboratory rodent under prolonged anesthesia necessary for some in vivo imaging techniques. Additionally, problems associated with particular types of in vivo imaging will be addressed, and methods to solve or alleviate obstacles suggested.

With the advent of genetic manipulation, many disease models are generated in rodent species. Body size and different drug reactions among species and strains represent two of the biggest challenges for selection of appropriate anesthesia methods for rodent imaging procedures. Body size influences not only the selection of equipment and method of anesthesia, but also how the animal is monitored during and after a procedure (Colby 2004). To compound this problem, imaging often requires that the animal is remote from the technician and inaccessible during the image acquisition. For this reason, digital technology is used to remotely monitor respiratory rate, heart rate, core body temperature, ECG, PaO₂, end-tidal CO₂, pulse rate, and blood pressure. Although the technology for rodent physiological monitoring lags behind the recent technical advances in small animal imaging, new products are emerging and in use.

The technical challenges involved with imaging rodents requires both the scientist and veterinarian to carefully plan the entire imaging procedure from start to finish, as well as consider the post procedural care of animals. It is important for the investigator to know their imaging goals and the information they hope to gain from imaging while designing an...
experiment, and consultation with an imaging expert likely invaluable. Due to the specialized
equipment and level of expertise often needed to perform imaging procedures, there is
usually a cost imposed on the investigator for the generation of imaging data and possibly
for data analysis and storage. Careful planning will maximize the investigator’s investment
and experience with in vivo imaging and minimize the expenses accrued due to a poorly
planned study.

It is imperative to identify the equipment and skill sets required while designing an imaging
experiment since a successful study depends on a number of factors. Maintaining the
animal in physiological homeostasis during imaging is important so that the effects of
anesthesia do not skew the imaging data. Additionally, if the animal is to be recovered and
imaged repeatedly over time, deleterious effects of prolonged anesthesia must be prevented
to minimize the risk of post-procedural complications. For long-term experiments one must
consider the increased anesthetic risk to older or diseased animals and obtaining repeated
intravenous access. Genetically engineered mice are often smaller than their wild type
controls and this amplifies the technical challenges of imaging these valuable animals.
Although it is important to monitor as many physiological parameters as possible, it is often
not feasible, depending on the imaging study. For example, artifacts from ECG leads may
interfere with a study of muscle tissue. Additionally, the choices of anesthetics and
monitoring devices are critical to a functional study’s success.

**Injectable Anesthetics**

Many investigators have developed skills in the use of injectable agents when performing
short anesthetic procedures on rodents. These agents can be administered in a variety of
ways: intraperitoneal, intramuscular, subcutaneous or intravenous injection. These drugs
can be convenient to use since many are readily available as non-controlled substances and
require no special equipment; however, one problem with chemical anesthesia is the
inability to adjust the animal’s dose once given. In some animals, the standard dose may be
insufficient to achieve a stable plane of anesthesia while in others the same dose may be
excessive and lead to mortality. This is especially true when drugs with a narrow therapeutic
range and margin of safety are used (pentobarbitol).

Advantages (such as familiarity and convenience) of injectable anesthetic agents may justify
their use for short procedures (20 minutes), but when longer anesthesia is needed,
injectable anesthesia can be problematic. Repeated doses of some drugs may lead to
delayed toxicity, or more acutely overdose and death. Especially challenging is the tissue
redistribution of drugs in overweight animals making proper anesthesia difficult. Some drugs
may be used as a continuous infusion (propofol, alpha-chlorolose) allowing adjustment of
the anesthetic as needed. Although this method provides greater control over the animal’s
anesthetic plane, it is not without some cost to convenience. Alpha-chlorolose can be
administered through an intra-peritoneal line, but is still dependent upon absorption from the
peritoneal cavity. The animal’s hydration status, cardiac output, and systemic blood
pressure will all play important roles in the success of this drug. Propofol is an ultra-short,
rapidly metabolized anesthetic agent that is administered intravenously. Anesthetic levels
can be rapidly adjusted, but venous access is necessary. It can be technically challenging to
place an intravenous catheter in a rodent and often difficult to maintain its patency. The
obstacle of consistently obtaining intravenous access in a rodent is not trivial and is often
prohibitive, thus eliminating some classes of drugs from consideration by some
investigators.
Prolonged recovery time is another problem with injectable anesthesia. These agents must be metabolized and eliminated through hepatic and renal pathways. Many physiological factors, which are often adversely impacted by the drug itself (cardiac output), influence the pharmacokinetics of these drugs. Because of the time required to metabolize the drugs, recovery time is frequently 2-3 times longer than the anesthesia time. The investigator must plan for additional time to properly recover the animal and provide post-procedural care. Before the animal is fully recovered and the injectable drugs are completely metabolized, it is possible for the animal to “re-narcotize” and become sedated and immobile. An investigator may believe that animal is awake enough to move around and maintain body temperature, but when not stimulated, the rodent may stop moving, sleep again, and possibly die from hypothermia. If animals are returned to the housing room before fully awake, they are sometimes found dead the next morning secondary to hypothermia. One way to partially avoid this problem is to select anesthetic drugs that are reversible, but this does not alleviate the need to properly monitor the animal during the recovery period.

Many factors such as gender, strain, age, and health status influence an animal’s response to a drug, and various strains of laboratory mice respond quite differently to the same anesthetic agent (Gaertner et al 2008). Additionally, sub-clinical infections of rodent pathogens may affect anesthetic outcomes. It is prudent to test a few animals with the proposed anesthetic before beginning the experimental study with valuable animals.

Inhalant Anesthesia
When considering anesthesia for in-vivo imaging, length of time required for the imaging modality is an important factor. Some types of in vivo imaging are rapid and generate data within minutes (optical imaging, radiographs, ultrasound). Injectable anesthetics with appropriate recovery may be used for these types of procedures with great success. Other types of imaging such as micro Computed Tomography (CT) and magnetic resonance imaging (MRI) require the animal to be immobilized for prolonged periods of time (30 min to 3 hours). Additionally, the animal is inaccessible to the anesthetist during imaging. It is usually safer to anesthetize the animal with an inhalant anesthetic for these longer procedures. These agents can be adjusted rapidly to maintain appropriate anesthesia, are minimally absorbed, and the recovery period is short. A precision vaporizer should be used to administer the anesthetic. The delivery of anesthesia can be adjusted remotely without the need to manipulate the animal. Once an animal is optimally positioned for an imaging procedure, it is not often practical to manipulate the animal in order to administer maintenance anesthetics; hence, another advantage of inhalational anesthesia over injectable anesthesia.

Inhalant anesthesia for in vivo imaging can be delivered by three methods: endotracheal tube (ET), face-mask, or imaging chamber. Control of an anesthetized animal’s airway with an ET is beneficial so that in the event of cardiac or respiratory arrest, the animal can be artificially ventilated. However, all factors must be considered when deciding on a delivery method. In a single tube (such as a trachea) the resistance is inversely related to the radius of the tube to the 4th power \(R \propto 1/r^4\). So decreasing the airway diameter with an ET increases the resistance of the airway, making it more difficult for an animal to spontaneously move air into its lungs. For this reason, it is preferable to use the largest ET that will fit the animal’s trachea without causing trauma. This is obviously problematic in rodents whose tracheal diameters may only be 2 mm. Oral insertion of a rodent ET requires technical skill that is difficult to master. Difficulty in fully opening the oropharynx and the small diameter of the trachea make oral intubation difficult. The rat’s laryngeal opening can be visualized with a modified laryngoscope (Weksler 1994, Schaefer 1984); however, oral
intubation of the mouse is challenging and difficult no matter what method is used (Brown 1999, Spoelstra 2007, Rivera 2005).

Attempts to intubate small animals, even by experienced personnel, may traumatize the oropharynx, larynx, trachea, and at times, esophagus. Despite the lack of mucous producing cells in lower airways of rodents, airway obstruction in anesthetized mice and rats occurs frequently due to their small size and respiratory secretions. The animal’s vital signs and physiological parameters must be diligently monitored while under anesthesia to avoid complications. To overcome airway resistance when using ET in rodents and avoid the variability of tidal volumes between individuals, use of a pressure driven ventilator is strongly recommended. Ventilator assisted spontaneous ventilation works well for rodents when using an ET. Alternatively, the rodent can be paralyzed with a muscle relaxant and ventilation completely controlled by the investigator, but it must be remembered that the ET is not cuffed and the airway is not completely controlled. Physiologically stable rat ventilation is a fairly robust procedure (Strohl 1997); however, reliable noninvasive methods are still being developed for the mouse.

Delivery of inhalant anesthesia by face-mask or imaging chamber is easy, but not without inherent problems, and may preclude the use of some devices such as a bite bar for stabilization of the head during brain imaging. Unskilled anesthetists can easily master the placement of a face-mask without respiratory trauma. Use of a face-mask or chamber does not facilitate ventilation, and there is no control of the airway should an emergency occur. Because animals typically breathe spontaneously when using a face-mask, the possibility of hypoxia must be considered if the animal is not properly maintained under anesthesia. Administering inhalant anesthesia through an imaging chamber is the easiest method, but does not allow positioning of the animal once anesthetized, and the same limitations apply to the chamber as with the face-mask. If either of these methods is selected, it is important to consider the method of scavenging exhaled anesthetic gasses to prevent an occupational health hazard.

Anesthetic Monitoring
Monitoring physiological parameters of anesthetized animals is critical in every procedure, but especially during imaging since the animal is frequently at some distance from personnel. Many physiologic parameters are used to adequately determine the level of anesthesia, as well as try to maintain physiological homeostasis necessary for good data acquisition. It is common to monitor pulse and/or heart rate, blood pressure, respiratory rate, blood oxygenation, arterial blood gases, and body temperature in larger animals. The small size of mice and rats, coupled with their high heart rates and small respiratory volumes, present unique challenges to the veterinarian and technician. Equipment required to monitor these physiologic parameters may not exist or may require an invasive procedure in order to place sensors that accurately evaluate the animal’s status (Flegal 2004).

The selection of appropriate physiological monitoring equipment requires careful consideration of several factors, such as, what procedures the animal may have had previously, the length and type of the imaging procedure and capabilities of available monitoring equipment. The technical expertise of the staff using the equipment is important because rats, mice and other small rodents often require different types of monitoring equipment compared to larger species. The type of imaging equipment is a limiting factor with regard to what monitoring equipment can be used. Some imaging devices have no route for equipment lines (ECG leads) to pass from the imaging chamber to the outside for observation. Other monitoring equipment can create image artifacts that interfere with
accurate data interpretation. Also, the magnetic environment of MRI precludes the use of ferromagnetic devices. Some non-ferromagnetic devices become inactivated within the magnetic field; therefore, it is important to have safe and accurate monitoring equipment when using MRI.

**Body Temperature.** Hypothermia is a major concern for an inactive rodent due to their small size, their rapid metabolic rate and high body surface area to body weight ratio. Monitoring body temperature during anesthesia is critical to avoid hypothermia and secondary complications such as high mortality rates in post-procedural rodents. Rodents must be fully recovered before being returned to their home cages. Most anesthetic agents depress the thermoregulatory centers of the brain, and the longer the animal is anesthetized, the greater the risk of lowering core body temperature. Many types of commercial devices are available to monitor core body temperature, but a rectal temperature probe is most commonly used. A feedback system can be set up so that when the animal’s body temperature drops below a set point, the heat delivery system turns on and sends heat to the animal. Heat can be delivered by a variety of methods, and the type of method used is dependent on the imaging device. Disposable hand warmers are useful for small rodents, but contain iron particles that make them incompatible with MRI due to the magnetic effect. Additionally, iron obscures the images during CT imaging. Circulating warm water pads and heated air are alternative methods for heat delivery during imaging. Some imaging devices have warmed imaging platforms (optical, ultrasound) to facilitate maintaining warmth in an anesthetized animal.

**Respiratory Rate.** In a spontaneously breathing animal, respiratory rate helps the anesthetist evaluate anesthetic level and adequate ventilation. Adequate ventilation ensures sufficient delivery of oxygen ($O_2$) to the tissues and removal of carbon dioxide ($CO_2$). Homeostatic blood levels of $O_2$ and $CO_2$ are carefully controlled by sensors in the brain that increase or decrease respiratory rate to help adjust blood gas levels. There are secondary metabolic compensatory mechanisms, but ventilation is the fastest and simplest method of adjustment. In a normal animal, if $CO_2$ rises, the animal will increase its respiratory rate to increase alveolar gas exchange to “blow off” more $CO_2$. If the anesthetic level is too deep, this reflex may be suppressed. Assessment of the entire animal is important since an increase in respiratory rate could indicate an insufficient anesthetic level.

Monitoring spontaneous respiratory rate can be as simple as counting breaths visually, or by using a monitoring device. Some devices translate the movement of respiration via an external pressure sensor and sends a signal to the monitoring device. The respiratory waveform is displayed on a visual monitor and the signal can be used for gated imaging. Respiratory gating technique eliminates image artifact associated with the movement of breathing.

**Electrocardiogram (ECG).** ECG measures the electrical activity of the heart, but not cardiac output or other aspects of cardiac performance. Serious circulatory problems may be present with a normal ECG, such as low cardiac output; therefore, assessing the entire animal with several physiological parameters is vital. Obtaining rodent ECG tracings that give diagnostic information about the animal’s cardiac conductivity while imaging can be difficult. Although ambient electrical noises from imaging or monitoring equipment and room lights often obscure P and T waves, ECG is still useful for detecting arrhythmias and changes in heart rate. Cardiac arrhythmia can occur due to a variety of cardiac problems, metabolic disorders, or physiological imbalances that may occur with an animal under
prolonged anesthesia. Commercial devices are available to measure rodent ECG within the magnetic field of MRI and can be used to facilitate cardiac gated imaging.

In simple terms, if there is a regular QRS complex, there is a heartbeat and the animal is alive. A change in heart rate (HR) may be an early indication of improper anesthesia before other biological indicators are observed. If heart rate decreases in a normothermic animal, the anesthesia level may be too deep and the animal is at risk of dying; conversely, an increase in HR may indicate that the animal’s anesthesia level is too light. Rapid assessment and reaction to early changes in HR can prevent catastrophic events such as the animal waking up during imaging.

Heart rate indirectly reflects cardiac output (CO); CO is defined as heart rate per minute times stroke volume. Higher heart rates often mean greater cardiac output; however, an increased heart rate may be in response to other factors such as decreased oxygenation, insufficient anesthesia, hypovolemia, hypotension, or hyperthermia. An extremely high heart rate can result in insufficient time for the left ventricle to fill with blood between contractions, therefore lowering cardiac output.

Pulse Oximetry. This device provides a broad assessment of an animal’s cardiopulmonary function since it measures both pulse rate and the saturation percentage of oxygenated hemoglobin (SaO₂) in the blood. SaO₂ above 90% reflects adequate ventilation with delivery of oxygen to the tissues. If an animal is maintained under anesthesia with 100% oxygen, values greater than 95% are considered normal; lower values indicate problems with ventilation or perfusion resulting in possible hypoxemia. Placing the device on an adequately vascularized area is a challenge: the pressure of “clothes pin” type sensors, tolerated by larger animals, can interfere with tissue perfusion in smaller animals and render the sensor inaccurate. The heart rates of mice (325-780 bpm) and rats (250-450 bpm) (Hrapkiewicz 1998) are normally high compared to larger species, so the use of human and veterinary devices was limited and impractical. Pulse oximeters capable of accurately monitoring rodent heart rates have recently become available.

Blood Pressure. All anesthetic agents affect systemic blood pressure (BP) in some way through various mechanisms such as decreased cardiac output, vasodilatation, or decreased peripheral resistance. Monitoring blood pressure helps the anesthetist maintain an appropriate level of anesthesia while maintaining adequate blood flow to vital organs and tissues. Although many devices exist to measure blood pressure in rodents, the techniques are not always robust, and often are not practical when the animal is located at a distance or within an imaging device. The rodent’s small size requires specialized equipment and a need for amplification of the detected signal. Additionally, anesthetized rodents lose body heat quickly when immobile, so peripheral vasoconstriction secondary to hypothermia may prohibit pulse detection by indirect methods. Direct blood pressure measurement involves the use of an arterial catheter attached to a pressure transducer or optical sensor that converts pressure changes on its detector into electrical impulses for computerized display. Fiber optic technology provides new devices for invasive BP measurements in rodents. There are technical challenges involved with placing arterial lines in rodents, and the amplification of the blood pressure signal can be masked by interference with ambient electrical signals from other equipment and room lights.

Capnography. Use of the respiratory circuit for both CO₂ and anesthetic gas measurement is common in larger animals and reliable in rats. Sample gases taken from the respiratory circuit are analyzed for the concentration of each monitored gas. Typically, CO₂ is displayed
as inspiratory and expiratory which reflects the animal's breathing pattern. Expiratory CO₂ (end-tidal) can provide important information about the animal's physiological status (i.e. acid base balance) or provide problematic information such as possible leaks in the respiratory circuit or improper ET placement. By observing the respiratory rate along with end-tidal CO₂, one can assess anesthetic depth and ventilation status. If end-tidal CO₂ is high, the animal may be improperly ventilated or there is a problem in gas transfer between the circulatory and respiratory systems. If end-tidal CO₂ is low, the animal may be excessively ventilated (or not producing CO₂ due to death). Changes in levels of CO₂ also affect blood pH and normal homeostasis. As the blood level of CO₂ rises, and the buffering capacity of the blood is overwhelmed, the blood pH decreases and the animal becomes acidic. If this state continues, the body has metabolic compensatory mechanisms to try to correct the acidosis, but respiratory intervention is the quickest and easiest method to correct this imbalance.

**Imaging Specific Considerations**
Different imaging techniques have unique procedures or technical issues that must be considered during an in vivo study. MRI has the most stringent requirements regarding image acquisition and experimental planning. The high magnetic fields produce no known deleterious effects on the animal, but any equipment in close proximity to the magnet must be non-ferromagnetic and MR compatible. Monitoring equipment must be carefully checked for compatibility in the magnetic environment, and must be capable of working accurately in that setting without interfering with image quality. Traditional computer monitors are adversely affected by magnetic fields and should be replaced with flat-screen monitors. Non-ferrous metals such as needles, ECG leads and temperature probes may produce undesirable artifacts, but the field of view and need for metallic devices must be considered. Recent advances made in fiber optic technologies are facilitating the ease of monitoring physiological parameters in the magnetic fields. Despite the magnetic environmental limitations, anesthetic monitoring equipment should be utilized during image acquisition due to the isolation of the animal. Additionally, imaging procedures can range in time from several minutes to several hours depending on the imaging parameters.

**Summary**
With the expansion of in vivo imaging technologies available for small animals and the increase in animal models of human disease, diagnostic imaging will likely become a routine procedure. The physiological stress imposed by the restraint of small laboratory animal species necessitates the use of general anesthesia to optimize image acquisition. Some imaging procedures may be lengthy in duration; therefore, it is imperative that investigators plan the anesthesia and recovery carefully. Furthermore, it is imperative that the investigator monitors physiologic parameters of the anesthetized animals since the animals may be located at a distance from the anesthetist and, consequently, be unavailable for manipulation. Careful attention to the animal’s vital signs during imaging can prevent mortality and morbidity as well as increase the validity of data. Each imaging technique has a set of unique limitations or requirements that must be considered to complete a successful study. Careful investigator planning and collaboration with experienced imaging personnel will produce the best results.

**References**


For details about ventilation and pulmonary function, this interactive website is good: <http://sprojects.mmi.mcgill.ca/resp/index.htm>