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Overview
Clinical Considerations in Rodent Bioimaging

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Imaging modalities such as micro-computed tomography (micro-CT), micro-positron emission tomography (micro-PET), high-resolution magnetic resonance imaging (MRI), optical imaging, and high-resolution ultrasound are rapidly becoming invaluable research tools. These advanced imaging technologies are now commonly used to investigate rodent biology, metabolism, pharmacokinetics, and disease in vivo. Choosing an appropriate anesthetic regimen as well as monitoring and supporting the animal's physiologic balance is key to obtaining images that truly represent the biologic process or disease state of interest. However, there are many challenges in rodent bioimaging such as limited animal access, small sample volumes, anesthetic complications, strain and gender variability, and the introduction of image artifacts. Because each imaging study presents unique challenges, a thorough understanding of the imaging modality used, the animal's health status, and the research data desired is required. This article addresses these issues along with other common laboratory animal clinical considerations such as biosecurity and radiation safety in in vivo rodent bioimaging.

Preclinical in vivo small animal imaging modalities such as micro-computed tomography (micro-CT), micro-positron emission tomography (micro-PET), high-resolution magnetic resonance imaging (MRI), optical imaging, and ultrasound, are becoming valuable research tools for investigating biology and disease. Recent applications also include phenotyping (10, 41) and studying gene expression (16, 65). Reduced equipment costs, improved image quality, faster data processing, and increased user-friendliness have contributed to their increasing popularity and use. However, the benefits of these technologies also present new challenges to the laboratory animal professional. Delivering adequate and appropriate anesthesia of up to several hours in duration, maintaining physiologic homeostasis during functional imaging, limited patient access (Fig. 1 and 2), and remote monitoring of small rodents are major issues that must be addressed. The small size of rodents necessitates the use of specialized monitoring equipment. Frequently encountered obstacles include obtaining vascular access, limited sample volumes, dead space in anesthesia circuits, rapid development of hypothermia, strain variation or study-related alterations in anesthetic sensitivities, image interference, and prolonged or repeated anesthetic episodes.

One advantage of non-invasive imaging is the ability to obtain data from individual animals at multiple time points and to use an animal as its own internal control, thereby reducing the number of animals required for a given study (13). However, the physiologic impairment or unexpected death of an animal can be profound because it may effectively remove multiple experimental observations during a study.

With many imaging devices (MRI, CT, and PET), common means of monitoring and interacting with an anesthetized animal are not always possible because equipment design or safety issues can dictate equipment selection and animal manipulation. For example, because of the high magnetic field constantly present around an MRI unit, no items containing ferromagnetic metals (iron-containing metals that are influenced by a magnetic field) can be used within close proximity because such items can become projectiles. Therefore, all potentially hazardous items including medical (e.g., anesthesia and monitoring machines,

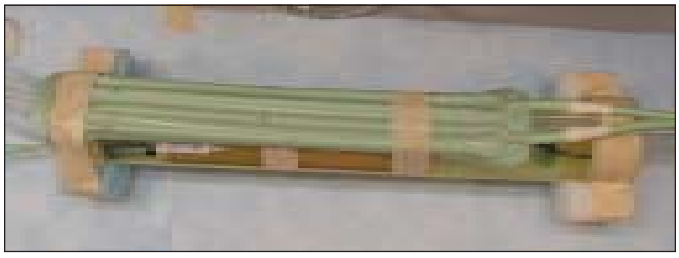

Figure 1. Rat positioned within an RF (radiofrequency) coil prior to placement in the bore of an MRI unit. Note that only the tail tip is visible.

Figure 2. Mouse placed on the bed of a CT scanner and insulated to help maintain body temperature.

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needles, catheters) and personal (e.g., keys, jewelry, pens) should be screened for ferromagnetic metals before entering an MRI suite. In addition, previously performed procedures should be reviewed to ensure that no ferromagnetic metals (e.g., metal sutures, clamps, implants, catheters) have been implanted or affixed to the animal.

Many vendors now provide MRI-compatible equipment including anesthesia machines and monitoring equipment. If standard equipment must be used, it should be located in a separate room, shielded from the magnetic forces. Penetration panels are positioned in the intervening wall through which MRI-compatible tubing and monitoring equipment lines can be passed. Even if equipment is firmly secured in an imaging room, it may still be negatively affected by the magnetic field. In addition, only flat screen monitors should be used because conventional monitors can both influence and be influenced by the magnetic field.

Multi-modal Imaging

Algorithms have been developed for rodent bioimaging that superimpose image information from multiple modalities such as PET, MRI, and CT to give additional spatial and quantitative information (42, 58). Obtaining such data necessitates that the animal remain in the exact position during multiple imaging sessions. This is best accomplished by placing the animal on a universal imaging bed that can be moved between and is compatible with the desired modalities. The animal, anesthesia tubing, and monitoring cables must be securely fixed to this bed during transport. Several manufacturers are developing multifunctional imaging technologies that would eliminate the need for transport (33).

Anesthesia

Although anesthesia is essential for invasive procedures and immobilization during imaging, anesthetics may introduce variables by altering cardiovascular, respiratory, and central nervous functions (25, 44). Further, serial imaging often requires repeated anesthesia, each episode of which can extend up to 6 h. Metabolic and physical stressors that accompany anesthesia, such as hypothermia, pulmonary atelectasis, hypercapnia, acidosis (71), hypoxia, hepatic toxicity (73), and tracheal irritation from repeated intubations, can vary depending on the length of the anesthetic episode and the anesthetic protocol used. Because these consequences may profoundly affect research results and the health of the animal, imaging frequency, anesthesia length, and anesthetic regimen, should be considered pre-emptively.

Anesthesia should be easily administered and produce anesthesia for the duration of the scan or allow for easy, often remotely administered supplementation (79). Although intramuscular or subcutaneous anesthetics have been used, inhalant anesthetics delivered through a non-rebreathing circuit (71) and intravenous anesthetics allow remote adjustment of anesthetic depth. Prolonged inhalant anesthesia is relatively safe for rodents when delivered with a precision vaporizer (78). Commonly used anesthetics are not without risk or complications that could interfere with research. For example, immunomodulatory effects have been documented in mice for up to 9 days following three 40-min weekly exposures to sevoflurane (19). Consequently, the time period between imaging sessions needs to be carefully considered and anesthetic effects fully understood.

It is important that the anesthetists and investigators have an understanding of how the prescribed anesthetic protocol will affect individual animals. Strain differences in response to anesthetics also have been documented (30, 68). Some genetically engineered rodents have been shown to vary in susceptibility to anesthesia-associated morbidity and mortality (21, 27, 30, 68, 69). Gender and age may also influence physiologic responses to anesthetics (76). It is important to note that researchers using the same drugs in previously published studies may not have considered their potential effect on the animals selected for imaging studies (21). Performing pilot studies prior to imaging may be advisable to assess proposed anesthesia protocols.

Functional Imaging

Functional imaging is designed to detect a physiologic event or series of events in response to an experimental action. This may include the distribution or metabolism of an agent, changes in blood flow or blood volume, or alterations in organ function. Maintaining an animal within a stable and narrow physiologic range is key to measure physiologic responses related to the experimental action and minimize responses elicited by animal manipulation (14). As implied earlier, anesthesia can markedly influence functional imaging procedures by affecting such parameters as blood flow, tissue oxygenation, and diffusion of test substances (5, 25, 44). The ability to detect and manipulate variation between animals allows one to manage and decrease the impact of natural variables between animals or groups of animals (81).

Numerous modalities can be employed for functional imaging including MRI, PET, magnetic resonance spectroscopy (MRS) (51), and optical imaging. For example, by detecting hemodynamic changes within the brain (54), functional magnetic resonance imaging (fMRI) can detect regional brain activity (60) or drug–receptor interactions (81). However, the distribution and intensity of brain activity can be influenced by factors affecting cerebral blood volume, cerebral blood flow, and blood oxygenation levels (47, 81). Therefore, one must consider and compensate for physiologic responses induced by the administration of fluids, anesthetics (47), analgesics, and test substances, and by artificial ventilation (8), alterations in blood oxygenation, body temperature, and blood pressure (60, 81).

PET can quantify cerebral glucose metabolism and approximate regional neuronal activity. Cerebral glucose metabolism can be estimated by administering the radiotracer 2-[^{18}F]fluoro-2-deoxyglucose (FDG) intravenously, allowing time for the substance to be taken up by the brain, and then imaging the brain to determine the location and intensity of FDG uptake (55, 74). With PET, the same precautions regarding physiologic responses noted for fMRI must be considered. However, one must also consider the activity level of the animals after FDG administration. The glucose analog, FDG, is not specific for brain tissue and can be absorbed by other tissues that utilize glucose as an energy source (24). If an animal is very active after FDG administration, high levels of FDG can be detected in muscle tissues and proportionally decreased levels may be detected in the brain (29).

Monitoring During Imaging

Requirements for monitoring and physiologic support depend on many factors including the health of the animal, the anesthetic employed, and the objective of the imaging procedure. Healthy animals anesthetized with an inhalant anesthetic and undergoing a 5-min MRI to determine the size of a subcutaneous

tumor may require only minimal monitoring and thermal support. However, more intensive monitoring and support would be indicated for an animal weakened by prior experimental manipulations and undergoing a prolonged functional imaging procedure to determine the diffusion of an agent within the brain.

Physiological monitoring of rodents can be challenging due to their size and rapid heart (mouse, 328 to 780 bpm; rat, 250 to 600 bpm) and respiratory (mouse, 80 to 230 rpm; rat, 66 to 114 rpm) rates (2, 70). Monitoring equipment originally used for larger animals has been redesigned for rodent bioimaging. However, compatibility with the given modality must be considered when selecting monitoring equipment. Factors to consider when purchasing rodent monitoring equipment have been described (23).

Body temperature. Rodents are highly susceptible to hypothermia due to their large surface-area-to-body-mass ratio and rapid metabolism (3). In addition, anesthetic agents may suppress the thermoregulatory centers of the brain, inhibiting response to decreased body temperature (28). Hypothermia may develop in rodents even during relatively brief (10- to 15-min) procedures (22). Besides affecting health, hypothermia may affect parameters such as metabolism and heart rate (59). Therefore, an animal's core body temperature must be closely monitored and maintained.

Body temperature can be monitored with probes including rigid or flexible thermistors. Depending on the imaging modality being employed and the physical properties of the instrument, some probes may be impractical, produce image distortions, or present a safety hazard. Probes are available in multiple sizes allowing their use with small rodents including mice. Temperature probes most frequently are placed in the rectum. However, esophageal temperature probes may be used if their diameter permits passage through the thoracic inlet without vascular compression. Although many probes are constructed of metal, fiberoptic probes are also available. Although markedly more expensive, they will not induce image artifacts when used with MRI or CT. If a probe must be used that may induce an image artifact, attempts can be made to position it outside of the imaging field. For example, when a CT scan of the abdomen is performed, the probe may be placed in the axillary area. Although this will prevent precise determination of core body temperature, major alterations can still be detected.

A self-regulating heating device can be safe and easy to use (59). They generally consist of three units: a probe, a temperature controller, and a warming source (such as a warm water recirculating blanket). The temperature controller integrates with the probe so that the animal's body temperature is continually monitored. Changes in the body temperature are then automatically adjusted for by altering the temperature of the warming source, thereby maintaining the animal within a very narrow temperature range. With MRI, CT, or PET, a rigid warm-water re-circulating pad may be preferable over a flexible blanket, as use of the latter may introduce image artifacts as the flexible blanket inflates and deflates in response to the cycling of the water pump contained within the heating unit. However, water pads of any design may induce image artifacts during MRI.

Other compatible heating devices include warm-water re-circulating blankets (independent of a temperature controller); isothermal heating pads (which should not be placed in direct contact with the animal due to the risk of thermal burns); heated slide warmers; heat lamps (that must be placed at least one meter from the patient and actively monitored to prevent burns [11]); medical warm air blowers (59); and portable, disposable hand warmers (61). Portable handwarmers used by outdoor enthusiasts also can be used to directly or indirectly heat the animal. For example, when wrapped around the anesthetic tubing immediately proximal to the animal, handwarmers can heat the animal indirectly by increasing the temperature of inspired gases.

Attempts should be made to insulate the animal from the environment or to control the environmental temperature to decrease heat loss. With select optical imaging equipment, temperature within the imaging chamber can be regulated. In addition, with most imaging modalities, it is possible to cover or wrap the animal with a drape or bubble wrap to decrease radiant heat loss. Care should be taken during insulation to include the tail as it is a vascular structure with a proportionally large surface area (11).

Hydration. Rodents undergoing lengthy imaging procedures under anesthesia may become dehydrated. Therefore inspired gases should be humidified by bubbling them through a water bath. Parenteral administration of warmed fluids also may help to prevent or treat dehydration (3, 64). However, there is controversy over the advisability of fluid administration in select rodent studies. Zuurbier and colleagues (83) found that administering intravenous fluids to different strains of mice who were anesthetized and mechanically ventilated helped to maintain blood pressure, but increased organ water content, decreased total hemoglobin, and increased acidosis.

Respiratory System

Anesthetics are known to depress respiration (53). Therefore, it must be monitored and appropriately supported in order to maintain health and to reduce variables introduced by poor ventilation including hypoxia, hypercapnia, and acidosis (63). For example, these changes can affect results, especially during functional imaging, by altering drug metabolism or cerebral blood flow (25, 44, 74). Some genetically engineered strains are highly susceptible to respiratory complications during anesthesia as mentioned above (63). Hypoxia can occur even if a high concentration of oxygen is administered, because of anesthetic-induced respiratory depression, airway obstruction, or pulmonary atelectasis during prolonged anesthesia (34).

It has become increasingly common to intubate rodents during prolonged imaging procedures (9, 21). Intubation facilitates delivery of inhalant anesthetics, provides a means to deliver positive pressure ventilation, and facilitates respiratory monitoring and gating. It has the added advantage of providing a secure connection between the animal and anesthetic machine so there is no disruption in the supply of delivered air.

Rodent intubation sets are available commercially, although many individuals still adapt common medical equipment. For example, intravenous catheters can double as endotracheal tubes. Because of the elasticity of the rodent trachea, a cuffed endotracheal tube is not required to obtain a tight seal. A pediatric laryngoscope with a modified Miller blade can be used to visualize the laryngeal folds in rats, thereby facilitating tube placement. High-intensity, small-diameter light sources can be used to transcutaneously illuminate the mouse trachea during tube placement. Alternatively, a dissecting microscope, a fiberoptic scope, or other lighted source of magnification may be

used to directly visualize the rat or mouse larynx. Regardless of method, proper positioning, including the use of intubation boards, facilitates intubation. For terminal procedures, the endotracheal tube can be placed after a tracheotomy (61).

Once an animal is intubated, artificial ventilation can be provided. It will improve ventilatory efficiency and prevent respiratory depression or apnea (36). Recommended methods for performing and monitoring artificial ventilation in rodents are well established (62, 63). Artificial ventilation may also be used to reduce variability within a study. For example, it would be useful to optimize and standardize ventilation among mouse strains that may display differing levels of sensitivity to hypoxia or hypercapnea (63, 72). If an animal is ventilated for prolonged periods, it may be necessary to wean it from the ventilator to stimulate resumption of spontaneous respiration (18).

Use of artificial ventilation is not without risk; its use should only be employed if the user is familiar with the system and is prepared to address the potential complications that may arise (18). Common complications include hyperinflation of the lung resulting in barotrauma and pressure-induced hemodynamic abnormalities. Positive-pressure ventilation can also decrease cardiac output and blood pressure, resulting in inadequate tissue perfusion.

Monitoring methods independent of the ventilator (e.g., capnograph, pulse oximeter, respiratory pillow) should be used to confirm adequate ventilation and to help interpret visual observations. For example, an animal may attempt to breath out of synchrony with the ventilator. This may occur due to one of two reasons: the animal may not be in a sufficient plane of anesthesia, or the animal may be under-ventilated and thus not have the opportunity to adequately eliminate CO_2. It is important to correctly identify the cause of resistance for proper correction. For example, if the anesthetic plane is too light, the dose of delivered anesthetic should be increased. If an animal is under-ventilated, the ventilatory rate or the tidal volume should be increased; increasing the anesthetic dose would be contraindicated. Such conditions can be differentiated with concurrent use of a capnograph.

Often in bioimaging, there is considerable distance between the animal and anesthesia equipment. Therefore, an anesthesia system must minimize deadspace in the breathing circuit (36). The mixing of fresh and expired air must be minimized so that the animal does not rebreathe air high in carbon dioxide and low in oxygen or gas anesthetics (if present). Endotracheal tubes and connectors as well as monitoring equipment should be designed to minimize deadspace (35, 36). Non-rebreathing anesthetic systems suitable for use in rodents also have been described (49).

Respiratory rate can be approximated through chest movement detected by a small compressible pillow (P-resp Pneumatic Pillow, SA Instruments, Stony Brook, N.Y.) integrated with a pressure transducer. The animal's respiratory movement compresses the pillow and affects the pressure transducer that is linked to a computer that provides a graphical display of movement and the calculated respiratory rate. Respiratory movement, per se, does not indicate successful air exchange. An animal will initially display respiratory movement even if a full or partial obstruction occurs within the breathing circuit. Therefore, additional monitoring methods, such as with use of a capnograph, should be used to confirm adequate ventilation.

A CO_2 monitor, which measures the level of CO_2 in expired gas, can facilitate generation of a capnograph to display the concentration of expired CO_2 as a function of time. The location of the sample chamber is important. Only CO_2 monitors with a mainstream sample chamber should be used for rodents. Mainstream sample chambers, in contrast to sidestream chambers, are positioned inline with the breathing circuit (immediately adjacent to the endotracheal tube) so that no fresh gases are diverted from the animal. Unless designed for use with rodents, they may add a considerable amount of deadspace to the breathing circuit. Sidestream sample chambers are designed to divert expired gases from the breathing circuit for sampling. When used with large animals, the volume of gases diverted is relatively insignificant. However, with rodents whose tidal volumes may range from approximately 0.5 to 6 cc per animal (35), the volume of gas sampled is actually a mixture of fresh and expired gases. As a result, sidestream monitors prevent the animal's access to adequate fresh gases and may dilute the expired gas, leading to underestimation of end-tidal CO_2 (11, 31, 35, 63).

End-tidal CO_2 is used to approximate the level of arterial CO_2 and assess the adequacy of ventilation (11). The shape of the capnograph waveform can indicate conditions such as circulatory shock, rebreathing CO_2, a leak in the breathing circuit, improper endotracheal tube placement, and airway obstruction (23, 31). Carbon dioxide monitors also can be used to determine respiratory rate as well as its end-tidal CO_2.

Serial measurements of arterial blood gases are the gold standard for assessing respiratory function (63). Body temperature at the time of sample collection should be provided to the blood gas analyzer so that temperature corrections can be made. The partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2) and the pH of the blood can be measured from a single sample. From these, the blood bicarbonate concentration (HCO_3^-) and base excess can be calculated. The Siggaard-Andersen chart is useful in interpreting blood gas results (52, 67). Treatment for disrupted acid–base balance includes identifying and correcting the cause (e.g., hyper- or hypoventilation, excessive fluid therapy, drug administration). In addition, disruption can be compensated for by manipulating respiration or administering sodium bicarbonate or select electrolytes (17).

Blood gas analyzers that require very low sample volumes have been developed, facilitating their use with rodents. Therefore, multiple samples can be collected during a prolonged scan. However, the cumulative sample volume should be limited to prevent hematological variability that may distort results. Continuous intra-arterial blood gas monitoring that does not require blood to be withdrawn is available for rats (56).

Cardiovascular system. Electrocardiogram (ECG) and blood pressure devices are available to monitor the cardiovascular system of rodents. Care must be taken when selecting machines for use with rodents (23). An ECG system must be able to detect the rapid heart rate of mice and rats, and a blood pressure device must be able to accommodate their small size. Monitoring equipment in close proximity to the animal must be compatible with the imaging modality.

Electrocardiograms frequently are used during imaging to monitor heart rate and rhythm, and to help detect conduction defects, myocardial ischemia, and metabolic disturbances. Electrocardiographs can also be used to synchronize heart rate with image acquisition (discussed below). Because electromechanical dissociation can occur, in which there may be inefficient cardiac pumping action despite a normal ECG tracing, multiple monitor-

ing methods should be used to obtain an accurate assessment of overall physiologic condition. For instance, use of a capnograph (which reflects both respiratory and cardiovascular function) can compliment electrocardiography. During electromechanical dissociation, a capnograph would detect high levels of exhaled CO_2 resulting from poor circulation.

Needle or patch electrodes are used most frequently for electrocardiography in rodents. However, the composition of the electrodes must be compatible with the imaging modality. Alligator clips should not be used unless they have been properly modified to prevent soft tissue injury. With CT, MRI, or PET, electrodes should be placed outside of the region of interest to prevent image artifacts. Titanium electrodes are non-ferromagnetic and can be used with MRI. ECG boards with integral electrodes are commonly used with ultrasound or optical imaging systems. Therefore, an animal's paws can be placed in direct contact with the electrodes. Sufficient conduction gel must be applied to ensure a sound connection between the paws and the electrodes.

Because of the influence of an MRI's magnetic force on electrical signals, MRI units may distort the ECG waveform obtained from a conventional electrocardiograph. This can negatively influence cardiovascular monitoring and cardiac gating (4, 46).

The cardiovascular system is also frequently assessed during imaging through direct or indirect blood pressure measurements (43, 50, 75). Direct measurements are more accurate (11) as they can detect the pressures exerted within a catheterized artery such as the femoral or carotid artery. However, this method requires arterial catheterization (77) which can be technically challenging, time-consuming, and difficult to maintain. Indirect measurement typically employs an inflatable tail cuff, a pressure sensor, and a probe to detect arterial blood flow (32). Indirect measurements are less accurate than direct measurements and are intermittent rather than continuous (43). A review of direct and indirect methods to assess rodent arterial blood pressure has been published (75).

Pulse oximeters measure oxygenated hemoglobin (SaO_2) in the blood and evaluate tissue oxygenation when blood gas analysis is not available. The use and limitations of pulse oximeters have been described (31). The small size and rapid heartbeat of rodents make their use difficult in these species. Only pulse oximeters proven to be accurate with a given species should be used. Pulse oximeters are available for rats; however, to the authors' knowledge, no systems have been validated for mice. Although they are useful for monitoring an animal with a high SaO_2 and a correspondingly high partial pressure of oxygen (PaO_2), they may underestimate a significant decrease in PaO_2 as the SaO_2 level falls below 90% (31). In addition, inaccurate results will be obtained if blood vessels near the pulse oximeter probe undergo vasoconstriction.

Gating. MRI, CT, and PET can image a body area or organ that is under continuous movement as a result of the heartbeat or respirations. However, blurring of the image can occur if gating is not used. Gating eliminates motion artifacts and improves image quality by synchronizing image acquisition with precise periods during the respiratory or cardiac cycle (40). As a result, images are only collected or analyzed for a fraction of time during each heartbeat or respiration. Images are then averaged over a constant position within the cardiac or respiratory cycle. Although this results in slightly longer scans, image definition is greatly enhanced (12) (Fig. 3).

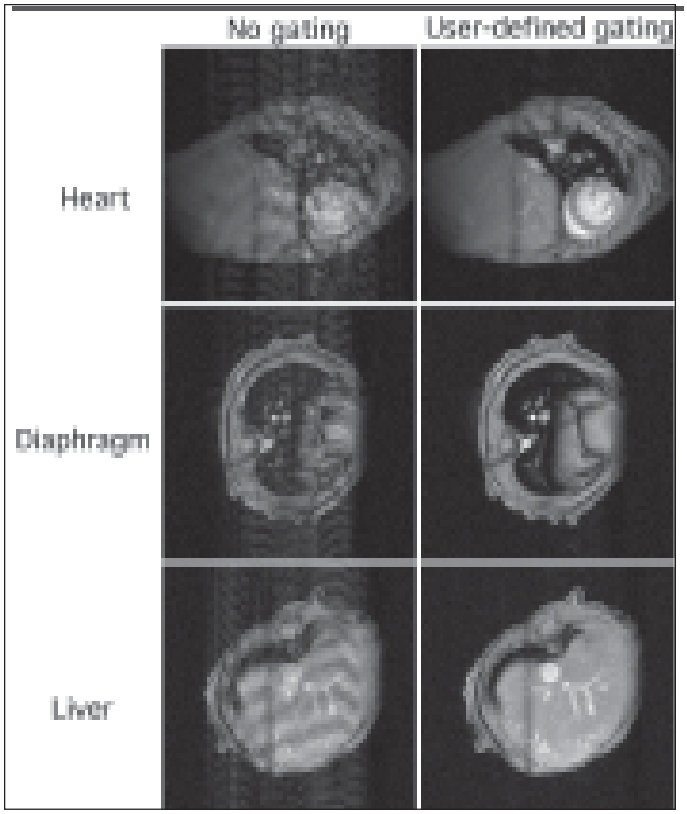

Figure 3. Illustration of improved image quality obtained from an MRI scan with and without using a motion gating system. Images are obtained through the heart, diaphragm, and liver. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., from the article "Assessment of motion gating strategies for mouse magnetic resonance at high magnetic fields," by P. J. Cassidy, J. E. Schneider, S. M. Grieve, C. Lygate, S. Neubauer, and K. Clarke. ©2004.

(i) Respiratory gating. Respiratory gating systems synchronize image acquisition with respiratory movement. Artificial ventilation simplifies imaging as the ventilator can be integrated into the gating system (36). A frequently used direct respiratory detection method for spontaneously ventilating animals employs the compressible pillow coupled to a pressure transducer as described above. Other direct methods utilize an ECG signal, an impedance pneumograph, or motion sensors (48). Triggers for respiratory gating also can be detected indirectly. In one indirect method, images are obtained throughout all phases of the respiratory cycle. Post-processing selects images obtained during a specified phase of the cycle for analysis. Another indirect method examines previously obtained images and then times future image acquisition based on the prediction as to when the next optimal imaging period will occur (12).

With select imaging modalities such as ultrasound or optical imaging, use of a high-frequency oscillatory ventilator may eliminate the need for respiratory gating. It delivers very low tidal volumes at a rapid rate, keeping the lungs under constant positive pressure and the alveoli continually expanded, which results in limited respiratory movement. Unfortunately, it cannot be used with MRI or CT because the ventilator uses a strong magnet to deliver oscillatory ventilation. Under any circumstance, its applicability would depend on the imaging modality's spatial resolution and susceptibility to artifacts (36).

(ii) Cardiac gating. Cardiac gating synchronizes image acquisition with the cardiac cycle. One method synchronizes image acquisition with a specific time point within the electrocardiographic RR interval (40). However, the electromagnetic force generated by a MRI magnet may distort the ECG waveform, making accurate detection of R waves unreliable. To decrease or prevent distortion, gating systems have been developed that incorporate a fiberoptic esophageal stethoscope which is unaffected by an electromagnetic field. The stethoscope is positioned near the heart, each heart-beat detected through esophageal compression, and the resulting interruption of the optic signal transmitted by the fiberoptic cable. Image acquisition can then be synchronized to the heart's movement (1, 4).

Imaging of Conscious Animals

With most rodent imaging, general anesthesia is required to reduce motion artifact. As emphasized in previous sections, use of anesthetics can complicate data interpretation (38, 66). Recent advances have enabled imaging of conscious animals. These systems incorporate stereotaxic restraint into traditional rodent imaging modalities or attach miniaturized imaging devices directly to the animal (7, 66, 80). Their use shows great potential especially in neurophysiology and fMRI. However, there is some debate over the potential for restraint-induced stress to influence experimental results (15). Optical imaging systems also have been developed for imaging freely moving conscious animals (82).

Radiation Exposure

Radiation exposure during serial in vivo imaging (e.g., micro-PET and micro-CT) depends on tracer dosages and imaging parameters. Exposure during micro-CT typically ranges from 10 to 50cGy (26, 57). Even at these low-levels, stimulation of DNA repair, free-radical detoxification, stimulated immune responses to neoplastic cells, and apoptosis have been noted (20). In addition, doses as low as 1 cGy have reduced lymphoma tumor volume in mice (6). PET tracer doses typically range from 0.5 to 2.0 mCi for rats and from 50 to 200 μCi in mice (15). Total doses acquired following frequent serial imaging of rodents are clinically significant although they do not typically result in cumulative lethal levels of 5-6Gy (16, 39).

Radioactive Tracers

Common radiotracers used in micro-PET imaging are relatively short-lived, with half-lives ranging from 122 sec for O^{15} to 110 min for F^{18} (15). However, they do leave animals briefly radioactive (37). Therefore, care must be taken to ensure that animal care workers, animal handlers, investigators, and veterinarians are trained appropriately in handling radioactive materials. Radioactive animals should be physically separated from other animals until background levels are measured. Radioactive animal waste and bedding can usually be left overnight to decay 7 to 9 half-lives; they then are disposed of as non-hazardous waste.

Biosecurity

Imaging facilities frequently are challenged with issues related to biosecurity. Methods to exclude infectious organisms from imaging facilities as well as pathogen containment should be well established and understood by all users. This can be especially challenging for centrally located or shared imaging facilities. Numerous strategies may be employed by imaging facilities to control health risks inherent in consolidating animals from multiple sources. For example, animals may be accepted into a core imaging facility but then euthanized and not allowed to return to the original facility. Unfortunately, this strategy eliminates one of the main advantages of imaging, the ability to perform sequential scans on an individual animal. Alternatively, animals may be released from the imaging facility but then quarantined prior to re-introduction to the original colony. This could be associated with an increased expense due to the cost of a health surveillance program and an additional risk of spreading a contaminant between facilities. Another strategy entails placing the animals in a mobile, self-contained chamber for the duration of time it is in the imaging suite. The chambers must be equipped with an appropriate means to prevent the airborne spread of contaminants and to provide adequate air quality and temperature levels. Currently, the authors are not aware of commercial sources for such isolation chambers, although individuals are actively exploring their use with micro-CT and MRI (unpublished data).

Regardless of the flow of animals between or within facilities, it is advisable to disinfect equipment (including anesthetic equipment) and work surfaces appropriately between individual animals or groups of animals. Care must be taken to avoid damaging sensitive and expensive equipment by use of corrosive agents or through exposure to water or heat. If possible, equipment that cannot be adequately disinfected should be protected by a disposable, water impermeable cover to reduce contamination. It is also advisable to maintain a daily log detailing imaging equipment usage time and animal information (responsible investigator, animal identification, and animal housing locations before and after the scan). A log could be invaluable in tracking the source of an outbreak as well as identifying other animals who may have been exposed.

Conclusion

Obtaining accurate and consistent images of animal models is one of rodent bioimaging's greatest strengths, but also its greatest challenge. Success relies heavily on choosing the appropriate anesthetic regimen, maintaining metabolic homeostasis, and obtaining suitable monitoring equipment. Because each study is likely to present unique conditions, a thorough understanding of the imaging modality, the animal's health status, and research aim is required by everyone involved, especially the laboratory animal professional. With that background information and some imagination, many of the clinical challenges encountered in rodent bioimaging can be addressed successfully. Despite great strides in rodent imaging, this recent and rapidly advancing field holds abundant opportunities for research and discovery.

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References

1. **Ahmad, I. M., R. G. Wise, G. A. Gresham, G. Bronns, T. A. Carpenter, L. D. Hall, and C. L. Huang.** 2002. Non-invasive magnetic resonance imaging assessment of myocardial changes and the effects of angiotensin-converting enzyme inhibition in diabetic rats. J. Physiol. **538:**541-553.
2. **American Association for Laboratory Animal Science.** 2002. Laboratory animal data: quick reference guide for researchers, p.23, 35. American Association for Laboratory Animal Science, Memphis, Tenn.
3. **Balaban, R. S. and V. A. Hampshire.** 2001. Challenges in small animal noninvasive imaging. ILAR J. **42:**248-262.
4. **Brau, A. C., C. T. Wheeler, L. W. Hedlund, and G. A. Johnson.** 2002. Fiber-optic stethoscope: a cardiac monitoring and gating system for magnetic resonance microscopy. Mag. Reson. Imaging **47:**314-321.
5. **Beckmann, N., T. Mueggler, P. R. Allegrini, D. Laurent, and M. Rudin.** 2001. From anatomy to the target: contributions of magnetic resonance imaging to preclinical pharmaceutical research. Anat. Rec. **265:**85-100.
6. **Bhattacharjee, D. and A. Ito.** 2001. Deceleration of carcinogenic potentially by adaptation with low dose gamma irradiation. In Vivo **15:**87-92.
7. **Brevard, M. E., T. Q. Duong, J. A. King, and C. F. Ferris.** 2003. Changes in MRI signal intensity during hypercapnic challenge under conscious and anesthetized conditions. Mag. Reson. Imaging **21:**995-1001.
8. **Broux, C., I. Tropres, O. Montigon, C. Julien, M. Decorps, and J. F. Payen.** 2002. The effects of sustained hyperventilation on regional cerebral blood volume in thiopental-anesthetized rats. Anesth. Analg. **95:**1746-1751.
9. **Brown, R. H., D. M. Walter, R. S. Greenberg, and W. Mitzner.** 1999. A method of endotracheal intubation and pulmonary functional assessment for repeated studies in mice. J. Appl. Physiol. **87:**2362-2365.
10. **Budinger, T. F., D. A. Benaron, and A. P. Koretsky.** 1999. Imaging transgenic animals. Annu. Rev Biomed Eng. **1:**611-648.
11. **Cantwell, S. L.** 2001. Ferret, rabbit, and rodent anesthesia. Vet. Clin. North Am. **4:**169-191.
12. **Cassidy, P. J., J. E. Schneider, S. M. Grieve, C. Lygate, S. Neubauer, and K. Clarke.** 2004. Assessment of motion gating strategies for mouse magnetic resonance at high magnetic fields. Mag. Reson. Imaging **19:**229-237.
13. **Chatham, J. C. and S. J. Blackband.** 2001. Nuclear magnetic resonance spectroscopy and imaging in animal research. ILAR J. **42:**189-208.
14. **Chen, Y. C. I., W. R. Galpern, A. L. Brownell, R. T. Matthews, M. Bogdanov, O. Isacson, J. R. Keltner, M. F. Beal, B. R. Rosen, and B. G. Jenkins.** 1997. Detection of dopaminergic neurotransmitter activity using pharmacologic MRI: correlation with PET, microdialysis and behavioral data. Mag. Reson. Med. **38:**389-398.
15. **Cherry, S. R.** 2001. Use of positron emission tomography in animal research. ILAR J. **42:**219-232.
16. **Cherry, S. R.** 2004. In vivo molecular and genomic imaging: new challenges for imaging physics. Phys. Med. Biol. **49:**R13-48.
17. **Day, T. K.** 2002. Blood gas analysis. Vet. Clin. North Am. Small Anim. Pract. **32:**1031-1048.
18. **Drellich, S.** 2002. Principles of mechanical ventilation. Vet. Clin. North Am. Small Anim. Pract. **32:**1087-1100.
19. **Elena, G., N. Amerio, P. Ferrero, M. L. Bay, J. Valenti, D. Colucci, and N. R. Puig.** 2003. Effects of repetitive sevoflurane on immune response, select biochemical parameters and organ histology in mice. Lab. Anim. **37:**193-203.
20. **Feinedegen, L. E. and M. Pollycove.** 2001. Biologic responses to low doses of ionizing radiation: detriments versus hormesis. J. Nucl. Med. **42:**17N-27N.
21. **Flecknell, P. A.** 1993. Anaesthesia of animals for biomedical research. Br. J. Anaesth. **71:**885-894.
22. **Flecknell, P. A.** 1996. Laboratory animal anaesthesia, p. 161-171. Academic Press, San Diego, Calif.
23. **Flegal, M. C. and S. M. Kuhlman.** 2004. Anesthesia monitoring equipment for laboratory animals. Lab Anim. (N.Y.) **33(7):**31-36.
24. **Gambhir, S. S.** 2002. Molecular imaging of cancer with positron emission tomography. Nature Rev. Cancer **2:**683-693.
25. **Gjedde, A. and M. Rasmussen.** 1980. Pentobarbital anesthesia reduces blood-brain glucose transfer in the rat. J. Neurochem. **35:**1382-1387.
26. **Goertzen, A. L.** 2003. Development of a combined microPET and microCT system for mouse imaging. PhD dissertation. University of California, Los Angeles.
27. **Gong, D., Z. Fang, P. Ionescu, M. J. Laster, R. C. Terrell, and E. Eger.** 1998. Rat strain minimally influences anesthetic and convulsant requirements of inhaled compounds in rats. Anesth. Analg. **87:**963-966.
28. **Graham, S. and E. S. Lin.** 2003. Metabolism and temperature regulation, p. 475-493. *In* C. Pinnock, T. Lin, and T. Smith (ed.), Fundamentals of anaesthesia. Greenwich Medical Media Ltd., London.
29. **Green, M. V.** 2004. Personal communication.
30. **Groeben, H., S. Meier, C. G. Tankersley, W. Mitzner, and R. H. Brown.** 2003. Heritable differences in respiratory drive and breathing pattern in mice during anesthesia and emergence. Br. J. Anaesth. **91:**541-545.
31. **Hackett, T. B.** 2002. Pulse oximetry and end tidal carbon dioxide monitoring. Vet. Clin. North Am. Small Anim. Pract. **32:**1021-1029.
32. **Hartley, C. J., G. E. Taffet, A. K. Reddy, M. L. Entman, and L. Michael.** 2002. Noninvasive cardiovascular phenotyping in mice. ILAR J. **43:**147-158.
33. **Hasegawa, B. H., K. H. Iwata, M. C. Wu, A. J. Da Silva, H. R. Tang, W. C. Barber, A. H. Hwang, and A. E. Sakdinawat.** 2002. Dual-modality imaging of function and physiology. Acad. Radiol. **9:**1305-1321.
34. **Haskins, S. C.** 1996. Monitoring the anesthetized patient, p. 409-424. *In* J. C. Thurmon, W. J. Tranquilli and G. J. Benson (ed.), Lumb and Jones' veterinary anesthesia. Lippincott Williams and Wilkins, Philadelphia.
35. **Hedlund, L. W., G. P. Cofer, S. J. Owen, and G. A. Johnson.** 2000. MR-compatible ventilator for small animals: computer-controlled ventilation for proton and noble gas imaging. Mag. Reson. Imaging **18:**753-759.
36. **Hedlund, L. W. and G. A. Johnson.** 2002. Mechanical ventilation for imaging the small animal lung. ILAR J. **43:**159-174.
37. **Herschman, H. R.** 2003. Micro-PET imaging and small animal models of disease. Curr. Opin. Immunol. **15**:378-384.
38. **Hoffman, R. M.** 2002. Green fluorescent protein imaging of tumor cells in mice. Lab Anim. (N.Y.) **40(4):**34-41.
39. **Holdsworth, D. W. and M. M. Thornton.** 2002. MicroCT in small animal and specimen imaging. Trends Biotechnol. **20:**S34-S39.
40. **Jelicks, L. A., J. Shirani, M. Wittner, M. Chandra, L. M. Weiss, S. M. Factor, I. Bekirov, V. L. Brauntein, J. Chan, H. Huang and H. B. Tanowitz.** 1999. Application of cardiac gated magnetic resonance imaging in murine Chagas' disease. Am. J. Trop. Med. Hyg. **61:**207-214.
41. **Johnson, G. A., G. P. Cofer, S. L. Gewalt, and L. W. Hedlund.** 2002. Morphologic phenotyping with MR microscopy: the visible mouse. Radiology **222:**789-793.
42. **Klabbers, B. M., J. C. de Munk, B. J. Slotman, H. A. Langendijk, O. S. Hoekstra, R. Boellaard, and A. A. Lammertsma.** 2002. Matching PET and CT scans of the head and neck area: development of method and validation. Med. Phys. **29:**2230-2238.
43. **Krege, J. H., J. B. Hodgin, J. R. Hagaman, and O. Smithies.** 1995. A noninvasive computerized tail-cuff system for measuring blood pressure in mice. Hypertension **25:**1111-1115.
44. **LaManna, J. C. and S. I. Harik.** 1986. Regional studies of blood-brain barrier transport of glucose and leucine in awake and anesthetized rats. J. Cereb. Blood Flow Metab. **6:**717-723.
45. **Lasbennes, F., P. Lestage, P. Bobillier, and J. Seylaz.** 1986. Stress and local cerebral blood flow: studies on restrained and unrestrained rats. Exp. Brain Res. **63:**163-168.
46. **Laudon, M. K., J. G. Webster, R. Frayne, and T. M. Grist.** 1998. Minimizing interference from magnetic resonance imagers during electrocardiography. IEEE Trans. Biomed. Eng. **45:**160-164.

47. **Lee, S. P., T. Q. Duong, G. Yang, C. Iadecola, and S. G. Kim.** 2001. Relative changes of cerebral arterial and venous blood volumes during increased cerebral blood flow: implications for BOLD fMRI. Mag. Reson. Med. **45:**791-800.
48. **Lemieux, S. K. and G. H. Glover**. 1996. An infrared device for monitoring the respiration of small rodents during magnetic resonance imaging. J. Mag. Reson. Imaging **6:**561-564.
49. **Lerche, P., W. W. Muir, III, and R. M. Bednarski.** 2000. Nonrebreathing anesthetic systems in small animal practice. J. Am. Vet. Med. Assoc. **217:**493-497.
50. **Lorenz, J. N.** 2002. A practical guide to evaluating cardiovascular, renal, and pulmonary function in mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. **282:**R1565-R1582.
51. **Magistretti, P. J.** 2000. Cellular bases of functional brain imaging: insights from neuron-glia metabolic coupling. Brain Res. **886:**108-112.
52. **Martin, L.** 1999. All you really need to know to interpret arterial blood gases. Lippincott Williams and Wilkins, Philadelphia.
53. **McDonell, W.** 1996. Respiratory system, p. 115-147. *In* J. C. Thurmon, W. J. Tranquilli, and G. J. Benson (ed.), Lumb and Jones' veterinary anesthesia. Lippincott Williams and Wilkins, Philadelphia.
54. **Menon, R. S.** 2001. Imaging function in the working brain with fMRI. Curr. Opin. Neurobiol. **11:**630-636.
55. **Moore, A. H., C. L. Osteen, A. F. Chatziioannou, D. A. Hovda, and S. R. Cherry.** 2000. Quantitative assessment of longitudinal metabolic changes *in vivo* after traumatic brain injury in the adult rat using FDG-microPET. J. Cereb. Blood Flow Metab. **20:**1492-1501.
56. **Pakulla, M. A., D. Obal, and S. A. Loer.** 2003. Continuous intra-arterial blood gas monitoring in rats. Lab. Anim. **38:**133-137.
57. **Paulus, M. J., S. S. Gleason, S. J. Kennel, P. R. Hunsicker, and D. K. Johnson.** 2001. High resolution x-ray computed tomography: an emerging tool for small animal cancer research. Neoplasia **2:**62-70.
58. **Phelps, M.** PET: The merging of biology and imaging into molecular imaging. 2000. J. Nucl. Med. **40:**661-81.
59. **Qui, H. H., G. P. Cofer, L. W. Hedlund, and G. A. Johnson.** 1997. Automated feedback control of body temperature for small animal studies with MR microscopy. IEEE Trans. Biomed. Eng. **44:**1107-1113.
60. **Reese, T., B. Bjelke, R. Porszasz, D. Baumann, D. Bochelen, A. Sauter, and M. Rudin.** 2000. Regional brain activation by bicuculline visualized by functional magnetic resonance imaging. Time resolved assessment of bicuculline-induced changes in local cerebral blood volume using an intravascular contrast agent. NMR Biomed. **13:**43-49.
61. **Schimel, D.** 2004. Personal communication.
62. **Schuessler T. F. and J. H. Bates.** 1995. A computer-controlled research ventilator for small animals: design and evaluation. IEEE Trans. Biomed. Eng. **42:**860-866.
63. **Schwarte, L. A., C. J. Zuurbier, and C. Ince.** 2000. Mechanical ventilation of mice. Basic Res. Cardiol. **95:**510-520.
64. **Seeler, D. C.** 1996. Fluid and electrolyte therapy, p. 572-589. *In* J. C. Thurmon, W. J. Tranquilli and G. J. Benson (ed.), Lumb and Jones' veterinary anesthesia. Lippincott Williams and Wilkins, Philadelphia.
65. **Sharma, V., G. D. Luker, and D. Piwnica-Worms.** 2002. Molecular imaging of gene expression and protein function in vivo with PET and SPECT. J. Mag. Reson. Imaging **16:**336-351.
66. **Sicard, K., Q. Shen, M.E. Brevard, R. Sullivan, C. F. Ferris, J. A. King, and T. Q. Duong.** 2003. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions. J. Cereb. Blood Flow Metab. **23:**472-481.
67. **Siggaard-Andersen, O.** 1971. An acid–base chart for arterial blood with normal and pathophysiological reference areas. Scand. J. Clin. Lab. Invest. **27:**239-245.
68. **Sonner, J. M., D. Gong, and E. I. Eger.** 2000. Naturally occurring variability in anesthetic potency among inbred mouse strains. Anesth. Analg. **91:**720-726.
69. **Sonner J. M., D. Gong, J. Li, E. I. Eger, and M. J. Laster.** 1999. Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. Anesth. Analg. **89:**1030-1034.
70. **Suckow, M. A., P. Danneman, and Brayton.** 2001. The laboratory mouse: a volume in the laboratory animal pocket reference series. CRC Press, New York.
71. **Szczesny G., A. Veihelmann, S. Massberg, D. Nolte, and K. Messmer.** 2004. Long-term anaesthesia using inhalatory isoflurane in different strains of mice—the haemodynamic effects. Lab. Anim. **38:**64-69.
72. **Tankersley, T. G., R. S. Fitzgerald, and S. R. Kleeberger.** 1994. Differential control of ventilation among inbred strains of mice. Am. J. Physiol. **267:**R1371-R1377.
73. **Topal, A., N. Gul, Y. Ilcol, and O. S. Gorgul.** 2003. Hepatic effects of halothane, isoflurane, or sevoflurane anaesthesia in dogs. J. Vet. Med. A **50:**530-533.
74. **Toyama, H., M. Ichise, J. S. Liow, D. C. Vines, N. M. Seneca, K. J. Modell, J. Seidel, M. V. Green, and R. B. Innis.** Evaluation of anesthesia effects on [18F]FDG uptake in mouse brain and heart using small animal PET. 2004. Nucl. Med. Biol. **31:**251-256.
75. **Van Vliet, B. N., L. L. Chafe, V. Antic, S. Schnyder-Candrian, and J. P. Montani.** 2000. Direct and indirect methods used to study arterial blood pressure. J. Pharmacol. Toxicol. Methods. **44:**361-373.
76. **Waterman, A. E. and A. Livingston.** 1978. Effects of age and sex on ketamine anaesthesia in the rat. Br. J. Anaesth. **50:**885-889.
77. **Waynforth, H. B. and P. A. Flecknell.** 1992. Experimental and surgical technique in the rat, p. 212-236. Academic Press, Inc., San Diego, Calif.
78. **Wiersema, A. M., R. Dirksen, W. J. G. Oyen, and J. A. van der Vliet.** 1996. A method for long duration anaesthesia for a new hindlimb ischaemia-reperfusion model in mice. Lab. Anim. **31:**151-156.
79. **Wood, A. K., A. M. Klide, S. Pickup, and H. L. Kundel.** 2001. Prolonged general anesthesia in MR studies of rats. Acad. Radiol. **8:**1136-1140.
80. **Woody, C., A. Kriplani, P. O'Connor, J. F. Pratte, V. Radeka, S. Rescia, D. Schlyer, S. Shokouhi, S. Stoll, P. Vaska, A. Villaneuva, N. Volkow, and B. Yu.** 2004. RatCAP: a small, head-mounted PET tomography for imaging the brain of an awake rat. Nucl. Instrum. Methods Phys. Res. A. **527(1-2):**166-170.
81. **Xu, H., S. J. Li, J. Bodurka, X. Zhao, Z. X. Xi, and E. A. Stein.** 2000. Heroin-induced neuronal activation in rat brain assessed by functional MRI. Neuroreport **11:**1085-1092.
82. **Yang, M., E. Baranov, P. Jiang, F. Sun, X. Li, L. Li, S. Hasegawa, M. Bouvet, M. Al-Tuwaijri, T. Chishima, H. Shimada, A. R. Moossa, S. Penman, and R. M. Hoffman.** 2000. Whole-body optical imaging of green fluorescent protein expression. Clin. Exp. Metastasis **15:**547-552.
83. **Zuurbier, C. J., V. M. Emons, and C. Ince.** 2002. Hemodynamics of anesthetized ventilated mouse models: aspects of anesthetics, fluid support, and strain. Am. J. Physiol. Heart Circ. Physiol. **282:**H2099-H2105.