Anesthesia and Monitoring of Animals During MRI Studies

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Abstract

The use of imaging represents a major impact on the refinement and the reduction of in vivo studies in animal models, in particular for allowing longitudinal monitoring of the onset and the progression of disease within the same animal, and studying the biological effects of drug candidate and their therapeutic effectiveness. But the use of imaging procedures can affect animal physiology, and the need to anesthetize the animals for imaging entails potential health risks. During anesthesia, there is an inevitable autonomic nervous system depression which induces cardiovascular depression, respiratory depression, and hypothermia. Also other procedures associated with imaging such as animal preparation (e.g., fasting, premedication), blood sampling, and dosage/contrast agent injections can also affect physiology and animal welfare. All these factors are likely to have confounding effect on the outcome of the imaging studies and pose important concerns regarding the animal's well-being, particularly when imaging immune deprived animals or diseased animals. We will discuss these challenges and considerations during imaging to maximize efficacious data while promoting animal welfare.

Key words

Anesthesia
Physiological monitoring
Animal welfare
3Rs

1. Introduction

The use of imaging technologies is instrumental in biomedical research due to their great scope for noninvasively studying biochemical and biological processes in the living animal. Their application represents a major impact on the "3Rs" principles particularly in the reduction of animals used for in vivo studies, allowing longitudinal monitoring of the onset and the progression of disease within the same animal through serial imaging and also to test the therapeutic effectiveness of new treatment. Preclinical magnetic resonance imaging (MRI) remains the most versatile imaging modality which has been extensively used for anatomical, functional, and physiological characterizations of tissues/organs [1]. Current MRI systems can routinely achieve a spatial resolution of 100 μm in all dimensions in living animals providing high-quality anatomical detail. But MR techniques can also provide information on chemical composition (spectroscopy) and other parameters such as cardiovascular function [2] and neuronal network alterations [3]. Its nonionizing 3D imaging functionality is truly noninvasive avoiding any exposure of the animals to any ionizing radiation. This is especially important during serial imaging giving an advantage over modalities such as CT, SPECT, and PET. A challenge is the higher spatial resolution required with rodents compared to

humans. In order to segment each body into the same number of voxels, the rodent voxel volume must be approximately 3000 times smaller than that of a human. In MRI, the answer to this challenge lies in higher magnetic fields system (e.g., 11.7 T), strong magnetic field gradient systems (e.g., 1000 mT/m) [4] and preclinical-specific radio-frequency coils and cryogenic probes [5]. Overall, MRI methodology provides a major preclinical refinement and reduction tool, delivering clinically relevant outcomes in a minimally invasive manner in a living organism, allowing for repetitive monitoring in the same animal, maximizing biological relevance, thus delivering a rapid, efficacious, and reasonably cost-effective use of animal disease models. A major difference with human MRI remains which is the need to anesthetize the animals for imaging, which per se entails potential health risks in the imaged animals. Indeed, during anesthesia there is inevitable autonomic nervous system depression which induces cardiovascular and respiratory depression and hypothermia. All these factors have a profound effect on the animal's homeostasis and may thereby confound the image quality and interpretation, or statistically speaking lead to bias and increased variability. In addition, other experimental procedures occasionally performed along with imaging such as fasting, premedication, blood sampling and the administration of contrast agents or imaging biomarkers can also affect physiology and animal welfare. Furthermore functional pharmacological blood oxygen level dependent (BOLD) MRI can be markedly affected by anesthesia due to its effects on blood flow, blood oxygenation levels, and cardiac and respiratory functions [6]. Similarly, the need for lengthy acquisition times for ultra-high resolution MRI also poses challenges for adequate physiological monitoring. All these factors are likely to have confounding effects on the outcome of the imaging studies and pose important concerns regarding the animal's well-being, particularly when imaging disease-modeling animals [7].

In the remainder of this chapter we will discuss the challenges associated with setting up and carrying out preclinical MRI procedures, addressing the protocols related to animal handling, induction and maintenance of anesthesia and physiological monitoring, to promote appropriate animal care and to minimize potential confounding effects on imaging outcomes.

2. Methods

2.1. Planning and Setting Up an MRI Study

When thinking on undertaking a preclinical MRI study, it is important to allocate enough time for planning, addressing all the animal regulatory guidelines and defining all scientific objectives. The following protocol may be used as guidelines.

1. Define specific targets and procedures
    - What are the scientific objectives and how are they relevant to the clinical scenario and/or in vivo mechanistic modeling? Include a clear hypothesis to be tested via the primary (and optionally secondary) objectives.
    - What is the rationale for using imaging, and in particular MRI?
    - Which stage of disease models will be used and why? Define why the animal species and model is being selected.
    - Describe the rationale for the specific imaging timeframes used (onset and/or progression of disease and/or response to treatments).
    - Define imaging outcomes (both qualitative and quantitative) and data analysis algorithms.
    - Describe how data quality control is handled (e.g., imaging system calibration).
    - Draw up prospective plans for further imaging sessions and/or co-registration with other imaging modalities.
2. Ensure that all animal procedures are approved by the specific institutional animal welfare body (AWERB-UK [8]; IACUC-USA [9]) and are conform to regulatory frameworks (EU Directive

2010/63/EU [10]; US-PHS "The Guide for the Care and Use of Laboratory Animals; Health Research Extension Act of 1985 [11]) for the care and use of animals.

3. For budget preparation, include costs for animal purchase, transport, housing, time for technical and veterinarian support, imaging acquisition, data storage, processing and analysis.

4. Define experimental procedures, ensuring (1) the animal model can be set up and specific MRI experiments can be done in the facility, (2) biosafety requirements are fulfilled (e.g., need for quarantine), (3) general and specific housing capabilities are available, and (4) supporting staff is trained and available.

5. Design the study including the number of experimental and control groups, steps taken for randomization and blinding, a workflow to work out the experimental procedures that will be carried out within the same animal for severity assessment. Check that this is all in accordance with the ARRIVE guidelines [12].

The experimental protocol should include:

- Health surveillance/general check prior to study.
- Selection of animal strain, sex, age, weight (range), and genotype (e.g., knock-out or transgenic).
- Plan specifics for animal housing (individual/in groups); IVC caging; isolators; specific SPF housing; specific bedding; cage enrichment.
- Define experimental group, including appropriate any sample size calculation used and number of independent replications of each experiment.
- Selection of statistical methods to be used for each analysis, including data normalization and a measure of precision (e.g., standard error or confidence interval).
- Pretreatment: fasting, specific feeding, premedication, contrast agents including dose and route of administration.
- Anesthesia regime: selection of drug, dosage and route of administration.
- Physiological monitoring (for depth of anesthesia and imaging monitoring) with clear definition of adverse effects, care procedures, and humane endpoints.
- Record keeping: reporting all the animal procedures, usage, and discharge records according to regulatory requirements.

2.2. Transport of Animals to the Imaging Unit and Acclimatization

Animals often have to be transported from general holding rooms to the imaging facility. One consequence is that this will require full compliance with the institutional health screening controls to minimize disease transfer to any other animals in the facility. A second consequence is that for the transported animals themselves experience stress leading to an increase of the glucocorticoid level, a loss in body weight and immune response suppression which have been observed for up to 48 h after transport in rodents [13]. Therefore an acclimatization period, typically between 3 and 7 days depending on the procedure and transport duration, is required after animal transport. Imaging facilities should make housing capacity available to allow for the acclimatization of transferred animals and reduce stress before imaging takes place. Housing in the imaging facility is also useful for monitoring animal recovery after anesthesia. A sufficiently long recovery period should also be included after imaging, depending on the type of anesthetic, the length

of anesthesia, and any experimental procedures carried out. If an imaging unit is integrated within a centralized animal unit, transport time and acclimatization may be considerably reduced.

2.3. Animal Preparation for Imaging

Different procedures will be required depending on the animal model used and the in vivo experimental protocol required prior to imaging. This may include procedures such as fasting, specific treatments/dosing, and behavior assessments. Such additional procedures need to be well planned ahead and appropriately monitored. As regulated procedures, these need to be properly reported as per specific regulatory guidelines (Note, fasting is a regulated procedure). Regular assessments of the animals include regular body weight measurements and standard clinical checks on such aspects like fur status, general behavior, and peer interaction, alertness, good food/water uptake, feces control and absence of diarrhea, and face expression including healthy eyes. Passing these criteria, animals can be accepted into the study. Then, these measurements and assessments prior to any imaging serve as physiological baseline values.

On the day of imaging, animals may also require more specific interventions such as vessel cannulation, implantation of ECG probes, and tracheal intubation. These need to be carried out according to institutional guidelines for aseptic surgery [14] with good thermoregulation control and a suitable analgesic and anesthesia regime to ensure minimal physiological impact of these interventions. The pre-anesthetic exam should contain, but is not limited to, confirmation of animal's identification, sex, age, body weight, body/fur condition, hydration/color of mucous membranes status, heart rate and rhythm, respiratory rate, signs of diarrhea, evidence of normal food and water consumption and normal production of urine and feces in the cage. The body condition of the animal will impact on anesthesia induction, maintenance, and recovery (e.g., obese animals may respond more slowly to anesthetics).

Prior to imaging acquisition animals will be placed on the "imaging bed" which will need to be fitted with anesthesia supply, thermoregulation equipment for the anesthetized animals and physiological monitoring sensors. Anesthesia regimes and monitoring systems are discussed below.

2.4. Induction of Anesthesia

Anesthesia is generally required during imaging to ensure humane restraint of the animals. Most anesthetics induce a certain degree of autonomic nervous system depression, which triggers cardiovascular depression (e.g., reduction in cardiac output, blood pressure) and respiratory depression (e.g., hypoxia, hypercapnia) and induces hypothermia, affecting whole body metabolism. Such effects can be critical in laboratory rodents which have a small body size, high body surface-to-weight ratio, and high metabolic rate, compromising the pharmacological efficacy of anesthetic agents and their ability to thermoregulate. It is important to ensure that anesthetized animals remain in a stable physiological state with consistent cardiovascular, respiratory function and body temperature during imaging.

The experimental protocol should include:

- Animal transport—ensuring biosafety based on health surveillance program.
- Acclimatization period (3–7 days; shorter if integrated imaging & housing units).
- Animal preparation and pretreatments (e.g., dosing/fasting).
- Regular general health status monitoring (body weight) with good record keeping.

2.5. Anesthesia Regimes

Injectable and inhaled anesthetics are commonly used in preclinical imaging. Gas anesthetics are most suitable and recommended for imaging due to their rapid onset and recovery times, and faster elimination. Inhaled anesthetics with medical air or medical oxygen as gas carrier allow for a better control of depth of anesthesia and degree of oxygenation than injectables [15]. This is particularly critical when undertaking

long-term imaging procedures due to the risk of developing hypoxia, respiratory depression, hypercapnia, and acidosis. Dosing with injectables remains challenging due to the lack of interventional management and when additional dosing is required which can lead to overdosing and intermittent changes on the depth of anesthesia. Injectables also often cause a prolonged recovery time, in particular opioids with their strong residual effect [16]. Some injectables can be administered via infusion which may allow to achieve a more steady plasma concentration over the course of imaging.

Anesthetic dose rates for injectable agents will depend on species used, administration route, age, sex, strain, body condition, environment, experimental setup, previous drug treatments, and the level of anesthesia required. During the initial period of use, it is important to monitor animals closely and make any adjustments necessary in the protocol for subsequent experiments.

2.5.1. Injectables Anesthetics

The most commonly used injectable anesthesia agents are listed in Table 1 with their individual advantages and disadvantages and typical dosing regimes.

Table 1

Commonly used injectable anesthesia agents

Agent	Advantages	Disadvantages	Dosing
Fentanyl/fluanisone (Hypnorm™) based combination	• Good analgesic • Sedative • Need to "top up" for long-term anesthesia. • Sedative effect can be reverse with buprenorphine to speeds up recovery time	• Cardiovascular and respiratory depression • Poor muscle relaxation alone • Prolonged recovery time • Hypersensitivity to noise	Mouse: 10 mL/kg; rat: 2.7 mL/kg (i.p.) Hypnorm™/Hypnovel™ (midazolam)/water mixture (1:1:2 vol) (120–140 min sleep time) HypnormTH top up 0.3 mL/kg (mouse) 0.1 mL/kg (rat) i.p. (30–40 min sleep time)
Ketamine based combination	• Analgesic effects • Light sedation • Wide safety margin • Can increase blood pressure	• High muscle rigidity unless combined with other agents. • Increases intracranial pressure. • Recovery often with ataxia and hyper responsiveness.	Mouse and rats: Ketamine + medetomidine: 75 mg/kg + 0.5–1 mg/kg i.p. Ketamine + xylazine 75–100 mg/kg/10 mg/k i.p. (60–120 min sleep time) Atipamezole: 1 mg/kg i.p. (reverse agent)
Alfaxalone (Alfaxan)	• Minimal respiratory/cardiovascular depression. • Rapidly metabolized: good for repeat dosing • Suitable for long-term anesthesia	• Administration route IV (rodents, cats) or IM (primates)	15–20 mg/kg (mouse) 10–12 mg/kg (rat) iv (10–15 min sleep time after bolus) 0.25–0.75 mg/kg/min iv infusion (long term)
Propofol (Rapinovet®, Diprivan®)	• Rapidly metabolized, • Good for continuous infusion for long-term anesthesia. • Rapid recovery • High safety: can be used in animals with hepatic or renal impairment.	• IV use only • No analgesic properties • Severe respiratory depression: risk of apnea	26 mg/kg (mouse) 10–12 mg/kg (rat) iv (10–15 min sleep time after bolus) 2–2.5 mg/kg/min iv infusion (long term-mouse) (0.5–1 mg/kg/min iv infusion (long term-rat)
Barbiturates products	• Sedative effect • Hypnotic • Reasonable muscle relaxation	• No analgesic properties • Severe respiratory depression and hypotensive • Easy to over does • High metabolites accumulation • Caustic substances: use only iv route	Pentobarbitone: 40–50 mg/kg i.p. (mouse) (120–180 min sleep) Thiopentone: 30 mg/kg iv 15 min sleep) (rat)

Agent	Advantages	Disadvantages	Dosing
Chloral hydrate	• Sedative effect • Hypnotic • Minimal CVS and respiratory depression	• No analgesic properties • Paralytic noted in rats • Terminal/non-recovery work only	300–400 mg/kg i.p. (1–2 h sleep time) (mouse and rats)
a-Chloralose	• Sedative effect • Hypnotic. • Suitable for long-term anesthesia. • Minimal CVS and respiratory depression	• No analgesic properties • IV use only • Slow induction and recovery associated with involuntary excitement • Terminal/non-recovery work only	50–60 mg/kg i.p. (rats) 120 mg/kg i.p. (mouse) (8–12 h; for non-recovery only) 50 mg/kg iv bolus followed by 25–40 mg/kg/hr (rats)
Urethane	• Suitable for long-term anesthesia. • Minimal CVS and respiratory depression	• Carcinogenic: only allowed to be used with special justification! • Terminal/non-recovery work only	0.8–1.3 g/kg, i.p. (mouse and rats) duration of action 8–10 h (nonrecovery only)
Avertin® (tribromoethanol)	• Wide safety margin • Good muscle relaxation • Rapid induction and recovery	• Local irritation/peritonitis • Handling and storage safety issues • Toxic effects • Pharmaceutical-grade TBE (e.g., Avertin) is no longer available: IACUC/AWERB	0.015 mL/g body wt of 2.5% i.p. 30 min—supplemental doses of anesthesia: minimum of one-half of the initial does up to 1 mL max vol per animal

2.5.2. Inhalation Anesthesia

Inhalation anesthesia is the recommended method for imaging laboratory animals as this provides a rapid induction and recovery and since inhalation anesthesia agents are safe, nonirritant, and nonexplosive. Medical oxygen is commonly used as the carrier with a flow rate between 0.5 and 1.5 L/min. Non-rebreathing circuits are usually used to ensure minimum dead space and resistance, and resulting waste/excess gas is removed by a scavenger system to protect lab staff. The most commonly used anesthetic circuit type for laboratory rodents is Bain's coaxial T-piece coupled with an open facemask system mounted to the imaging bed.

The most commonly used inhalation anesthesia agents are listed in Table 2 with their individual advantages and disadvantages and typical dosing regimes.

Table 2

Commonly used inhalation anesthesia agents

Agent	Advantages	Disadvantages	Dosing
Halothane	• Potent anesthetic • High therapeutic index • Rapid induction and recovery (1–3 min) • Adequate muscle relaxation • Nonirritant, nonflammable nor explosive • Easy to vaporize	• Highly metabolized (hepatotoxic) • Cardiovascular depressant • Moderate hypotension: reduction in cardiac output and peripheral vasodilatation) • Respiratory depressant • Halothane sensitizes the heart to catecholamines (sympathetic stimulation)	Induction: 3–4% Maintenance 1–2% (rats and mice)

Agent	Advantages	Disadvantages	Dosing
Isoflurane	• Similar physical properties to halothane • Rapid induction and recovery • Low toxicity and metabolic activity: highly safe • Suitable for high frequency and log-term anesthesia • Minimal cardiovascular depression • Moderate respiratory depression • Good muscle relaxation	• Decreases arterial blood pressure (vasodilatation) • More expensive than halothane • Strong smell; aversive response for repetitive use • More potent respiratory depressant than halothane	Induction: 3–4% Maintenance 1–2% (mice); 1.5–2.5% (rats)
Sevoflurane	• Faster induction and recovery times (3 × times faster than isoflurane) • Less respiratory depression that isoflurane • Less struggling and excitement during induction • Metabolism similar to isoflurane: good for repetitive and/or long-term anesthesia • Blood glucose homeostasis is better maintained • Method of choice for PET FDG myocardium uptake studies	• Expensive but cost coming down • Costly specific vaporizers • Provides an equally reliable anesthesia in laboratory mice.	Induction: 1–8% Maintenance: 3–5% (mice and rats)

It is important to appreciate the variation in response to anesthetics between different animal strains and thus, to reassure and adjust the anesthesia protocol to the particular needs of a given strain and experimental setup.

2.6. Monitoring and Impact of Anesthesia During Imaging Procedures

Most anesthetic drugs will impact on respiration, the cardiovascular system, and/or thermoregulation. It is important to monitor the animals during imaging and provide any physiologic support to ensure animal welfare and also to minimize any confounding effects on data acquisition. The recommended approach is to monitor respiratory function and body temperature, as the minimum standard applicable during imaging to control depth of anesthesia. Cardiac function is also highly recommended. Direct visualization is not possible and it is greatly suggested to use the existing monitoring equipment for laboratory rodents which is tailored to their small body size, fast cardiac and respiratory rates, and importantly in the case of MRI, is non-ferromagnetic to avoid any interference with the magnetic field [17, 18]. Most MRI-compatible systems are based on fiber-optic or carbon fiber equipment and power source or batteries are adequately filtered/isolated to avoid magnetic interference [19].

While protocols do not specifically define how frequently one should monitor the animals during anesthesia, it is obvious that the more invasive the procedure and/or the longer time under anesthesia, the more likely it is to interfere with normal homeostasis and thus greater the need for more frequent/constant monitoring. Also it is instrumental to support the acquisition of gated imaging, minimizing the effects due to biological motion and also targeting specific imaging sequences, in a given respiratory or cardiac phase.

The position of the animals and the imaging bed systems used are also crucial, to ensure that the neck and head are well positioned to avoid restricting the breathing, and also the body and extremities to avoid restricting the circulation and to avoid any bruising, strains or avulsion in the body structures.

2.6.1. Temperature

Most anesthetic agents profoundly depress thermoregulation [20]. Rodents are highly susceptible to hypothermia due to their large surface area-to-body mass ratio and rapid metabolism. This is particularly critical when imaging nude or hairless mice. Hypothermia will have confounding effects on glucose metabolism and heart rate, which can significantly affect, e.g., FDG-PET studies and echocardiography outcomes. Therefore monitoring body core temperature is crucial. This is mostly done using rectal

thermometers or thermocouples which are well fitted to the MRI systems and generally do interface with an external heat source such as circulating hot water blankets or blowing air systems that help to maintain the animal's body temperature. Hyperthermia is as dangerous as hypothermia, thus the external heat source should be "thermostatically controlled" and linked to the core temperature reading. Heat loss must also be minimized during the animal preparation before imaging (e.g., hair removal, alcohol application) and fluid replacement, if required, should be warmed to 37 °C.

The eyes should be protected both from drying off and exposure keratitis by regular application of ophthalmic ointment.

2.6.2. Respiratory System

Most anesthetics are known to cause respiratory depression. Therefore it is important to reduce variability due to poor ventilation including hypoxia, hypercapnia, and acidosis [21, 22]. Respiratory monitoring is generally carried out via detecting the breathing motion registered as compressions of a respiratory sensor placed in contact with the animal's chest. These systems are extensively used during imaging and highly compatible with MRI. Motion artifacts due to breathing can be eliminated from the images by employing gated imaging. Typically the most prominent motion of the diaphragm and abdomen occurs during inspiration, and acquisitions are generally carried out during expiration.

Other more advanced approaches include the use of arterial blood gas analysis, which is very valuable during functional MRI. The partial measurements of oxygen (pO_2) and carbon dioxide (pCO_2) and the pH of the blood are detected from a single blood sample allowing levels of oxygenation, imbalance of CO_2 production and acid-base balance to be monitored. Impaired gas exchange, or hyper- or hypoventilation can be corrected by changing the anesthetic regime, and when critical through using artificial ventilation. Indeed, some studies will require the animal to be mechanically ventilated and it is important to ensure that the animal does not develop hypercapnia or hypoxia. Other advanced respiratory monitoring systems include digitized systems such as capnographs that measure the CO_2 level through a highly sensitive infrared spectroscopy CO_2 sensor in the inhaled and exhaled gas, based on the CO_2 values in the venous return to the heart and the efficacy of breathing. This equipment provides a very good indicator of the respiratory function by continuous measurements of the CO_2 level. Capnographs that measure the CO_2 level in the inhaled and exhaled gas between inspiration and expiration at the endotracheal tube connector or face masks level are very good indicator of the respiratory function [23].

2.6.3. Cardiovascular System

Basic cardiovascular monitoring, including heart rate and blood pressure, is highly recommended. Electrocardiograms (ECG) are generally used to monitor heart rate and rhythm and help to detect arrhythmias, myocardial ischemia, or metabolic disorders. ECG measurements are also used to synchronize the heart rate to the image acquisition, during gated imaging. Non-ferromagnetic electrodes, MRI–compatible needles or patch electrodes are mostly used. Pulse oximeters are also very useful to monitor arterial oxygenation and pulse during anesthesia, detecting any changes long before the animal becomes cyanosed. The system provides real-time continuous measurements of arterial O_2 saturation, pulse strength, breathing rate, blood flow and effort to breathe. The systems available for rodents are not invasive and are MRI compatible [24].

Blood pressure measurements are also used during imaging [25]. The mean arterial pressure (MAP) is the overall judge of the state of the circulation, being the best indicator on how well tissues are perfused. As the MAP falls, vital organ auto regulation and perfusion is quickly compromised. Measuring MAP stability is very important during functional MRI or when assessing the perfusion of contrast agents through specific tissues/organs during contrast enhanced MRI. The direct blood pressure measurement involves placing a catheter in an artery and connecting it to a transducer. The procedure is generally invasive as the artery, femoral or carotid usually, has to be exposed surgically. Indirect methods typically use an inflatable tail cuff pressure sensor to detect the arterial blood flow [26] but are less accurate than direct ones and generally data acquisition is intermittent.

2.6.4. Repeated and Long-Term Anesthesia

Some imaging protocols may require long acquisition times and/or high frequency imaging with considerable impact on cumulative time of anesthesia. In these circumstances it is recommended to use minimally metabolized volatile anesthetics like isoflurane or sevoflurane, which allow for a quick induction, good surgical anesthesia and fast recovery, so that animals can regain full physiological functions quickly. Repeat imaging is likely to induce some hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system responses to the chronic stress with prolonged elevation of corticosterone and impairments to homeostasis [27]. It is of utmost importance to monitor body temperature. Hypo- or hyperthermia will interfere with many electrophysiological outcome measurements. The extremities and tail should be covered whenever possible. Long imaging procedures with lengthy anesthesia may seriously compromise the hydration statues of the animals. Therefore it is important to compensate for the fluid lost and prevent animal dehydration. Parental administration of warmed fluids (0.9% saline or lactated Ringer's) may be applied and ideally also the inspired gases may be humidified to avoid desiccation via the airways. Eye protection may also be used to help maintain good lubrication of the cornea and also to protect the eyes from exposure keratitis.

2.6.5. Ventilation

Some studies will require the animal to be mechanically ventilated, which may have important effects on thoracic hemodynamics and may also override the autonomic reflex control of breathing, which normally maintains blood gas homeostasis. When monitoring a ventilated animal, ideally, one should have oxygen/carbon dioxide monitoring equipment (e.g., pulse oximeter, end tidal capnograph). The ventilation settings can then be adjusted to try to maintain physiological levels of O_2/CO_2. Blood gas and electrolyte analysis are useful but confer intermittent monitoring, and a local analyzer is needed.

The most commonly observed physiological effects and health risks during anesthesia and troubleshooting management are listed in Table 3.

Table 3

Commonly physiological effects and management during anesthesia

	Heath risks	**Troubleshooting issues**
Respiration	Hypoxia, hypercapnia and acidosis Effect exacerbated during prolonged Impact on HbO_2/02	*High breathing rate:* • Increase anesthesia dose • Check anesthetic system
		Low breathing rate/apnea: • Animal may be too deep: Lightening or reversing anesthesia • Hypothermia: Check temperature • Hyperventilation in oxygen • Short term reaction to injectables (e.g., thiopentone)
		Respiration fails: • Manual ventilation if animal is intubated • Gently massage the chest side to side-not very effective (if animals is accessible outside the MRI magnet) • Supply O_2 even if using injectable anesthesia • Use specific reverse agents
Cardiac function	Rodents high heart rate (×10 faster than humans) and short circulation time Profound effect of anesthetics Heart rate, rhythm and pulse intensity	*Tachycardia:* • May be a pain response: Too "light" anesthesia, increase anesthesia • Correct any fluid deficit (check for any bleeding)

	Heath risks	Troubleshooting issues
		Bradycardia: • Animal may be too deep: Lightening or reverse anesthesia • Animal too cold: Control body temperature • Can be surgically induced by vagal reflexes or by anesthetic drugs • Atropine may be indicated
		Cardiac arrest • Gently massage the chest side to side-not very effective • Stop anesthesia and transfer to O_2 supply only • Some drugs are effective in larger animals (adrenalin) but seldom practical for emergency procedures in rodents
Risk of anesthesia overdose	Heart rate (high or slow at critical stage) Arrythmias Respiration (high and/or shallow)-diaphragmatic Pulse weak Membranes pale	• Turn off gas anesthetics/administer reversal anesthetic agent. • Maintain animal with O_2 only (or ventilate with oxygen) • Administer isotonic fluids. • Warm up the animal slowly to increase body temperature/metabolism

2.7. Materials

Table 4 lists physiological parameters and exemplary MRI-compatible systems for monitoring. Respiration and heart rate signals can be fed into the MR console for gated imaging, e.g., imaging only during exhale phases. Temperature readings can be used as input to a feedback loop for temperature control.

Table 4

Exemplary systems for MRI-compatible physiological monitoring

Physiological parameter	Examples
Respiration monitoring and gating, ECG monitoring and gating	BioVet (m2m Imaging Corp., USA) Low-cost solution Uni Jena [28] Model 1030 monitoring and gating system (Small Animal Instruments, Inc., USA) Physioguard II (Minerve, France)
Temperature monitoring	BioVet (m2m imaging corp, USA) Imaging cells (Minerve, France) Model 1030 monitoring and gating system (Small Animal Instruments, Inc., USA) OTG-M (Opsens, Canada)
Heating systems	Hot air fan with feedback loop (Small Animal Instruments, Inc., USA) Hot air tubes built into animal bed (Minerve, France) Hot water tubes built into animal bed (aspect imaging, Bruker) Resistive heating system Oxford Inst. Radiation oncology [28]
Pulse oximetry, heart rate monitoring	MouseOx (STARR Life Sciences Corp., USA) MouseSTAT (Kent Scientific Corp., USA)
Respiratory CO_2 monitoring	Capnograph V9004 (Harvard Apparatus, Ltd., UK) Capnoscan (Kent Scientific Corp., USA)
Blood pressure monitoring	Samba Preclin 420/360 transducer (Harvard Apparatus, Ltd., UK) TSD104A blood pressure transducer (Biopac Systems, Inc., USA)

2.8. Recovery

Monitoring should be carried out until the animal has fully recovered from anesthesia, i.e., until the animal regained full consciousness with essential physiological functions back to normal. Checks should include in particular respiratory and cardiovascular function and the ability to control body temperature. Only then the animal may be transferred back to its housing cage.

During recovery, it is important to maintain the core temperature of the animal. Good practice is to place the animals on their right side or in sternal recumbent position in a warm recovery cage with no sawdust or suchlike bedding that may be inhaled. If the animals have been under anesthesia for a relatively long time, it may be worth giving oxygen, fluids and nutrition supplement. This can include oral or parenteral support, e.g., high-energy moist foods such as nutrient agar, jelly or crushed rodent pellets mixed with water, presented in a Petri dish or other manner that does not require the animal to reach up high. Additional analgesia should be provided if the animal has undergone a painful procedure or if there appears to be any sign of pain [28].

3. Conclusions and Outlook

Imaging technologies have dramatically increased the efficiency of preclinical studies, providing a powerful, noninvasive, and clinically translatable means of monitoring disease progression and therapeutic response. The noninvasive acquisition of detailed in vivo anatomical and functional data represents an important milestone in refinement and reduction in the use of animal models. However, it is important to continue refining all the rodent bio-imaging protocols including appropriate anesthetic regimen and monitoring systems to ensure the animal's well-being and to minimize stress-related responses that would compromise the imaging outcomes.

It is important to consolidate good protocols for handling, anesthesia and monitoring the animals suitable to all the specific needs of a wide range of imaging experiments. This is a rapidly advancing field that holds great opportunities for further research and technology development. Technological challenges are addressing faster acquisitions, faster analysis software, and more versatile integrated multimodality imaging. Imaging companies continue developing and integrating physiological monitoring systems in preclinical equipment, and strategies are needed to continue supporting further investment and developments in the field. Similarly, further studies on the impact of frequent and repetitive anesthesia/imaging are needed to ensure the appropriate severity assessments and its impact on lifetime experience for each studied animal and to improve their welfare.

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