International Journal of Pain Research
2026, Vol. 2, No. 3, pp. 75–81
https://doi.org/10.11648/j.ijpr.20260203.11

Research Article

Anesthesia in Laboratory Animals: A Practical Overview

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Abstract

There is a strong demand for standardized, reliable anesthetic protocols to support scientific research involving laboratory animals. To date, no universally accepted unified anesthesia scheme has been established for any single laboratory animal species, and animal welfare protection is a mandatory requirement that must be fully considered throughout all experimental perioperative procedures. Given the widespread lack of systematic anesthesia guidance for novice technical staff, this review was compiled with the primary purpose of sorting out complete perioperative anesthesia management strategies for common laboratory animals. This review covers the classification, application characteristics and applicable scenarios of injectable anesthesia and inhalant anesthesia, and further elaborates detailed perioperative care specifications including preoperative preparation, intraoperative vital sign monitoring, postoperative recovery antagonism and standardized euthanasia procedures. Meanwhile, it also summarizes differentiated anesthetic schemes for special experimental scenarios such as surgical operations, fMRI, PET and endoscopic modeling, and analyzes the existing limitations of current animal anesthesia techniques. The overall content is expected to provide comprehensive operable reference for technicians with limited practical experience in laboratory animal anesthesia. In conclusion, laboratory animal anesthesiology needs to balance experimental accuracy, animal physiological tolerance and welfare ethics; future studies should focus on personalized species-specific anesthesia protocols and minimally invasive precise monitoring technologies to make up for the deficiency of current monitoring means and individual difference research.

Keywords

Experimental Surgery, Laboratory Animal, Anesthesia, Analgesia, Animal Welfare

1. Introduction

Russell and Burch first described anesthesia in 1959 as "the greatest single advance in humane technique" and "virtually indispensable for the advance of experimental biology" [1]. *Croft's 1960 An Introduction to the Anaesthesia of Laboratory Animals provided practical injection and drug-selection guidance. Green's Animal Anaesthesia* [2] (1979), the first dedicated book on the subject, established safe anesthetic principles that remain applicable today. The 2020 ARRIVE 2.0 guidelines were subsequently introduced to address persistent poor reporting quality in animal research [3]. For staff with limited veterinary anesthesia training, accessible, uncomplicated anesthetic guides remain essential. Here, we outline the principal anesthetic methods for small laboratory animals.

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Received: 2 June 2026; **Accepted:** 29 June 2026; **Published:** 17 July 2026

2. Common Anesthetic Agents Used in Laboratory Animals

Human general anesthesia comprises immobility/analgesia, loss of consciousness, and amnesia. Laboratory animal anesthesia endpoints parallel these and can be achieved through various methods depending on study purpose and animal model. Injectable anesthetics and analgesics are the primary agents for small animals; neuromuscular-blocking agents are additionally relevant for large animals.

2.1. Inhalational Agents

Volatile anesthetics offer rapid induction, adjustable depth, and suitability for both brief and prolonged procedures. Disadvantages include the need for sophisticated vaporizers, waste-gas scavenging, and personnel exposure risk. Prolonged delivery requires endotracheal intubation, which is technically challenging in small animals. Early agents (ether, halothane) have been phased out due to flammability, explosion risk, and organ toxicity [4]. Isoflurane and sevoflurane are currently the most widely used inhalational anesthetics in animal research; sevoflurane's lower blood-gas partition coefficient enables faster induction and recovery. In China, isoflurane is extensively employed in mice, rats, rabbits, and dogs, particularly for prolonged experiments requiring precise depth control.

2.2. Common Injectable Anesthetics

Chloral hydrate, formerly used extensively, produces weak analgesia at hypnotic doses and is now considered inadequate as a sole surgical agent. Urethane provides stable, prolonged deep anesthesia (up to 7+ hours) [5] and remains employed in non-recovery physiological and pharmacological studies in rodents. Ultra-short-acting barbiturates such as sodium pentobarbital, once widely used intraperitoneally [6], are now primarily used for euthanasia [7]. During the COVID-19 pandemic, Japanese researchers demonstrated non-pharmaceutical-grade sodium pentobarbital 5% aqueous solution as a short-term alternative when pharmaceutical-grade agents were unavailable [8]; however, regulatory controls have since markedly reduced pentobarbital use [9].

Propofol, a rapid-acting sedative-hypnotic with short duration and quick recovery, is frequently used for anesthetic induction or maintenance in brief procedures involving rodents, rabbits, dogs, and pigs. Alfaxalone, with a similar mechanism, is commonly employed in dogs, cats, and mice [10], and was recently identified as suitable for brain electrophysiology in Western painted turtles [11].

2.3. Miscellaneous Injectable Drugs and Drug Combinations

Ketamine, a dissociative anesthetic with rapid onset and potent analgesia [12], is the single injectable agent providing the most properties of a complete anesthetic, though direct myocardial depression limits its ubiquitous use [13]. Rational drug combinations are therefore employed to mitigate adverse effects. Zoletil-50 combines tiletamine and zolazepam (1:1) [14, 15]. Xylazine, an alpha-2 receptor agonist, is typically combined with other anesthetics [16], and ketamine is commonly used for premedication or adjunctive administration [17]. The ketamine-xylazine (KX) protocol is applicable to mice, rats, rabbits, and sheep [15]. Ketamine combined with dexmedetomidine produces fewer cardiovascular side effects than the KX protocol [18].

2.4. Narcotic Analgesics

Analgesia is integral to the "Refinement" principle of the 3Rs. As early as 1984, Flecknell emphasized that general anesthetics alone cannot eliminate postoperative pain and distress, recommending adjunctive narcotic analgesics during invasive procedures [19]. Nevertheless, a 2005 follow-up study revealed that up to 70% of authors who omitted analgesic reporting had never administered analgesics, indicating a widespread deficiency [20]; the ARRIVE 2.0 guidelines subsequently reinforced the necessity of analgesic protocols.

Key analgesics include opioids (buprenorphine, morphine) and NSAIDs (meloxicam). Morphine is a potent classic analgesic but carries numerous side effects [21]. Buprenorphine, with 40-fold greater analgesic potency than morphine, offers minimal respiratory depression and low addiction potential [22], leading to its widespread adoption. Meloxicam is suitable for mild to moderate pain across diverse species, with long duration of action and a favorable safety profile [23].

2.5. Neuromuscular Blocking Agents

Neuromuscular blocking agents (NMBs) facilitate tracheal intubation and mechanical ventilation, improving surgical exposure, particularly in larger animals. Atracurium and rocuronium are commonly used [24]. Bellini L et al. [25] detailed atracurium use during porcine laparoscopy: intramuscular ketamine (7 mg/kg) with medetomidine (15 μg/kg), intravenous propofol induction, sevoflurane maintenance, intraoperative morphine (0.3 mg/kg), and neuromuscular blockade with atracurium (0.4 mg/kg IV). Notably, atracurium-induced neuromuscular blockade was significantly prolonged under permissive hypercapnia ($PaCO_2$ 60–70 mmHg), offering valuable insights for experimental and clinical practice.

If NMBs are administered under inadequate anesthesia, accidental awareness during general anesthesia (AAGA) may occur—a serious concern warranting vigilant attention [26].

3. Common Anesthetic Protocols for Laboratory Animals

Laboratory animal anesthesia aims to enhance biomedical data reproducibility while complying with animal welfare ethics [27]. No universally accepted anesthetic protocol exists,

even for specific animal models [12]. Beyond surgery, animal models are increasingly employed in medical imaging, ultrasonography, and endoscopy, making appropriate anesthesia selection critical [28].

3.1. Premedication

For small animals, most injectable anesthetics require dilution (occasionally to 10% solutions) to minimize overdose risk and improve dosing accuracy. Pre-induction anticholinergics such as atropine reduce salivary and respiratory secretions, preventing airway obstruction and bradycardia—particularly important in mice, dogs, and cats [29].

3.2. Anesthesia Induction and Endotracheal Intubation in Small Animals

Both inhalational and injectable agents are used for induction. Endotracheal intubation provides a secure, controllable airway for large animals but remains technically demanding in small animals due to anatomical variability. For mice, successful tracheal intubation itself is a significant challenge.

While ventilators and specialized equipment for large animals are widely available, small animals often require adapted clinical materials. For mice, a Welch Allyn fiber optic otoscope serves as a laryngoscope, a 20 G IV catheter as the endotracheal tube, and a thin wire as a stylet. The mouse is placed in an induction chamber with 3–5% isoflurane for 90–120 s until movement ceases; the otoscope visualizes the vocal cords, and the catheter-stylet assembly is advanced 5–8 mm while withdrawing the stylet. For rats, a size-0 Miller laryngoscope and a 20 G IV catheter are used. Injectable induction with ketamine (60 mg/kg) plus xylazine (5 mg/kg) is an effective alternative [31].

Endoscopic intubation benefits both research quality and animal welfare. Konno K et al. demonstrated that the TESALA AE-C1 endoscope system enabled simple, reliable, and minimally traumatic rat tracheal intubation [32], with the endotracheal tube advanced 1.5–2.0 cm past the glottis. Correct placement is confirmed via capnography or chest wall movement before mechanical ventilation.

A ventilator with a precise breathing circuit is essential for respiratory stability, particularly in prolonged survival experiments [30, 33]. When resources are limited, improvised systems—such as a three-way stopcock replacing the Mapleson E breathing circuit—have been successfully employed in rats [30]. High-performance animal anesthesia ventilators facilitate rapid emergence while preserving normal blood gases and avoiding lung injury [34].

3.3. Anesthetic Maintenance and Intraoperative Monitoring

No universally accepted anesthesia maintenance protocol exists for animal studies, and monitoring cardiovascular and respiratory function in small animals is challenging. Jalde et al. [35] administered intramuscular ketamine (10 mg/kg) and propofol (2 mg/kg) for sedation in pigs before intubation, observing that high-dose propofol could induce arrhythmias and severe hypotension. Comparing low-dose ketamine infusion with sevoflurane versus propofol, neurally adjusted ventilatory assist (NAVA) was feasible under both regimens, with sevoflurane demonstrating superior respiratory mechanics.

3.4. Anesthesia for Non-surgical Procedures

3.4.1. Functional Magnetic Resonance Imaging (fMRI)

fMRI measures changes in local blood flow, volume, and oxygenation (neurovascular coupling) rather than direct neural activity. Adequate sedation/analgesia is essential to prevent motion artifacts while the chosen agent must minimally perturb the neurovascular system [36]. Dexmedetomidine, a highly selective alpha-2-adrenoceptor agonist, produces sedation, analgesia, and anxiolysis via locus coeruleus activation—a mechanism closer to physiological sleep. Unlike isoflurane, dexmedetomidine suppresses cortical microcircuits and neurovascular coupling to a lesser extent; BOLD signal amplitude and functional connectivity under dexmedetomidine more closely resemble the awake resting state. Intravenous dexmedetomidine combined with low-dose isoflurane is a common regimen [36, 37], maintaining physiological stability while preserving neural activity and neurovascular coupling.

3.4.2. Positron Emission Tomography (PET)

PET provides insights into neuroreceptor and neurotransmitter dynamics. Tracer kinetics depend critically on standardized injection parameters: injection activity affects imaging sensitivity and count statistics, while molar activity determines specific binding efficiency. Ma Y et al. [38] investigated the effects of mechanical ventilation and anesthetics on PET tracer kinetics in rats. Mechanical ventilation reduced peak tracer levels and prolonged time-to-peak, while isoflurane accelerated the tracer equilibrium rate as reflected by the standardized uptake value ratio (SUVr).

3.4.3. Anesthesia for Endoscopic Animal Models

Endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) are key treatments for early upper gastrointestinal cancers; delayed bleeding occurs in 1.8–15.6% of post-ESD patients. Pigs are common models for endoscopic experiments. To prevent oral endoscope interference with the airway, meticulous airway management is essential [39]. Uehara S et al. [40] established a delayed bleeding model after endoscopic resection in 6-month-old Clawn miniature pigs (13–14 kg), using intramuscular ketamine (15 mg/kg) and xylazine (2 mg/kg) for induction, followed by tracheal intubation and isoflurane maintenance.

4. Considerations

4.1. Preoperative Preparation

Intramuscular injections are generally avoided in very small animals (e.g., mice) due to limited muscle mass and nerve injury risk. Rabbits are easily stressed and require gentle handling. An acclimatization period of 3 days to 2 weeks is recommended before procedures. Preoperative fasting may be considered for large animals, non-human primates, and species at risk of reflux/aspiration; rodents rarely vomit and typically do not require fasting [41, 42]. Healthy individuals are preferred for survival surgery, whereas non-survival surgery only requires meeting basic experimental criteria [43].

4.2. Intraoperative Monitoring and Maintenance

Hypothermia is a common and potentially fatal complication during animal anesthesia [44, 45], especially in small mammals with high surface area-to-volume ratios. Active warming via heating pads or lamps is critical [46]. Intraoperative fluid and blood loss warrant attention with appropriate supplementation. Rabbits have fragile spines and hindlimb bones, necessitating careful support during positioning.

Normal resting respiratory rate in mice is 80–230 breaths/min and heart rate 400–600 bpm; a 50% decrease in heart rate during anesthesia is generally acceptable [47]. Non-invasive blood pressure and oxygen saturation monitoring are often challenging in small rodents [48, 49]; direct observation of respiration and cardiac activity is primary. For larger animals, basic vital sign monitoring (blood pressure, heart rate, SpO_2) is feasible. In intubated animals, capnography, tidal volume, and airway pressure can be monitored, with EEG used in specific cases [50].

4.3. Postoperative Antagonism and Recovery

Postoperative analgesics and/or anesthetic antagonists may be administered based on surgical trauma and anesthetic depth. Li et al. [51] found that a GluN2A-selective positive allosteric modulator (PAM, 2 mg/kg) combined with nalmefene (0.1 mg/kg) and flumazenil (0.4 mg/kg) (PNF combination) significantly shortened the loss of righting reflex time in rats anesthetized with ketamine-fentanyl-dexmedetomidine, without affecting cardiac, hepatic, renal, or cognitive function. Kirihara et al. [52] reported that intravenous atipamezole (0.75 mg/kg) provided optimal antagonism of medetomidine-midazolam-butorphanol anesthesia in rabbits, with faster recovery than intramuscular injection. However, Layton et al. [53] noted that intramuscular atipamezole (0.12 mg/kg), while shortening recovery and improving thermoregulation in pigs, triggered adverse behaviors negatively impacting welfare.

4.4. Euthanasia of Laboratory Animals

Euthanasia, the humane termination of an animal's life with minimal distress through rapid loss of consciousness, is both a welfare requirement and part of the experimental protocol [54].

Common methods include CO_2 inhalation and barbiturate injection [7, 49]. CO_2 inhalation is preferred for small animals (rodents, rabbits) due to simplicity, safety, and low cost. Animals are placed in a closed chamber, with CO_2 introduced at 10–30% of chamber volume per minute; after loss of consciousness, flow rate may be increased (≤0.5 kPa). Cessation of respiration and heartbeat is confirmed, with observation continued for 2 min [55]. Intraperitoneal sodium pentobarbital (100–200 mg/kg) is also commonly used for rodent euthanasia [56].

5. Conclusion

This article systematically reviews the principles, drug selection, and techniques for anesthetizing common laboratory animals, providing an introductory guide for researchers. It sorts out the characteristics of various anesthetic agents and matched anesthesia protocols for surgery, imaging and endoscopic models, forming a complete perioperative operation framework that helps improve the stability and reproducibility of experimental data. Limitations include insufficient discussion of inter-strain individual variability and ongoing challenges in precise physiological monitoring, particularly in small animals.

Laboratory animal anesthesiology—a balance of animal ethics, scientific requirements, and physiology/pharmacology—will increasingly emphasize precision and humaneness. Future research should prioritize safer targeted anesthetics, personalized protocols tailored to species, strain, and experimental objectives, and non-invasive or minimally invasive monitoring technologies. These research directions are of great significance: they can further reduce perioperative pain and distress of experimental animals to fully implement the 3Rs refinement principle, while minimizing physiological interference induced by improper anesthesia to guarantee credible experimental outcomes. Continuous optimization of anesthetic strategies summarized in this review will maximize adherence to animal welfare ethics and elevate the overall standard of preclinical biomedical research in the long term.

Abbreviations

AAGA	Accidental Awareness During General Anaesthesia
ARRIVE	Animal Research Reporting of In Vivo Experiments
BOLD	Blood Oxygen Level Dependent
EEG	Electroencephalography

EMR	Endoscopic Mucosal Resection
ESD	Endoscopic Submucosal Dissection
fMRI	Functional Magnetic Resonance Imaging
IV	Intravenous
KX	Ketamine-Xylazine
NAVA	Neurally Adjusted Ventilatory Assist
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NMBs	Neuromuscular Blocking Agents
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PAM	Positive Allosteric Modulator
PET	Positron Emission Tomography
PNF	PAM-Nalmefene-Flumazenil
SUVr	Standardized Uptake Value Ratio
UFAW	Universities Federation for Animal Welfare

Acknowledgments

The author would like to thank the anonymous reviewers for their valuable comments and constructive suggestions, which have greatly improved the quality of this manuscript. We would also like to thank the Department of Anesthesiology at the Fourth Hospital of Hebei Medical University for their help with data collection and the writing of the manuscript.

Author Contributions

Binglin Peng: Conceptualization, Writing – original draft
Jing Zhang: Data curation, Writing – original draft
Huaqin Liu: Formal Analysis, Investigation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Field

Huaqin Liu: Clinical expertise: Anesthesia for elderly, critically ill patients, and cardiothoracic surgery. Research focus: Perioperative brain protection.