

Research Technical Note

Rodent Anesthesia Guidelines: Considerations for Choosing Agents for Hemodynamic Measurements

There is no single best choice for anesthetic agents as procedure, parameters of interest, and animal type all impact anesthesia choice. Always check what is currently available and allowed with your Institutional Animal Care & Animal Use Committee and make sure that the anesthetic agent is balanced with proper analgesics. It is important to note that the availability of anesthetic agents changes and is dependant on your institution and country.

Considerations Related to the Procedure

- Type of procedure
- Projected length of the procedure
- Amount and type of pain/distress anticipated
- Study goals (are important parameters influenced by certain drugs?)
- Survival or terminal study (agents associated with prolonged recovery or delayed effects may be approved for terminal studies while deemed inappropriate for survival procedures)
- Acute or chronic study

Considerations Related to the Animal

- Species and strain
- General condition and underlying health problems
- Age
- Sex
- Weight
- Previous Drug Exposure
- Nutritional Status
- Time of day as related to circadian rhythm
- Ability to maintain body temperature (preventing hypothermia due to heat loss)
- Numbers of animals to be anesthetized simultaneously

Note: There can be remarkable variation in response to anesthesia. Investigators should monitor anesthesia closely in each animal and make appropriate modifications in the anesthetic regimen when necessary

Considerations Related to the Drug(s) Used:

- Drug safety and ease of use
- Appropriateness for the procedure including administration method
- Appropriateness for the animal
- Side effects
- Equipment and training required for safe use
- Previous experience using the agent(s)
- Cost and status as controlled or uncontrolled drug

Summary: Anesthetic Agents Should:

- Provide an appropriate depth and length of anesthesia and analgesia without affecting important study parameters
- Be appropriate for the animal given its species, medical history and physical condition
- Have minimal side effects
- Be safe for both the animal and the personnel administering anesthesia

Rodent Anesthesia Guidelines Cont.

Cardiovascular Effects of Anesthetics

Many common anesthetics have a significant effect on cardiovascular measurements and can obscure or confound study results; sometimes over a longer period of time than anticipated. It is therefore necessary to choose an anesthesia protocol with care. For the purpose of cardiac experimental procedures general anesthesia is recommended, however dissociative anesthetics in combination with a sedative agent may be used as well.

During the experimental procedure, management of anesthesia has to be catered to any underlying or experimentally caused cardiovascular disorder. For example, experimentally induced aortic stenosis (trans-aortic banding or constriction) requires anesthesia which avoids systemic vasodilation and tachycardia while preserving sinus rhythm such as a synthetic narcotic based anesthesia.

INHALED (HALOGENATED ETHER) ANESTHETICS

It is known that inhaled anesthetics may cause circulatory depression at concentrations required to produce general anesthesia. In addition, each individual inhalation anesthetic has selective dose-dependent effects on cardiovascular function (sympathetic reflexes, intravascular volume status, vascular smooth muscle tone, myocyte contraction and relaxation, acid-base status etc.). For this reason, circulatory interactions of inhaled anesthetics might limit the anesthetic dose. Consequently, some laboratories combine inhaled anesthetics with sedatives or hypnotics to produce the necessary general anesthesia. Others empirically developed state of the art mono-anesthetic protocols using minimum amount of inhalation anesthetics to mimic close to fully-conscious state while collecting data.

Drop of blood pressure (BP) caused by inhalation anesthetics is a direct result of dose-dependent vasodilation accompanied by an afterload reduction and depression of myocardial contractility and an indirect result of attenuation of sympathetic nervous system. Decrease in BP during Isoflurane induced general anesthesia is so predictable that some laboratories often use this as a sign for assessing the depth of anesthesia.

Halogenated anesthetics decrease global LV systolic function at any given LV loading condition or at any given degree of underlying sympathetic tone. Experimental studies suggest that these agents cause minimal changes in LV diastolic compliance but impair LV diastolic relaxation in a dose-dependent manner. These agents have minimal direct effects on LV preload, but rather EDP may increase during anesthesia because of impaired diastolic filling and decreased cardiac output (CO).

The administration of inhaled anesthetics to experimental animals with cardiovascular diseases has some advantages. Most inhaled anesthetics are myocardial depressants with negative inotropic properties which decrease contractility and thus decrease myocardial oxygen demand. Arterial vasodilation combined with preserved coronary perfusion maintains oxygen delivery to the heart. Adequate oxygen delivery combined with a decreased demand for oxygen creates a more favorable myocardial oxygen balance in hearts with coronary insufficiency. Additionally, the vasodilating and antihypertensive actions of inhaled anesthetics effectively control an increase in BP in response to surgical pain.

Inhalation anesthetics have a proportionally greater negative inotropic effect on diseased myocardium compared with normal myocardium. In the case of an experimentally induced septic shock by injection of LPS or cecal puncture, profound ventricular dysfunction may not tolerate the cardiovascular depressant effects of inhaled anesthetics given in concentrations that are needed to produce the anesthesia. The pro-thrombotic side effect of sepsis causes decreased coronary perfusion pressure which prevents adequate oxygen extraction via Fick's principle. In this case cardiac oxygen demand exceeds the rate of consumptions (MVO₂) causing a negative oxygen balance which further depresses cardiac function.

Rodent Anesthesia Guidelines Cont.

RODENT ANESTHESIA BREATHING CIRCUITRY :

Open System is the traditional method of dipping ether or chloroform on gauze, later modernized by the Schimmelbusch mask and used until about 1950.

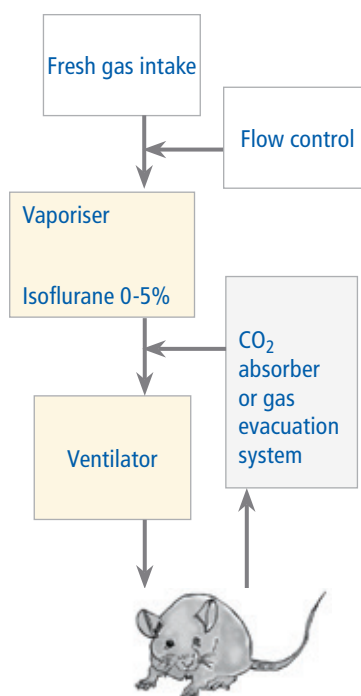
Semi-open System is commonly used today and includes all the Mapleson systems. This is typically used for animal anesthesia induction, usually a single branched system that uses a valve to control the pressure of the gas, and allows for waste gas to leave the system. This system can be further characterized by high fresh gas inflow in order to stop re-breathing of expired CO_2 .

Semi-closed and Closed Systems use a CO_2 absorbent and thus gases are re-circulated; the classification (semi-open vs closed) is defined by the amount of fresh gas flow. These systems are mainly used for maintenance of anesthesia following induction. Additionally, they can be used for anesthesia induction, but this is a slower process than using a semi-open system.

Expired gases from the animal pass through a container in the breathing system which contains a CO_2 absorbent to remove CO_2 from the expired gases. This method requires a high level of animal monitoring, especially levels of inspired and expired CO_2 and the anesthetic agent. This absorbent, by an exothermic chemical reaction removes the CO_2 , thus allowing an animal's expired gases to be re-breathed. Because of this exothermic chemical reaction, some warmth and humidity is added to the inspired gases. In this setting, the animal's expired gases are recirculated, allowing for a reduced inflow rate of additional fresh gas.

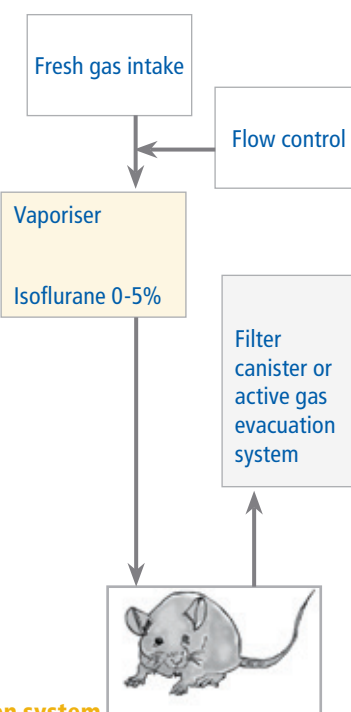
Breathing system components:

1. Fresh gas intake (O_2 , medicinal air etc.)
2. Adjustable pressure and/or volume limiting valve
3. Connection to animal (ventilator)
4. Waste gas connection tubing or anesthesia gas absorber



Semi-closed system

Schema of Isoflurane inhalation semi-closed or closed system for rodent maintenance anesthesia. Unidirectional valves permit pressure driven flow through the vaporizer into the inspiratory limb of circle system. Exhaled gases are routed into expiratory limb and recirculated through use of CO_2 absorber. A bidirectional valve positioned in the expiratory limb permits gases to be evacuated if needed (e.g. high pressure develops).



Semi-open system

Schema of Isoflurane inhalation semi-open circuit (gases are not recirculated). Unidirectional valves permit pressure driven flow through the vaporizer to the Anesthetizing Box; exhaled gases are routed into Filter canister (removal of excess of Halogenated gases) or into active gas evacuation system.

Rodent Anesthesia Guidelines Cont.

SEDATIVES-HYPNOTICS

This group of anesthetics include barbiturates, benzodiazepines, etomidate, propofol and ketamine. They are used for pre-surgical sedation, producing immediate loss of consciousness, to supplement the actions of the inhaled anesthetics, and to provide sedation in the immediate postoperative period. The circulatory effects of individual agents are an important consideration for subjects with CV disease. The sedative-hypnotics have direct effects on cardiac contractility and vascular tone in addition to indirect effects on autonomic tone.

Barbiturates (e.g. sodium pentobarbital, thiopental and methohexital) are anxiolytics, hypnotics, anticonvulsants and weak analgesics with negative inotropic effects. They produce dose-dependent decrease in dP/dt and the force-velocity relationship of ventricular muscle. Induction of general anesthesia with barbiturates is associated with a decrease in blood pressure (BP), heart rate (HR) and cardiac output (CO).

In comparison with barbiturates, propofol appears to cause less myocardial depression. Mean arterial pressure (MAP) decrease after propofol is attributed primarily to both arterial and venous dilatation. Propofol is well suited for continuous i.v. infusion for sedation because it has a short duration of action and can be titrated to effect. Propofol is usually combined with opioids (Fentanyl, Sufentanyl etc.) for its lack of analgesia.

Etomidate and ketamine are administered for rapid induction of general anesthesia in experimental animals with pre-existing hemodynamic compromise because they generally cause little or no change in circulatory parameters. Etomidate has virtually no effect on myocardial contractility even in diseased ventricular muscle. For its endocrine and neuroendocrine non-anesthetic interferences it is limited to short-term use as an i.v. induction agent.

Ketamine often increases HR and BP and causes bronchodilation because of its sympathomimetic properties. Ketamine has other beneficial effects including analgesia, anesthesia, and direct negative inotropic and vasodilatation effects

NARCOTICS (OPIOID) ANESTHETICS

Narcotic-based anesthetics offer the advantages of profound analgesia, attenuation of sympathetically mediated cardiovascular reflexes in response to pain, and have virtually no direct effects on myocardial contractility. Even though narcotics have little direct action on the heart, they may cause profound hemodynamic changes indirectly by attenuating sympathetic nervous tone while decreasing serum catecholamine levels, which may cause indirect cardiac depression.

In addition, other inconveniences encountered with narcotic-based anesthetics include difficulty estimating required dose, predicting the duration of postoperative narcotic-induced respiratory depression, and ensuring hypnosis during operation. Rapid administration of narcotics (Fentanyl) is also associated with muscle rigidity of the thoracic and abdominal musculature that may impede the ability to ventilate the patient immediately after the induction of general anesthesia.

Development of short-acting narcotic anesthetics may improve the ability to control anesthetic depth without prolonging recovery time. Ultra-short-acting narcotics (Remifentanyl) may have a unique niche in cardiac anesthesia because their effect is terminated immediately on stopping the drug infusion due to rapid in vivo ester hydrolysis.

ADVANTAGES OF INHALATION ANESTHESIA AS COMPARED WITH INJECTABLE ANESTHETICS

- Easily controllable cardiovascular depression
- Reduced impact on liver functions
- Reduced impact on kidney functions
- Encourages rapid recovery
- Allows superb control while on anesthesia
- Easy maintenance of surgical anesthetic depth
- Dose and volume can be easily adjusted
- Less stress on subject as compared to injections
- More predictable pharmacokinetics

Rodent Anesthesia Guidelines Cont.

CATEGORY	AGENT	SPECIES & DOSE (MG/KG)	ROUTE*	HEMODYNAMIC EFFECTS*	PMID CITATION
Anesthetics - Injectable	Alphaxolone (Alfaxan)	Mice: 15	IV	increased HR, decreased MAP	17319964
	Alphaxolone/Alphadolone (Saffan)	Rats: 18/6	IP	vasodilation	11098097, 11575348
	Chloral hydrate	Rats: 300 - 400	IP	minimal cardiopulmonary depression	8355479
	Alpha-chloralose ¹	Rats: 50-55	IP	minimal cardiopulmonary depression	19003937
	Fentanyl/Droperidol (Innovar-Vet)	Mice: 0.078/3.9	IM	vasodilation	15288130
	Fentanyl/Medetomidine	Rats: 0.3/0.3	IP	decrease HR, SV & CO, cardiorespiratory depression	22561119
	Propofol/Fentanyl/Medetomidine	Mice: 75/0.2/1 Rats: 100/0.1/0.1	IP	Vasodilation, cardiorespiratory depression	19001064, 20819392
	Propofol/Remifentanyl	Mice: 50-200/0.2-1	IP	Vasodilation, cardiorespiratory depression	17640460
	Ketamine	80- 200	IM	good HR & BP	18172330
	Ketamine/Diazepam (Valium)	Mice: 100/5 Rats: 40/5	IP	minor cardiorespiratory depression	7278122
	Ketamine/Xylazine (Rompun)	Mice: 80-150/7.5-16 Rats: 40-80/5-10	IP, IM	cardiorespiratory depression (MAP & CO), arrhythmia	15155266, 7278122
	Ketamine/Midazolam	Mice: 50-75/1-10 Rats: 60/0.4	IP	decreased MAP & CO	16174120
	Ketamine/Acepromazine	Mice: 100/5	IP	minor CV depression, hypotension	23382271
	Ketamine/Xylazine/Acepromazine	Mice: 100/2.5/2.5 Rats: 40/8/4	IP, IM	good MAP & HR	11924805
	Pentobarbital Na (Nembutal ²)	Mice: 30-90 Rats: 30-60	IV, IP	decreased CO, MAP & HR; increased ESV & EDV	15155266, 15027618
	Tiletamine/Zolazepam (Telazol)	Rats: 20 - 40	IM, IP	good CI, minor cardiorespiratory depression	17343357
	Thiamylal (SuritalR)	Rate: 25 - 50	IV, IP	cardiorespiratory depression, arrhythmia	1637605
	Thiopental Na (PentothalR)	Mice: 30-40	IV, IP	Cardiorespiratory depression, decreased BP	18172330
	Etomidate	Mice: 22-25	IP	decreased HR, good CO & MAP	7278119, 12814659
	Urethane ¹	Mice: 800 - 1300	IP	good MAP, & CO	15155266
Urethane/Etomidate/Morphine ¹	Mice: 750/20-25/1-2	IP	good MAP & CO	15604134	
Tribromoethanol ¹ (TBE or Avertin)	Mice: 250 Rats: 150	IP	moderate cardiopulmonary depression	16884172	
Anesthetics - Inhalant ³	Isoflurane (Forane)	Mice: 0.1-1.5% Rats: 0.25-2.5% in pure O ₂ maintenance	Inhalation	Vasodilation, decreased BP, good CO	18550865, 12003817, 22492676
	Desflurane	To effect (4-6%)	Inhalation	Vasodilation, decreased BP	22929732
	Sevoflurane	Rats: 3.5-4% in pure O ₂ maintenance	Inhalation	Vasodilation, decreased BP	21778336, 22167771

*SC=subcutaneous, IM= intramuscular, IP= intraperitoneal, PO=orally, IV=intravenous, HR = heart rate, SV = stroke volume, MAP= mean arterial pressure, CO= cardiac output, CI= cardiac index, ESV = end systolic volume, EDV = end diastolic volume,

1. Terminal Studies only.

2. Dilute stock solution to accurately dose animals

3. These agents should be used only in ways that prevent exposure to personnel.

Induce anesthesia in a closed container and maintain with a nose cone in an appropriately ventilated hood.

Rodent Anesthesia Guidelines Cont.

ANESTHESIA TIPS & CONSIDERATIONS

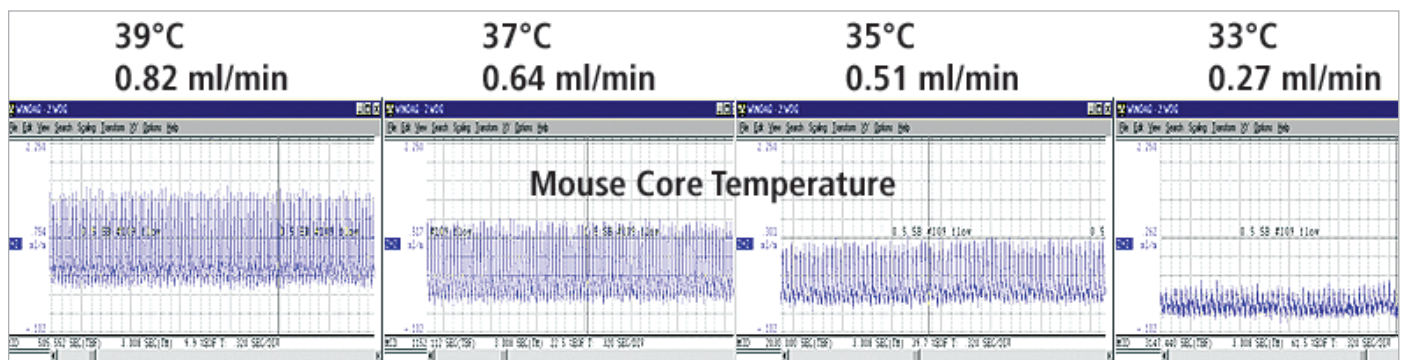
- When anesthetizing post-MI animals, maintenance of coronary artery pressure helps limit tachycardia.
- Induction of anesthesia can cause arrhythmias (junctional rhythms). Treat by reducing the dose of inhalation anesthetic or administering an anticholinergic.
- Halogenated volatile inhalation anesthetics (isoflurane) should be used in a vented hood to reduce operator exposure during procedures.
- It is advisable to monitor blood gases before, during and after anesthesia to ensure normal metabolism and prevent the development of alkalosis or acidosis.
- Anesthetized animals do not completely close their eyelids therefore they are at risk of corneal desiccation and ulceration. It is advisable to protect their eyes with sterile eye-lubricating ointment, especially in long-duration studies.
- It is recommended to use a single injection while delivering an injectable anesthesia to small rodents to reduce anxiety and ensure a stress-free induction and recovery. However, care must be taken when mixing agents for a single injection to ensure safety and efficacy.

CATEGORY	AGENT	DOSE (MG/KG)	FREQUENCY	ROUTE
Anticholinergics	Atropine	Both: 0.02-0.05	Both: once at induction	SC
	Glycopyrrolate	Both: 0.01-0.02	Both: once at induction	SC
Analgesic (NSAID)	Acetaminophen	Rats:100-300 Mice: 300	Rats: 4 hrs Mice: daily	PO
	Aspirin	Both: 100	Both: 4 hrs	PO
	Carprofen	Both: 5	Rats:12 hrs Mice: daily	SC
	Flunixin	Both: 1.1-2.5	Both: 12 hrs	SC, IM
	Ibuprofen	Both: 7.5	Both: daily	PO
Analgesic (Opiate)	Butorphanol	Rats: 0.05-2 Mice: 0.05-5.4	Both: 2-4 hrs	SC
	Meperidine	Both: 10 - 20	Both: 2-3 hrs	SC, IM
	Morphine	Both: 10	Both: 2-4 hrs	SC, IM
	Pentazocine	Both: 10	Both: 3-4 hrs	SC

Body Temperature

The high metabolic rate and high surface-to-volume ratio of mice means that they lose heat very quickly. It is therefore imperative to avoid anesthetics such as barbiturates, which alter the animal's ability to maintain core temperature.

Similarly, the animal should be warmed during operative procedures which open a body cavity and expose even greater surface area to ambient temperatures for heat loss. Body temperature should be monitored during heating to avoid increasing body temperature above 38°C.



Effect of Core Temperature On Femoral Blood Flow in a 22 gram CD-1 Mouse: As the effect of progressive lower core temperatures in the respective flow traces demonstrates, temperature has a profound effect on femoral blood flow and must be monitored.

Data, courtesy of M.F. Callahan, Dept. of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, NC

Rodent Anesthesia Guidelines Cont.

REFERENCES

Alves HC, et al. "Intraperitoneal propofol and propofol fentanyl, sufentanil and remifentanil combinations for mouse anaesthesia." *Lab Anim.* 2007 Jul; 41(3): 329-36

Blaudszun G, Morel DR. "Superiority of desflurane over sevoflurane and isoflurane in the presence of pressure-overload right ventricle hypertrophy in rats." *Anesthesiology.* 2012 Nov; 117(5): 1051-61

Gaertner, DJ, TM Hallman, FC Hankenson, MA Batchelder. 2008. Anesthesia and Analgesia in Rodents. Anesthesia and Analgesia in Laboratory Animals. Second Edition, Academic Press, CA.

Gargiulo S, et al. "Mice Anesthesia, Analgesia, and Care, Part I: Anesthetic Considerations in Preclinical Research." *ILAR J.* 2012; 53(1): E55-69

Hildebrandt IJ, et al. "Anesthesia and other considerations for in vivo imaging of small animals." *ILAR J.* 2008; 49(1): 17-26

Janssen BJ, et al. "Effects of anesthetics on systemic hemodynamics in mice." *Am J Physiol Heart Circ Physio.* 2004 Oct; 287(4): H1618-24

Roth DM, et al. "Impact of anesthesia on cardiac function during echocardiography in mice," *Am J Physiol Heart Circ Physiol* 2002; 282: H2134-40

Saha DC, et al. "Comparison of cardiovascular effects of tiletamine-zolazepam, pentobarbital, and ketamine-xylazine in male rats." *J Am Assoc Lab Anim Sci.* 2007 Mar; 46(2): 74-80

Zeller A, et al. "Mapping the contribution of β 3-containing GABAA receptors to volatile and intravenous general anesthetic actions." *BMC Pharm* 2007; 7(2)

IDEAL ANESTHETIC AGENT

- Reliable
- Wide safety margin
- Rapid onset/rapid recovery
- Easy to administer & control
- Nontoxic
- Causes no physical impairment
- Produces analgesia and muscle relaxation



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