Fundamentals of anesthesia for laboratory animals

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Effects of pain on the body

Pain is an important defense mechanism for the body, and stimulation of the sympathetic nerve helps escape from pain, and increased blood flow due to inflammation helps promote healing. However, for postoperative pain, this same mechanism has multiple adverse effects, slowing recovery from trauma and surgery and causing delayed wound healing.

1.Emotional: Causes low energy and anxiety, which further amplifies the pain.

2. Respiration: Causes decreased vital capacity, lung compliance and functional residual capacity, increased intrapulmonary shunt rate, and overall decreased gas exchange capacity.

3. Circulation: By tensing the sympathetic nerves, blood pressure rises, and heart rate and peripheral vascular resistance also increase. As a result, myocardial oxygen consumption increases and the burden on the heart increases.

4. Endocrine/Metabolism: Gluconeogenesis increases due to sympathetic tone, while insulin secretion decreases, resulting in decreased glucose tolerance. At the same time, renewal of body catabolism and increased proteolysis lead to delay in wound healing.

5. Others: It delays the recovery of appetite and motility, and causes an increase in the surgical wound infection rate.

Supervised by Nobuo Sasaki, Veterinary Clinical Anesthesiology, Gakusosha

Common Pain Misconceptions

- "Relieving pain causes animals to move and break sutures and fracture reductions."

  → It is unethical to use pain to control postoperative movement. If he needs to control activity, other means should be used

  (e.g., cage rest, gait restriction on lead, etc.)

- “You can‘t tell if you’re using analgesics to worsen your symptoms.”

→ Appropriate pain relief eliminates pain that can cause symptoms of worsening symptoms (eg, tachycardia).

- "Anesthetics are analgesics and therefore prevent pain.“

  → Many anesthetics (inhaled anesthetics, propofol, barbiturates) suppress pain perception but have no analgesic effect because nociception is still occurring in the unconscious state. Pain generated under general anesthesia is experienced with recovery from anesthesia.

WSAVA Global Veterinary Community

Pain identification, diagnosis and treatment guidelines
Journal of Small Animal Prac0ce © 2014 WSAVA

Guidelines for Perioperative Pain Management in Companion Animals (Veterinary Anesthesiology Society, Anesthesia and Pain Management Subcommittee)

1.Prediction of postoperative pain:

In general, the greater the degree of tissue damage, the greater the pain associated with surgery.

2.Anticipatory analgesia and multimodal analgesia:

Perform perioperative pain management (before, during, and after surgery) to enhance the therapeutic efficacy of postoperative pain and the safety of general anesthesia. (Omitted) We recommend the use of “anticipatory analgesia” and “multimodal analgesia” for perioperative pain management in companion animals.

3.Recognition and assessment of postoperative pain:

Animals feel pain, but their expressions vary according to age, species, and individual, and they may not express pain to protect themselves from external predators. (Omitted) Periodically determine the effectiveness of pain treatment and correct it (continue, add, cessation) should be performed and pain assessment is important. For postoperative pain management in companion animals, it is recommended to assess the degree of pain as regularly as possible as a vital sign using a pain scale or the like.

4. Appropriate Nursing:

“Pain” and “discomfort” and the unfamiliar environment of a veterinary hospital can cause anxiety in companion animals, and this anxiety increases pain. In hospitalized cases, provide the companion animal with comfortable soft bedding and a warm, clean environment, and minimize anxiety with appropriate nursing and gentle handling.

Animal Welfare

"Allowing humans to own and use animals while minimizing the pain and suffering they suffer."

(Hajime Ishikawa, "What is Animal Welfare?" 、『Japanese Society of Zoo and Wildlife Medicine』 Vol. 15 No. 1, 2010)

Act on Welfare and Management of Animals

(Methods, follow-up measures, etc. when providing animals for scientific use)

Article 41

2. When animals are to be used scientifically, they must be done in a way that causes as little pain as possible to the animal, to the extent necessary for the use.

Pain management in laboratory animals

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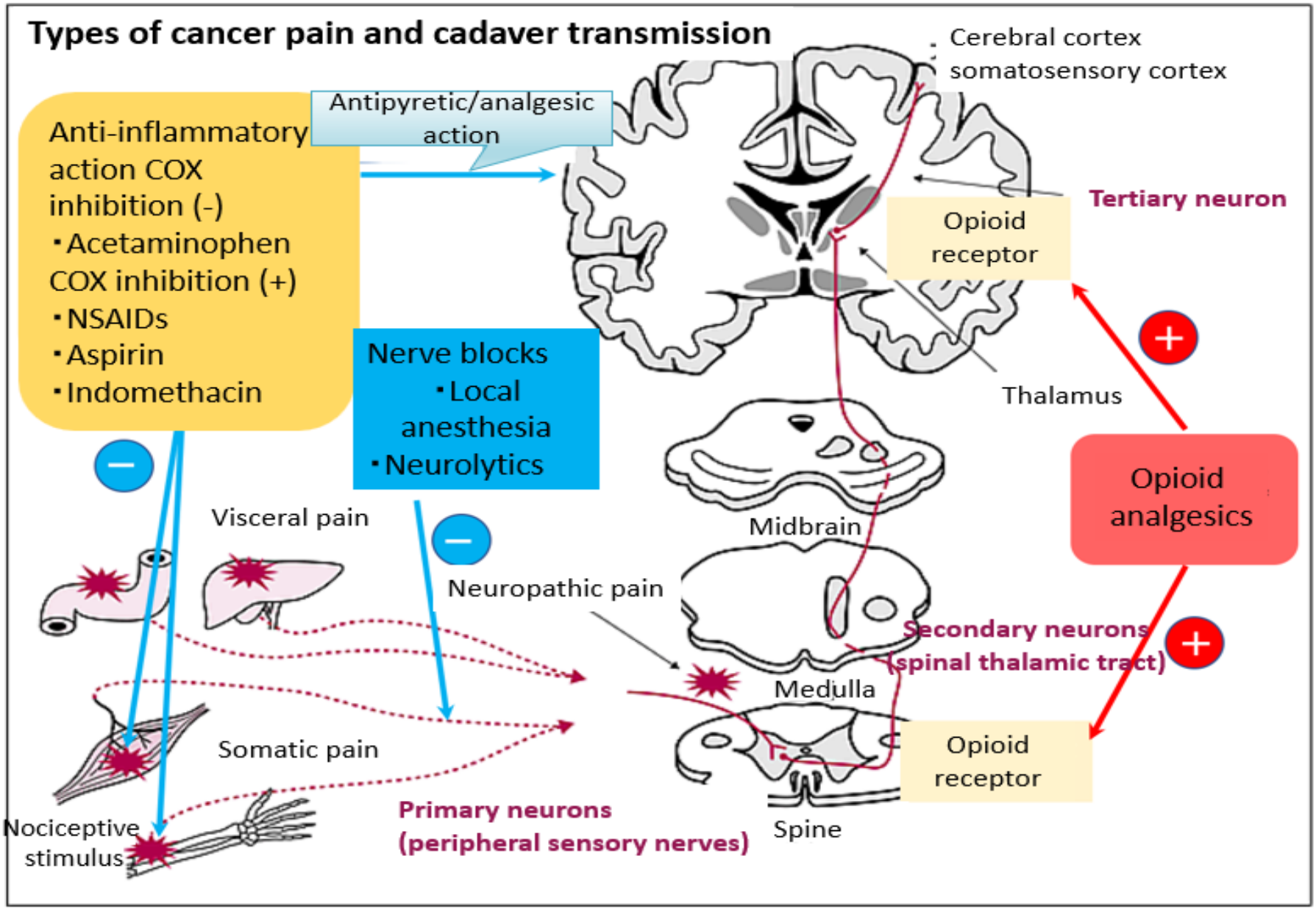
Companion Animal Pain Management



Partially modified from the 2010 version of Guidelines for Pharmacotherapy of Cancer Pain

What are opioids?

A general term for analgesics, narcotic analgesics and non-narcotic analgesics (excluding NSAIDs) that act as agonists on opioid receptors and have a strong central analgesic effect and are effective for most types of pain.

opioid	receptor agonism（μ、κ、δ）	action time	sedation	analgesic	reaction*
Narcotic					
Morphine	All receptors, mainly μ	3–6 hours	＋	＋＋	＋＋
Fentanyl	Mainly μ	10–45 minutes	—	＋＋＋	＋＋
Non-narcotic analgesic Butorphanol	Mainly κ	1–4 hours	＋/—	＋	＋/—
Buprenorphine	partially bound to μ	6–12 hours	＋/—	＋	＋

*Physiological functions of μ receptor: analgesia, respiratory depression, gastrointestinal motility inhibition, tolerance/dependence

・Narcotic analgesics have a strong analgesic effect, but a license is required to handle them due to problems such as euphoria and drug dependence.

・Non-narcotic analgesics have few side effects and are relatively easy to manage, but their analgesic effects are inferior to narcotic analgesics.

・While buprenorphine has a long duration of action (up to 12 hours), it takes a long time to develop its effect, and when side effects such as respiratory depression occur, it is difficult to antagonize the effect (psychotropic drug).

Narcotic analgesics for severe to moderate postoperative pain,
Non-narcotic analgesics recommended for moderate to mild pain

What are non-steroidal anti-inflammatory drugs (NSAIDs)?

A cyclooxygenase (COX) inhibitor that produces prostaglandins (PG) from arachidonic acid, it exhibits anti-inflammatory and analgesic effects by suppressing the production of PG, an analgesic substance in the periphery. It became clear that PG also plays an important role in pain transmission within the spinal cord, and new NSAIDs such as carprofen and meloxicam are also effective for postoperative pain. The main side effects are gastrointestinal disorders, renal disorders and blood coagulation disorders.

Prescription dose of NSAIDs

Drug	mouse	rat
Carprofen	5mg/kg sc, daily	5mg/kg sc, daily
Meloxicam	5mg/kg sc, daily	1mg/kg sc, daily

Paul Flecknell, Laboratory Animal Anaesthesia, Third Edition, Academic Press

Postoperative pain management

1.Initiate analgesic treatment in situations where pain is likely to occur, even if uncertain.
2. Provide preemptive (preemptive) analgesia before pain occurs. Pain becomes more intense with experience (memory).

What is general anesthesia?

Drug-induced reversible loss of analgesia, muscle relaxation, loss of consciousness, and loss of autonomic reflexes.

There is no ideal anesthetic that is safe for living organisms and has no adverse effects. Since each anesthetic does not have the same effect on all, it is usual to combine various sedatives, analgesics, etc. (balanced anesthesia) to obtain the necessary and safest systemic anesthetic for the procedure/surgery to be performed. A method that is as safe as possible and has no effect on the whole body is selected.

The four elements of general anesthesia: analgesia, sedation, muscle relaxation, and loss of noxious reflexes

→ If it lacks even one of these actions, it cannot be said to be an appropriate anesthetic.

- Inhalation anesthesia (isoflurane, sevoflurane, etc.)

Although the depth of anesthesia can be easily adjusted, it requires more preparation and manpower than injection anesthesia to secure the airway, prepare a dedicated vaporizer, and operate the anesthesia machine.

- Injectable anesthesia (ketamine/xylazine, mixed anesthetics, etc.)

It can be done easily without the need for dedicated equipment, but the depth of anesthesia cannot be adjusted after administration of the anesthetic.

Determination of surgical depth of anesthesia

Depth of general anesthesia

Phase 1: Also called the locomotor phase, it refers to the period from administration of an anesthetic to loss of consciousness.

Phase 2: Called the excitement phase, the period from loss of consciousness to phase 3 when the rhythm of breathing becomes constantPoint. Animals act violently in response to external stimuli.

Phase 3: This is called the appropriate period for surgical anesthesia. Respiration and heart rate are stable, laryngeal reflexes and eyelid reflexes are completely lost, sufficient muscle relaxation is obtained, and this is the appropriate time for many surgeries.

Phase 4: The central nervous system is severely depressed, breathing is weak or stopped, and blood pressure drops. If left in this state, death will result.

Determination of surgical depth of anesthesia:

First, confirm the disappearance of the righting reflex, and then confirm the disappearance of the reflex in response to stimuli to the toes and tail with hooked tweezers.

If breathing changes from steady to deep and slow, it means overly anesthetized. Conversely, rapid shallow breathing is a sign of waking up from anesthesia.

Inappropriate anesthesia of laboratory animals

- Inhalation anesthesia with diethyl ether

It has historically been widely used as an anesthetic, but has side effects such as flammability, airway irritation associated with excessive airway secretions, and pharyngeal spasms. This drug is no longer marketed as an anesthetic, but is sold as a reagent or industrial chemical, but is regulated by the Industrial Safety and Health Law and the Fire Service Law. Also, the use of non-pharmaceutical agents for anesthesia is ethically problematic.

- Pentobarbital sodium monotherapy

It has been widely used as an anesthetic and hypnotic, and is described in many textbooks as an injection anesthetic for laboratory animals. However, this drug has almost no analgesic effect, and it has been believed that surgical anesthesia can be obtained by making the patient unconscious due to its strong hypnotic effect. The dose that causes unconsciousness is close to the lethal dose, and it is known that many fatal accidents occur. Recently published laboratory animal anesthesiology textbooks (Fish, et al 2008, Flecknell, 2010) clearly state that general anesthesia with single administration of this drug is inappropriate. It is recommended as a euthanasia drug

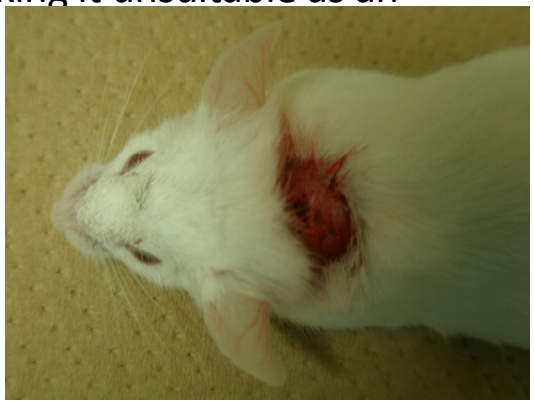
(AVMA Guidelines on Euthanesia, 2007).

Excerpt from Osaka University School of Medicine Laboratory Animal Medicine website, partially modified
http://www.med.osaka-u.ac.jp/pub/iexas/futekisetu3.htm

Inappropriate anesthesia of laboratory animals

・Anesthesia with non-pharmaceutical grade (reagent) agents (Avatine, tribromoethanol, etc.)

The use of pharmaceutical grade drugs ensures the elimination of unwanted side effects. Where pharmaceutical grade compounds are available, they should be used (ILAR Guide, 8th edition). Avatin is irritating at high doses, high concentrations, and repeated use, causing peritonitis and, in severe cases, death. Poor storage conditions can lead to lethal decomposition products, making it unsuitable as an anesthetic.



・ Urethane

It has been used in physiological studies from the perspective of an anesthetic that allows long-term immobility without lowering blood pressure with little depression of the cardiovascular and respiratory systems. However, this characteristic is due to sympathetic tone, and high concentrations of adrenaline and noradrenaline are secreted. In addition, urethane is classified as a mutagen (Group 2B suspected carcinogen to humans). Not only is it not applicable to arousal survival experiments, it is also dangerous to researchers and animal caretakers and is not recommended.

Excerpted and partially modified from the Commentary to the Standards for the Care and Keeping of Laboratory Animals and the Alleviation of Pain and Suffering

Recommended inhalation anesthetics for mice and rats (surgical anesthesia)

(NHI drug prices are calculated as of 2013)

- Isoflurane (Product name: Fallen, Distributor: AbbVie, drug price: 69.50 yen/mL)
  (Product name: Escaine, Distributor: Mylan Pharmaceutical, drug price: 30.10 yen/mL)
- Sevoflurane (Product name: Sevofuren, Distributor: Maruishi Pharmaceutical, Drug price: 63.40 yen/mL)
  (Product name: Sevoflurane “Mylan” Distributor: Mylan Pharmaceuticals Drug price: 49.10 yen/mL)

Common name	Diethyl ether*	Isoflurane	Sevofluorane
Boiling point (° C)	34.6	48.5	58.6
Explosive	+	-	-
blood/gas partition coefficient	12.0	1.4	0.63
Introduction/Awakening	slow	fast	faster
MAC (%) dogs	3.04	1.28	2.4
cat	-	1.63	2.58
airway stimulation	+ + +	+?	-
respiratory depression	-	+ +	+ +
muscle relaxation	+ + +	+ +	+ +
suppression of circulation	-	+	+
peripheral blood vessels	partialexpansion	Expansion	Expansion
blood pressure	ascension	descent	descent
heart rate	ascension	ascension	ascension

*Diethyl ether is not a recommended anesthetic.

Supervised by Nobuo Sasaki, Veterinary Clinical Anesthesiology, Gakusosha

What is MAC value (Minimum Alveolar Concentration)?

Anesthetic alveolar concentration (usually end-tidal concentration) at which 50% of animals showed no response to painful stimuli. It is an index of inhalation anesthetic strength. A concentration of 1.3 to 1.5 MAC is used for the surgical anesthesia period, and 2 MAC is used for deep anesthesia. MAC is decreased by hypothermia, administration of central depressant drugs, serious illness, and pregnancy.

Animal specific MAC value (%) for inhalation anesthetics

	Isoflurane (1.5MAC)	Sevofluorane (1.5MAC)
human	1.17	1.8
primates	1.28	-
dog	1.28	2.1-2.36
pig	1.45	3.5
sheep	1.58	3.3
cat	1.63	3.4
rat	1.38 (2%)	2.7 (4.05)
mouse	1.41 (2.1%)	2.5 (3.75)
rabbit	2.05	3.7

Paul Flecknell, Laboratory Animal Anaesthesia, Third Edition, Academic Press

- When anesthesia is induced, the concentration should be slightly increased.

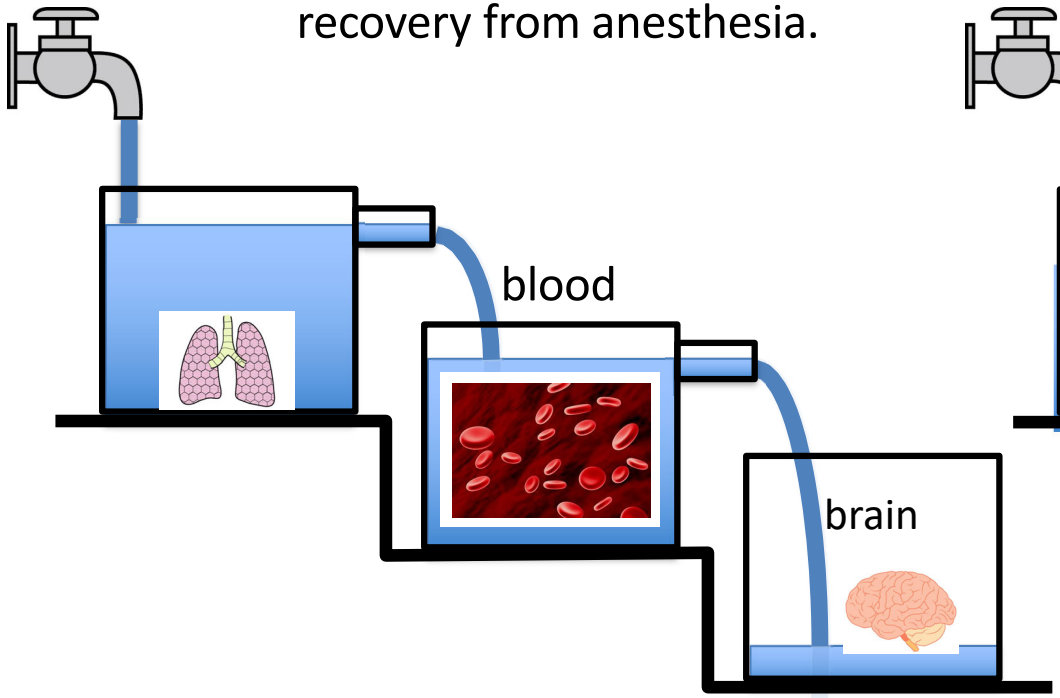
/Use a vaporizer dedicated to each anesthetic.

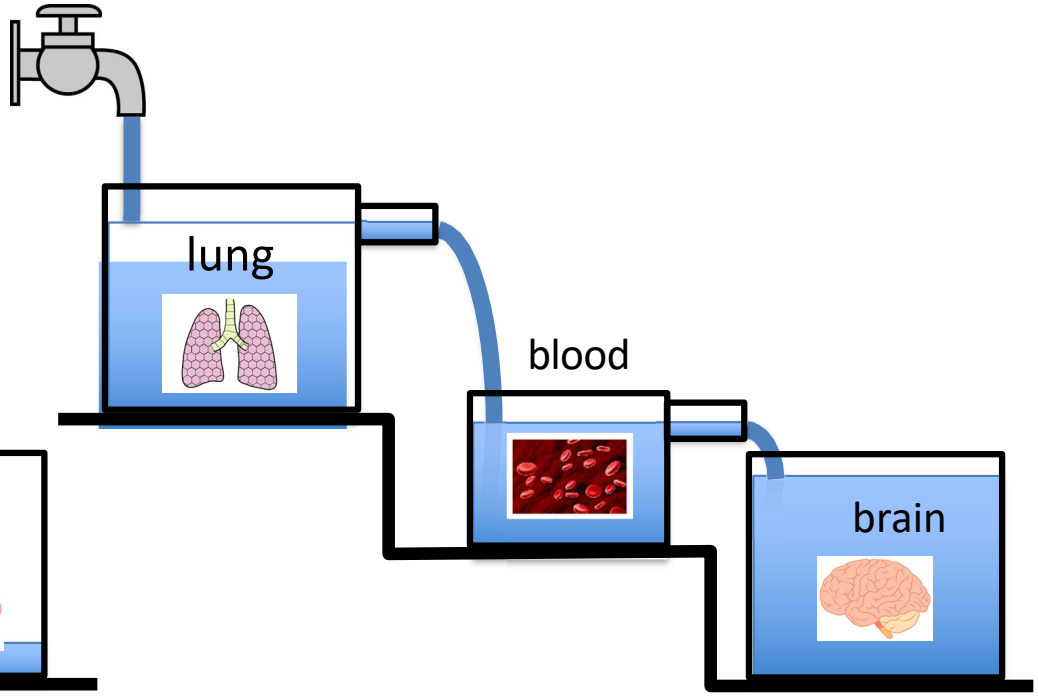
- Adjust the anesthetic concentration according to the condition of the animal.

What is blood/gas partition coefficient?

The ratio of the inhalation anesthetic concentration in the blood to the equilibrium inhalation anesthetic concentration.

The smaller this coefficient, the faster the induction of inhalation anesthetic and the recovery from anesthesia.



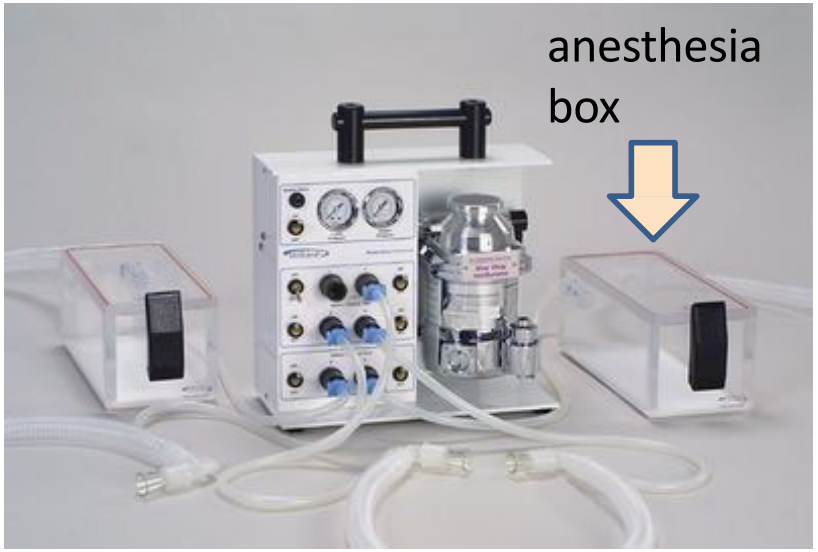


If the anesthetic is soluble in the blood, it will take longer for the blood to become saturated with the anesthetic, and it will take longer for it to reach the brain.

→ Slow induction and recovery of anesthesia.

If the anesthetic is difficult to dissolve in the blood, the blood becomes saturated with the anesthetic quickly, and it easily travels to the brain.

→ Induction and recovery of anesthesia is quick.



Inhalation anesthesia machine manufactured by VETEQUIP (Reprinted from HP of Hamley Co., Ltd.)

NARCOBIT

KN-1071
マウス･ラット等小動物実験用簡易吸入麻酔装置
NARCOBIT-E（Ⅱ型）

NATUME

The use of inhalational anesthetics during tail cuts, blood sampling, and euthanasia reduces animal pain and allows experimenters to perform experiments safely!

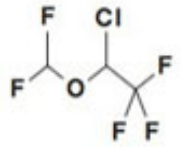
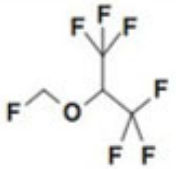
Inhalation anesthesia machine (Reprinted from the website of Natsume Seisakusho)

Research reagent

Isoflurane and Sevoflurane

Isoflurane and sevoflurane are anesthetic compounds that act on the central nervous system.

Caution! This product is a research reagent, not a pharmaceutical product. Not for human or animal medical use.

	Isoflurane	Sevoflurane
Structural formula		
CAS No.	26675-46-7	28523-86-6
Molecular weight	$C_3H_2ClF_5O$=184.49	$C_4H_3F_7O$=200.05
Introduction time	Fast	Faster than Isoflurane
Awakening time	Fast	Faster than Isoflurane
Tissue invasion (brain) ※1	High compared to sevoflurane	Low
Odor	Slightly pungent odor	Ether-like odor

※1 : Liang, G. *et al.:Anethesiology*.,112,1325(2010)

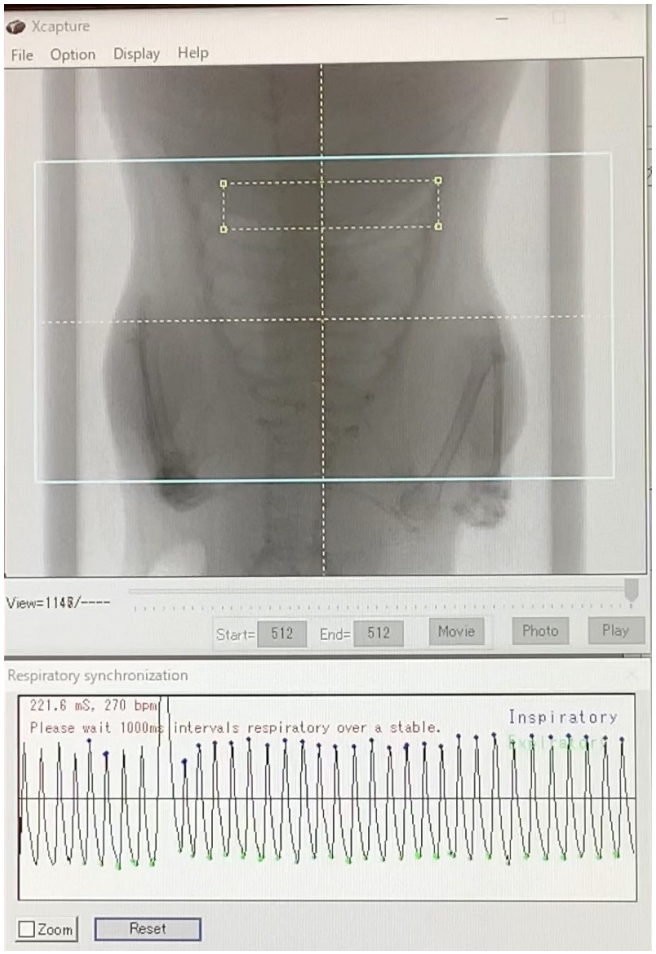
Code No.	Product name	Standard	Capacity	Desired purchase price (yen)
099-06571 095-06573	Isoflurane	for biochemistry	250ml 1L	17,000 57,000
193-17791	Sevoflurane	for biochemistry	250ml	14,500

Wako Reagents

塚本2

Effect of isoflurane inhalation anesthesia on respiratory function

1 %



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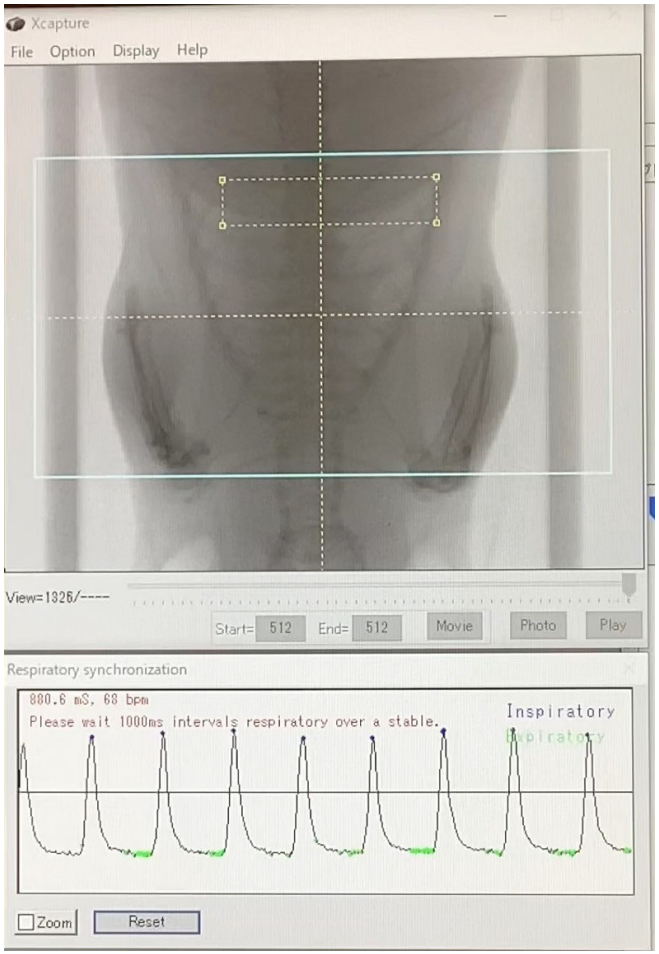

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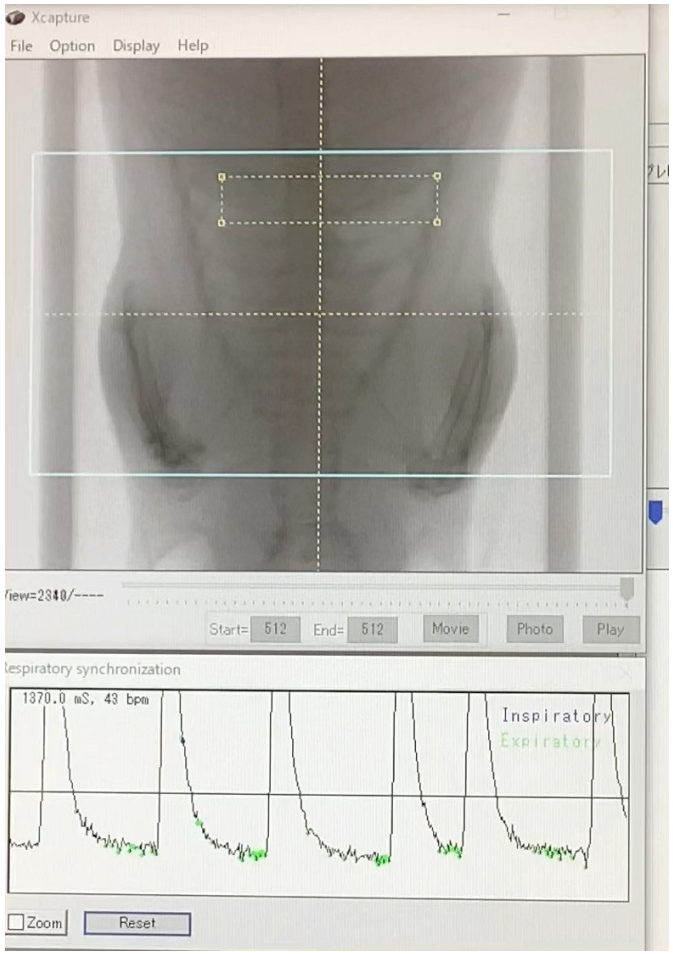

スライド 18

塚本2　「キャリアダッス」「ルームウエア」という単語

塚本 敦子, 2022/12/21

Simple anesthesia method for mice and rats

Absorbent cotton impregnated with a sufficient amount of anesthetic in a transparent anesthetic bottle(Kimwipe) and other absorbents are placed in advance, and after the anesthesia bottle is fully volatilized and the anesthesia bottle is filled, the mouse is placed in the anesthesia bottle and the lid is closed. At this time, isolate the mouse with a wire net so that the body wall of the mouse does not touch the absorbent. Since the concentration cannot be controlled, be careful of over-anesthesia.

In addition, since the concentration of volatile anesthetics increases as the temperature rises, it is necessary to pay attention to the temperature rise when using it continuously, and also consider the lack of oxygen in the bottle and the increase in carbon dioxide concentration.

The use of inhaled anesthetics during tail cuts, blood sampling, and euthanasia can reduce animal distress and

Experimenters can also experiment safely!

Recommended injection anesthetics for mice and rats (surgical anesthesia)

mouse

Drug name	dose	anesthesia time(minutes)	awakening time(minutes)
Medetomidine + midazolam + butorphanol*	0.3mg/kg + 4mg/kg + 5mg/kg ip	30	60
thiopental	30–40mg/kg iv	5～10	10～15
Propofol	26mg/kg iv	5～10	10～15
Ketamine + medetomidine	75mg/kg + 1mg/kg ip	20～30	60～120
ketamine + xylazine	80–100mg/kg + 10mg/kg ip	20～30	60～120

*: Immediate awakening with atipamezole 0.3 mg/kg ip additional injection.

Thiopental and propofol have weak analgesic effects.

rat

Drug name	dose	anesthesia time(minutes)	awakening time(minutes)
Medetomidine + midazolam + butorphanol*	**0.15mg/kg + 2mg/kg + 2.5mg/kg ip**	30	60
thiopental	30mg/kg iv	10	15
Propofol	10mg/kg iv	5	10
Ketamine + medetomidine	75mg/kg + 0.5mg/kg ip	20～30	120～240
ketamine + xylazine	75–100mg/kg + 10mg/kg ip	20～30	120～240

*: Immediate awakening with atipamezole 0.15 mg/kg ip additional injection

Kawai S. et al, *Exp. Anim.* 60(5), 481-487 (2011)
P. Flecknell Author, Supervised by Yuzuru Kurabayashi, Laboratory Animal Anesthesia, Gakusosha, Shigeru Hisawa, Edited by Laboratory Animal Science, Asakura Shoten

Precautions when anesthetizing rabbits

- Due to their high susceptibility to stress, care should be taken to minimize stress during treatment.

- Because animals rarely vomit or reflux gastric contents, fasting before surgery is not necessary.
- When anesthesia is performed using the open mask method, sedation or anesthesia should be induced with an injection in advance, as severe resistance or respiratory arrest may occur.

- Anticholinergics such as atropine are ineffective due to the presence of atropine esterase.

Rabbit Injectable Anesthetic Prescription Example

drugs	dose	usage	Anesthesia time (min)
Ketamine/Xylazine	35 mg/kg + 5mg/kg, im 10 mg/kg + 3 mg/kg, iv	surgical anesthesia	25–40 20–30
Ketamine/medetomidine	15 mg/kg + 0.25 mg/kg, im, sc	surgical anesthesia	20–30
Medetomidine/midazolam/butorphanol	0.5 mg/kg + 2 mg/kg + 0.5 mg/kg, ip	surgical anesthesia	60
Thiopental	30 mg/kg, iv	surgical anesthesia	5–10
Propofol	10 mg/kg, iv	light anesthesia	5–10

Precautions for anesthesia

・Use healthy SPF animals to prevent anesthesia accidents.

・After transportation, allow an acclimatization period of about 7 days.

・Mice and rats do not require preoperative fasting, as vomiting rarely occurs during the induction of anesthesia.Mice should be provided with drinking water prior to surgery.

・Guinea pigs should beShort-term fasting required.

・Genetically modified animals with unclear phenotypes should be given inhalational anesthesia, which provides greater control over the depth of anesthesia, than injection anesthesia.

・In mice, it is necessary to take measures to reduce body temperature during general anesthesia.

・For operations over 1 hour, inhalation anesthesia is performed by tracheal intubation.

・To avoid intraoperative hypoxia, it is desirable to use pure oxygen as the carrier gas and use a respirator.

There is no ideal anesthetic with no side effects, so it is important to consider the purpose of the experiment, the degree of invasiveness of the surgery, etc., and select an appropriate anesthetic! !