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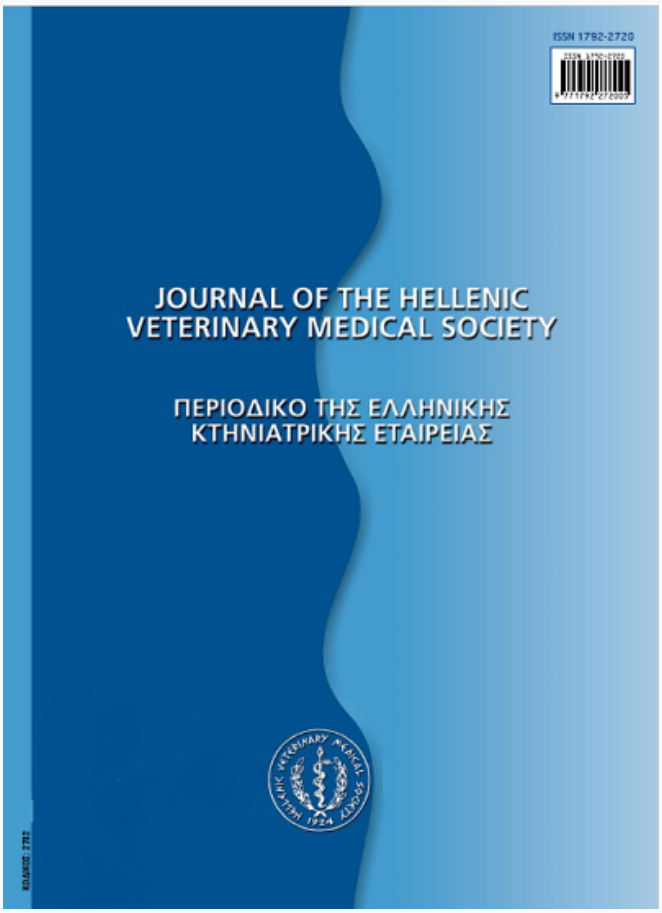

The mouse and the rat in surgical research. The anesthetic approach

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Review article
Ανασκόπηση

The mouse and the rat in surgical research. The anesthetic approach.

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Ο μυς και ο επιμύς στην πειραματική χειρουργική. Αναισθησιολογική προσέγγιση.

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ABSTRACT. The mouse and the rat are currently overwhelmingly preferred as laboratory animals. Surgical research on animals requires anesthesia and analgesia to obtain adequate immobility and to reduce stress and pain. Small rodent anesthesia is challenging for several reasons including the animals' size, metabolic rate, high risk of hypothermia and difficulty in monitoring. The purpose of this study is to create an overview of the information in the anesthetic practices for small rodents, in particular mice and rats.

Keywords: *analgesia; anesthesia; mouse; rat; surgery research; welfare*

ΠΕΡΙΛΗΨΗ. Ο μυς και ο επίμυς προτιμούνται κυρίως ως ζώα εργαστηρίου σε σχέση με τα άλλα ζωικά πρότυπα. Η πειραματική χειρουργική απαιτεί την αναισθησία και αναλγησία των ζώων, για την εξασφάλιση επαρκούς ακινητοποίησής τους, μείωσης του στρες και της αίσθησης του άλγους. Η αναισθησία στα μικρά τρωκτικά αποτελεί πρόκληση λόγω του μικρού μεγέθους τους, του υψηλού μεταβολικού ρυθμού τους, του κινδύνου υποθερμίας και της δυσκολίας στην παρακολούθηση των ζωτικών τους λειτουργιών. Ο σκοπός αυτής της μελέτης είναι η ανασκόπηση των αναισθητικών πρακτικών που εφαρμόζονται στα μικρά τρωκτικά και ιδιαίτερα στους μυς και επίμυς.

Λέξεις-κλειδιά: *αναισθησία, αναλγησία, επίμυς, ευζωία, μυς, πειραματική χειρουργική*

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INTRODUCTION

The mouse and the rat are the most preferred lab animals according to the seventh report of the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union (European Commission, 2013), representing 75% of the total number. This preference is due to their small size, and the fact that they are easy to house, maintain and adapt to new surroundings, while their genetic, biological, and behavioral characteristics closely resemble those of humans, replicating many symptoms of their conditions (Rosenthal and Brown, 2007). In addition, gene targeting technology, in order to generate a knockout/in rodent, can be easily applied to investigate the genetic basis of human physiology and disease (Scacheri et al., 2001).

The approach to anesthesia in surgical research has undergone significant changes during the last decade and its need and benefits have been clearly demonstrated (Kehlet and Dahl, 2003). For scientific quality, anesthetic techniques need to be reliable and safe, and the effects of the compounds used on the research animals must be well documented. Furthermore, in survival surgical studies, animals should recover quickly and should not to be allowed to suffer pain (Koch, 2006), which is highly significant for both the animal welfare and the quality of the study results. In addition, due to small body size, high body surface area/body weight ratio and high metabolic rate, their mechanisms of thermoregulation and the efficacy of injectable agents can be compromised (Tremoleda et al., 2012); therefore, high doses of these agents are required to induce unconsciousness, producing also detrimental effects on autonomic nervous system. Based on this knowledge, the veterinarian can promote humane animal-based research in the protocol review process, select the most appropriate anesthetic and analgesic agents, and provide assistance for troubleshooting problems (Robertson, 2001, Borchard et al., 1992).

Although the reporting of the administration of systemic analgesic drugs to laboratory rodents undergoing surgical procedures is increasing, the majority of papers that describe potentially painful procedures on laboratory rodents still do not report systemic analgesic administration. The absence of administration of analgesic agents to animals undergoing surgical procedures is against the refinement used to alleviate pain (Coulter C.A. et al., 2011, Stokes E.L. et al., 2009).

This article is a literature review of the anesthetic protocols in mice and rats during surgical procedures and it is divided in pre-operative, intra-operative and post-operative anaesthetic and analgesic management.

PRE-OPERATIVE MANAGEMENT

Pre-anesthetic Evaluation

Anesthesia of small rodents is particularly challenging mainly due to hypothermia, high metabolic rate and the lack of reliable clinical signs of respiratory and cardiovascular functions (Rembert et al., 2004), highlighting the importance of pre-anesthetic evaluation. This evaluation entails a thorough clinical assessment of the animal and precedes the delivery of anesthetic care for surgery. Although the physical status of the animals is documented during inspection, subsequent signs of actual disease are often discovered incidentally later, during the surgical experiment, anesthesia, or even after the recovery. For all of the above reasons, it is mandatory to ascertain, before the experimental study, the animals' behavioral patterns, body condition score, respiratory rate, food and water intake, as well as defecation, urination, absence of skin lesions, eyes and nose discharges, or perineal soiling (Cantwell, 2001). In addition, the physiological and behavioral response to stress affects a number of biological functions and systems. If stress is extreme or prolonged, substantial effort is required to maintain a state of equilibrium and the animal may, even develop major cardiovascular symptoms (Hildebrandt et al., 2008, Rottman et al., 2003).

Pre-operative fasting of animals is a general approach in surgical protocols, in order to prevent pulmonary aspiration of stomach contents during general

anesthesia. Several factors can predispose to aspiration of stomach contents including pregnancy, obesity, difficult airways management, full stomach and altered gastrointestinal mobility. In theory, increased fasting times could lead to decreased injury if aspiration occurs (Allman and Wilson, 2006); however, in rodents, this is generally deemed unnecessary because the emetic reflex is absent (Horn et al., 2012) and drinking water should be accessible until one hour prior to the induction of anesthesia (Luciano and Reale, 1992, Toth and Gardiner, 2000). Furthermore, in small animal practice nowadays there is a trend towards reducing the duration of preanesthetic fasting, because it does not guarantee an empty stomach and it lowers gastric content pH (Savvas et al., 2009).

Hypothermia is a major thermal disturbance; the relatively low body mass to high body surface ratio, combined with suppressed thermoregulatory mechanisms, allow the rapid escape of body heat. An increased risk of hypoglycemia, hydroelectrolytic and acid–base imbalances is also proposed, not only due to their size, but also to the immaturity of their thermoregulatory centers (Paddleford, 2000). Therefore, in order to prevent hypothermia, the animal should be laid on an insulated material, such as a clean surgical towel, along with circulating warm water or microwavable heating pad underneath to provide heat support; electric heating is avoided because of their irregular heating and potential thermal burns to the animals (Fueger et al., 2006). Also, it is essential to minimize soaking the body of the rodent during the scrubbing process and to avoid clipping excess hair (Bernal et al., 2009).

Accurate weighing of the animals ensures correct drug dosage calculations; obese animals present an altered biodistribution of lipophilic agents as well as a high incidence of hepatic dysfunctions and therefore are at high anesthetic risk due to hypoventilation and hypoxia. On the other hand, cachectic rodents present low plasma protein binding and might hide renal, hepatic, or cardiac deficiencies (Zuurbier et al., 2002). Additionally, age, sex, genetic and environmental factors, as well as inherent inter-individual variability contribute to anesthetic variability (Hildebrandt et al., 2008, Ciccone and Holdcroft, 1999). The small laboratory animals dehydrate much faster than other larger species; administration of 0.2 to 0.5 mL / 10 g body weight of isotonic fluids, such as 0.9% saline, warmed subcutaneously (Bazin et al., 2004) prior to anesthesia is recommended for survival procedures.

Pre-anesthetic Medication

The three components of anesthesia are analgesia (pain relief), amnesia (loss of memory) and immobilization. The advantages of preoperative sedation and analgesia include lowered patient and staff stress, ease of handling, and reduction of induction and inhalant anesthetic doses, most of which have dose-dependent adverse effects. The choice of pre-operative sedative and analgesic drugs depends, among others, on the animal's age, physical condition and the specific operative procedure (Flecknell, 1996). The categories of these pharmaceutical agents and their effects are shown below:

Anti-cholinergics (such as atropine sulfate, glycopyrrolate), inhibit parasympathetic nerve impulses by blocking the binding of the neurotransmitter acetylcholine to its receptor in nerve cells. Anticholinergics are divided into three categories in accordance with their specific targets in the central and/or peripheral nervous system: anti-muscarinic agents, ganglionic blockers, and neuromuscular blockers. Anti-cholinergics are administered selectively in combination with sedatives and analgesics, after the pre-anesthetic clinical examination of the animal, and according to the determined needs of the individual patient, the anticipated response to the anesthetic medication, and the tendency to develop bradycardia or excessive salivation (Zuurbier et al., 2002). The need for these actions is reduced or absent in rodents, eliminating the routine use of these drugs, while the rat rapidly metabolizes atropine due to a hepatic atropine esterase (Thurmon et al., 1996a).

Benzodiazepines (e.g., diazepam, midazolam or zolazepam) enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA A receptor, resulting in sedative, hypnotic

(sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant and muscle relaxant properties. They are CNS depressants, suppressing normal brain function without producing analgesia (Schaefer et al., 2005) with minimal respiratory and cardiac effects limiting the dosage by their ceiling effect (Flecknell, 1989). In general, benzodiazepines are safe and effective in the short-term, although in humans, cognitive impairments and paradoxical effects such as aggression or behavioral disinhibition occasionally occur (Broscheit and Kranke, 2008). In most applications, midazolam is preferred, as it can be injected intramuscularly and its action can be reversed by the GABA receptor antagonist Flumazenil.

Phenothiazines (e.g., promazine, acepromazine) are tranquilizers resulting in depressed alertness. Although the mechanism of action is still unknown, they have potent effects on the CNS and other organs, producing sedation, reducing the dose of drugs needed for general anesthesia, but also causing moderate hypotension and hypothermia (Tranquilli et al., 2007). Moreover, the incidence of ventricular arrhythmias and cardiac fibrillation is reduced, especially in those induced by epinephrine (Thurmon et al., 1996). Analgesia is not provided, but the animal's reaction to handling or pain via sedation and CNS depression is reduced (Thurmon et al., 1996); therefore, in painful procedures, they should not be used and analgesia is mandatory by other means. A sedative or tranquilizer must be administered when the animal is still in its cage and the drug must be allowed to take effect before move the animal to preparation area or operating room in order to reduce the animal's stress. In mice, acepromazine is used to potentiate and prolong ketamine and xylazine anesthesia (Arras et al., 2001), posing as the first choice among these agents, because of its long history of safe use in rodents (Messiha, 1991).

Alpha2-adrenergic agonists (e.g., xylazine, detomidine, medetomidine, dexmedetomidine) are a class of agents, which selectively stimulate alpha-2 adrenergic receptors, which produce analgesia and reduce hemodynamic stress through central and peripheral mechanisms (Buitrago et al., 2008) and with minimal irritation when injected intramuscularly or intraperitoneally. Disadvantages of α-2 agonists include cardiovascular depression (decreased heart rate, decreased cardiac output, and hypotension), which is controlled by the use of atropine or glycopyrrolate. Effects can be reversed using a specific antagonist, atipamezole, a highly selective and potent α2-antagonist that rapidly reverses sedation as well as other behavioral and physiologic effects (Vainio, 1997). Medetomidine is a newer compound alpha-2 receptor agonist with sedative and analgesic properties similar to xylazine with fewer undesirable side effects, providing excellent anesthesia in combination with ketamine. Dexmedetomidine is the newest of the alpha-2 agonist compounds that produces enhanced sedative and analgesic effects (Sinclair, 2003).

Pre-operative Analgesia

Administering a drug that blocks painful (nociceptive) input from entering the CNS before a surgical procedure attenuates the development of changes that manifest as increased pain at later time points (Kissin, 2005). Clinically, this strategy predicts not only less pain during the initial postoperative period, but also lowers the intensity of pain during the days after the procedure and is known as "preemptive" or "preventive" analgesia. The ideal goal is to prevent the initiation of stimulation so that central sensitization does not occur with prolonged effects (Schofield and Williams, 2002) (**Tables 1 & 2**).

The single most effective preemptive analgesic technique is local, regional or spinal infiltration and sensory blockade with **Local Anesthetics** (LA). The utility of local anesthetics without general anesthesia is not recommended due to humane concerns and chances of bite injury to humans (Fox et al., 2002). The two most useful injectable anesthetics for local or regional anesthesia are lidocaine and bupivacaine; the first has a proven fast onset of action with moderate duration of analgesia, is stable as a solution, and infiltrates through local tissues (Branson, 2001). A 0.5% lidocaine block of a surgical site will

Table 1: Drug Dosage –Analgesia in Mice

Drug(s)	Dose range	Route of administration	Frequency	Comments
NSAIDs*				
Flunixin meglumine	2.5 mg/kg	SC, IM	Every 12-24 hours	-
Carprofen	5 mg/kg	SC	Every 24 hours	-
Ibuprofen	40 mg/kg diluted in fresh water	PO	Daily in fresh water**	-
Ketoprofen	5 mg/kg	SC	Every 24 hours	-
Meloxicam	1-2 mg/kg	PO, SC	Every 12-24 hours	-
Opioids				
Buprenorphine	0.05-0.1 mg/kg	SC, IP	Every 6-12 hours	Or for major procedures, consider multi-modal analgesia with a NSAID (e.g. meloxicam)
Butorphanol	5 mg/kg	SC	Every 1-2 hours	If mild, pain of short duration is anticipated
Meperidine	10–20 mg/kg **or** 0.2 mg/ml of Demerol HCl syrup in water	SC, IM PO	Every 2-3 hours Daily in fresh water**	-
Morphine	10 mg/kg	SC	Every 2-3 hours	If severe, post-operative pain is anticipated
Pentazocine	10 mg/kg	SC	Every 2-4 hours	Mild to moderate pain; may develop analgesic tolerance with chronic administration
Tramadola	20-40 mg/kg **or** 1ml 5% solution in 150 mL of water	IP	Every 12-24 hours	Management of post-operative pain
Other				
Acetaminophen (Tylenol Pediatric Syrup) – analgesic / antipyretic[b]	1-2 mg/ml drinking water made fresh daily	PO	Daily in fresh water **	May be appropriate for procedures causing mild discomfort only; efficacy has been questioned in rodents

Sources: Flecknell, P.A., 2009; Schofield,C. J. & Williams, V., 2002.

[a] Smyj R. et al., 2013.

[b] Mickley, G.A. et al., 2006.

Notes: * Prolonged use may cause gastrointestinal, renal or other problems.

**Rodents may exhibit "neophobia" – always monitor for acceptance when adding medications to water or food.

Table 2: Drug Dosage –Analgesia in Rats

Drug(s)	Dose range	Route of administration	Frequency	Comments
NSAIDs *				
Flunixin meglumine	2.5 mg/kg	SC, IM	Every 12-24 hours	-
Carprofen	5 mg/kg	SC, PO	Every 24 hours	Oral doses may need to be increased
Ibuprofen	10-30 mg/kg	PO	Every 4 hours	-
Ketoprofen	5 mg/kg	IM, SC, PO	Every 24 hours	Oral doses may need to be increased
Meloxicam	1-2 mg/kg	SC, PO	Every 12-24 hours	-
Robenacoxib	0.25-4 mg/kg	SC	Every 24 hours	
Opioids				
Buprenorphine	0.05 mg/kg	SC	Every 6-8 hours	If mild to moderate, pain of increased duration is anticipated
Butorphanol	2 mg/kg	SC	Every 1-2 hours	If mild, pain of short duration is anticipated
Meperidine	10-20 mg/kg **or** 0.2 mg/ml of Demerol HCl syrup in water	IP, IM PO	Every 2-3 hours Daily in fresh water**	-
Morphine	10 mg/kg	SC	Every 2-3 hours	If severe, post-operative pain is anticipated
Pentazocine	10 mg/kg	SC	Every 2-4 hours	Mild to moderate pain of short duration; may develop analgesic tolerance with chronic administration
Tramadol a	5-10 mg/kg	IP	Every 12-24 hours	Management of post-operative pain
Other				
Acetaminophen (Tylenol Pediatric Syrup) – analgesic / antipyretic[b]	1-2 mg/ml drinking water made fresh daily	PO	Daily in fresh water**	May be appropriate for procedures causing mild discomfort only

Sources: Flecknell, P.A., 2009; Schofield,C. J. & Williams, V., 2002.
[a] Smyj R et al., 2013.
[b] Mickley, G.A. et al., 2006.
Notes: *NSAIDs may be used as the sole analgesic agent or they may be combined to provide multi-modal analgesia.
**Rodents may exhibit "neophobia" – always monitor for acceptance when adding medications to water or food.

et al., 2007) **(Table 3)**. Mainly lidocaine and to a lesser extent bupivacaine or counterclockwise stereoisomers ropivacaine and levobupivacaine, exert anti-inflammatory effects by changes in the normal immune response (Waite et al., 2010). Epidural and peripheral nerve blockade (Fairbanks, 2003) techniques have been widely used in experimental pain research utilizing mice and rats, and should be readily adaptable to clinical practice.

Effective systemic preemptive analgesic agents include **non-steroidal anti-inflammatory agents (NSAIDS)** and **opioids**, which can be used alone or in combination. There are several drugs in each category with different durations of action, but a careful choice should be made, taking into account the aim of the experiment (Ong et al., 2005). Ketoprofen, carprofen and meloxicam are the most widely used NSAIDS (Mathews, 2000) blocking peripherally the formation of inflammatory mediators associated with surgical injury and centrally secondary allodynia, when administered one hour or more before surgery (*Lee-Parritz, 2007;* Schafer, 1999). A neophobic response (the fear and rejection of new food) has been documented when adding drugs to water of rats, causing a temporary weight loss (Speth et al., 2001). The current recommended dose for ketoprofen and carprofen in rats is 5 mg/kg SQ. Dilution of ketoprofen for use in mice to provide accurate volumes of 0.05 ml or more is required; for meloxicam is 1 mg/kg PO or SC in rats and up to 10 mg/kg in mice. The dose interval for these agents has not been critically evaluated in small rodents, so it is implemented every 12-24 hours, as in other species. Ibuprofen has been recommended for use as a pain reliever with a wide ranging dose of 7.5 to 30 mg/kg (Jenkins, 1987) and, as a non-specific COX inhibitor resulting in decreased prostaglandin formation, it is well absorbed orally with the major proportion excreted in the urine and the minor through the stool within 24 hours of the last dose. Robenacoxib, is the first carboxyl, non-sulfur-containing COX-2-selective inhibitor to be developed for use in veterinary medicine. The data demonstrate that robenacoxib has potent analgesic, anti-inflammatory and antipyretic properties. Moreover, consistent with its weak activity as an inhibitor of COX-1, in the rat the gastric and intestinal tolerability of robenacoxib was significantly better than that of diclofenac, which non-selectively inhibits both COX-1 and COX-2 (King et al., 2009).

Opioids are classified as **alkaloids** such as morphine and codeine, **semisynthetic opioids**, such as hydromorphone, oxycodone and naloxone, **synthetic** such as fentanyl, buprenorphine and tramadol and finally as **peptide opioids** such as endorphine and enkephaline (Pasternak, 2014). Generally, the use of high-dose opioid anesthetic techniques produces the minimal depression of cardiac function and provides protection against cardiac arrhythmias (Schumann et al., 1994). Fentanyl, meperidine, and oxymorphone are added to anesthetic regimens, providing substantial analgesia and reducing the dose of the primary anesthetic (Thurmon et al., 1996a). Short-acting opioids enable rapid induction, optimal operative conditions, and quick recovery, with few side effects. Faster offset, easy titratability, and decreased accumulation are particularly use-

Table 3: Recommended Dosages of Local Anesthetics

Drug(s)	Mouse	Rat
Lidocaine	1-4 mg/kg or 0.4 mL/kg of a 1% solution	4 mg/kg (0.4 ml/kg of 1%, solution)
Bupivacaine	-	1–2 mg/kg (0.4–0.7 ml/kg of 0.25%, solution)

Sources: Flecknell, P.A., 2009; Heard, D.J., 2004.

ful for managing intraoperative responses during the maintenance of general anesthesia. Bradycardia, respiratory depression, excessive sedation, nausea, ileus and pica may be side effects (Cassidy et. al., 2010). Buprenorphine is commonly used in mice and rats offering significant advantages over other narcotics such as longer duration of action, safety and effectiveness for 6-12 hours (Gades et al., 2000). For procedures under anesthetics with poor analgesic properties, such as isoflurane, buprenorphine should be given at least one hour before incision (Heard, 2004). In rodents, oral administration of buprenorphine (0.5 mg/kg) in flavored-gelatin cubes is a commonly used analgesia method in laboratory animal medicine (Thompson et al., 2004). Tramadol is based on exerting influence on the μ-opioid receptor of the patient and also on increasing the activity of spinal descending inhibitory pathways (Ide et al., 2006 Leppert, 2009. Moreover, it provides postoperative pain relief comparable with that of pethidine and its analgesic efficacy can be further improved by combination with a non-opioid analgesic, without producing the constipation and dependence seen in equianalgesic doses of strong opioids (Grond & Sablotzki, 2004).

INTRA-OPERATIVE MANAGEMENT

General anesthesia is a state of unconsciousness achieved by injection or/and inhalation of substances that induce a reversible state of unconsciousness. For scientific quality, anesthetic techniques need to be reliable, safe, and the effects of the anesthetic and analgesic compounds must be well documented (Kohn et al., 1997).

Injectable Anesthesia

Injectable drugs can serve as the sole anesthetic agent, induce anesthesia before inhalation anesthesia, or be used as supplement in regional anesthesia. Although inhalation anesthetics are considered generally safer than injectable anesthetics, their use may be limited by a lack of equipment, facilities, or expertise of the anesthetists (Thurmon et al., 1996) (**Table 8**). The small diameter of the airways and the anatomy of the oropharynx prevent routine endotracheal intubation in mice and rats (Flecknell, 1996; Thurmon et al., 1996); therefore, injectable anesthetic agents tend to be the most preferred in the laboratory setting (Flecknell, 1996; Hedenqvist and Hellebrekers, 2003). To minimize the chance of drug overdose and to reduce drug-related tissue

Table 8. Advantages and Disadvantages of Inhalation Anesthesia

Advantages
• easy to administer
• accuracy over the depth of anesthesia
• provision of oxygen results in high oxygen concentration in the blood
Disadvantages
• specialized equipment is usually needed
• good ventilation and scavenging equipment required for the safety of personnel
• high cost of use

Source: Ludders J.W., 1999.

damage, anesthetic agents for smaller (<4 kg) laboratory animals may need to be diluted. Disadvantages of injectable anesthesia include the lack of precision in controlling anesthetic depth, prolonged recovery time, and physiologic changes such as hypotension, hypercapnia, and hypoxemia.

Intraperitoneal (IP) injection has been commonly used in laboratory mice and rats (**Table 4**) requiring minimal skill and distress (Flecknell, 1996), without causing lesions or sign of pain even when irritating drugs are administered (Morton et al., 2001). Intramuscular (IM) injection of irritant drugs (e.g. xylazine, ketamine) may cause swelling and lameness in the injected limb, resulting in self-mutilation (Smiler et al., 1990); therefore, it is avoided in small rodents. Intravenous (IV) injection into the tail vein is possible with the use of a restraining cage and if the personnel are well experienced.

The four critical factors to be considered when proposing injectable drug delivery to small laboratory rodents are: 1) the drug volume, 2) the site(s), 3) the irritant properties and 4) the method of administration. Parenteral anesthetics can be delivered by a single bolus injection, intermittent injection or continuous infusion (Waynforth, 1995). **Table 5** lists common injectable anesthetics, combination regimens and doses.

Dissociative anesthetics are (among others) ketamine and tiletamine. A combination of equal parts of tiletamine hydrochloride and zolazepam hydrochloride is evaluated as an injectable anesthetic for laboratory rodents. In low doses, ketamine provides chemical restraint with some analgesia, while in higher doses, it may provide short-term surgical anesthesia; in most instances, the dissociative anesthetics areused in combination with other injectable agents, such as xylazine, providing good immobilization with some degree of analgesia (Swalve, 2008). In combination with medetomidine, doses may need to be reduced for small rodents by a factor of 40-50% (Jang et al., 2009).

Other popular injectable anesthetic agents are **barbiturates** (like pentobarbital and thiopental). Although pentobarbital can be administered by the IM route, only IV or IP administration is recommended for thiopental because of its high histotoxicity (Zutphen et al., 1993). The analgesic effect of pentobarbital can be enhanced by balanced anesthesia with opioids, or ketamine **(table 5)**, but recovery is prolonged and hypothermia, respiratory depression, and hypotension are potential complications (Clemmesen and Hjalgrim-Jensen, 1980).

Tribromoethanol has been the standard anesthetic injectable agent in much mouse transgenic work, producing short-term (15-20 minutes) surgical anesthesia with good muscle relaxation and moderate respiratory depression (Zeller et al., 1998). Major drawbacks to this agent are the fact that it is cumbersome to prepare, the toxic by-products if not stored properly, the sensitization of some animals to subsequent exposure and idiosyncratic deaths in about 1% of naive mice, and the arguable inflammatory properties. (Zeller et al., 1998; Reid, 1999, Weiss & Zimmermann, 1999).

Table 4: Needle Sizes and Sites and Recommended Volumes for Injection

Species	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous
Mouse	Scruff, 2-3 ml, <20G	**Not recommended**. Can use quadriceps or caudal thigh, 0.05 ml, <23G	2-3 ml, <21G	Lateral tail vein, 0.2 ml, <25G
Rat	Scruff, back, 5-10 ml, <20G	**Not recommended**. Can use quadriceps or caudal thigh, 0.3 ml, <21G	5-10 ml, <21G	Lateral tail vein, 0.5 ml, <23G

Source: Hawk C.T. et al., 2005

Table 5: Common drugs and drug combinations used in Injectable Anesthesia for Mice and Rats

Drug(s)	Mouse	Rat
Pentobarbital (variable anesthetic depth)	(20-40 min. of anesthesia) 30-50 mg/kg, IP	40-50 mg/kg, IP (20-60 min. of anesthesia)
Pentobarbital + Ketamine	-	20 mg/kg, IP + 60 mg/kg, IP
Pentobarbital + Buprenorphine	-	36mg/kg, IP + 0.05 mg/kg, SC
Thiopental	30-40 mg/kg, IP	20-30 mg/kg, IP
Inactin (thiobutabarbital, EMTU)	-	80-100 mg/kg, IP (60-240 min. of anesthesia)
Tribromoethanol	240 mg/kg, IP (15-45 min. of anesthesia)	300 mg/kg, IP
Ketamine (only sedation)	80-120mg/kg, IP	80-120mg/kg, IP
Tiletamine + Zolazepam (only sedation)	40+80 mg/kg, IP	40+80 mg/kg, IP
Ketamine + Xylazine	80-120 mg/kg + 10-16 mg/kg, IP (20-40 min. of anesthesia)	80-100 mg/kg + 5-10mg/kg, IP (20-50 min. of anesthesia)
Ketamine + Medetomidine (The sex of mice and rats influences the pharmacokinetics and metabolism of ketamine)[a]	**female** 75+ 1mg/kg, IP **male** 50 + 1 mg/kg, IP	**female** 75+ 1mg/kg, IP **male** 50 + 1 mg/kg, IP
Ketamine + Dexmedetomidine	50-75+ 0.5-1mg/kg, IP	-
Atipamezole (for reversal of Xylazine and medetomidine)	1.0 mg/kg, IP	1.0 mg/kg, IP
Yohimbine (for reversal of Xylazine)	1.0 – 2.0 mg/kg, SC or IP	2.1 mg/kg IP, SC, or 1,05 mg/kg IP + 1,05 mg/kg SC
Ketamine + Xylazine + Acepromazine	100+10+ 3 mg/kg, IP	50 mg/kg + 5 mg/kg + 1 mg/kg, IP (30-45 minutes of anesthesia)
Ketamine + Acepromazine	100 + 5 mg/kg, IP (sedation)	75 +2.5 mg/kg, IP
Ketamine + Diazepam	100+5 mg/kg, IP	75-90+2.5-5 mg/kg, IP (in same syringe)
Ketamine + Midazolam	100+5 mg/kg, IP 100+3 mg/kg, IP 50+3 mg/kg, IP	75-90+4-5 mg/kg, IP (in same syringe)
Morphine	10 mg/kg, SC	2–10 mg/kg, SC
Meperidine	20 mg/kg, SC	-
Fentanyl	0.06 mg/kg, SC	0.3 mg/kg, IP
Fentanyl + medetomidine	Fatal	300μ g/kg + 300 μg/kg, IP
Buprenorphine	0.05-0.1 mg/kg, SC	0.01 - 0.05 mg/kg, SC or IP
Naloxone	0.01-0.05 mg/kg, IV, IM	0.01-0.1 mg/kg, IV, IM, or IP

Sources: Flecknell, P.A., 2009; Schofield,C. J. & Williams, V., 2002.; Hawk C.T. et al., 2005

[a] Hildebrandt et al., 2008.

Figure 1. Face mask (Low Cost Face mask, BRFAA), Balafas, E. et al., 2011.

Figure 2. Materials for endotracheal intubation in small rodents. Papastefanou, A.et al., 2014.

Inhalant Anesthesia

Inhalant anesthesia can be induced by an anesthetic chamber (NRC, 2011; Hau & Van Hoosier, 2003; Murray et al., 2000) or a face mask (Balafas et al., 2011), **(Figure 1)** and maintained using a face mask or an endotracheal tube (Kastl et al., 2004; Papastefanou et al., 2014) **(Figure 2)**. To prevent unwanted exposure of personnel to anesthetic vapors or waste gases, a method of 'scavenging' or removing the waste gases must be in place.

Inhalants are halogenated hydrocarbons (halothane), halogenated ethers (isoflurane, enflurane, desflurane, sevoflurane), or inorganic gases (N_2O). Inhaled anesthetics have a greater margin of safety, producing a more stable plane of surgical anesthesia, when used with a calibrated vaporizer, than injectable anesthetics. Since these anesthetics enter and leave the body through the respiratory system, the partial pressure of the anesthetic in the blood and brain can be changed immediately, thus easily adjusting the depth of anesthesia (Steffey, 1996; Brunson, 1997). Furthermore, animals undergoing inhalation anesthesia benefit from the fact that oxygen is most commonly used as the carrier gas, which improves tissue oxygenation. Most volatile agents are mainly eliminated by the lungs and undergo very little biotransformation, typically interfering only to a small extent with the liver function and metabolism of other drugs, which is especially important in pharmacology and toxicology research (Steffey, 1996). Minimum alveolar concentration (MAC) is a concept used to compare the efficiency or strength of anesthetic vapors and corresponds to the effective dose of an injectable anesthetic (**Table 6**) (Joo et al., 2001), depicting the concentration that produces immobility in 50% of subjects during noxious stimulation. MAC values are higher in neonates and decrease with age, but induction time may be prolonged (Hau et al., 2003).

All inhalant anesthetics depress cardiopulmonary function and renal blood flow in a dose-dependent manner (Steffey, 1996). The most popular inhalation anesthetics for laboratory animals include nitrous oxide and the halogenated compounds, halothane, isoflurane, and sevoflurane (**Table 7**). The utility and humane acceptability of CO_2 and O_2 combination as an anesthetic is an area of contention. Some studies have found that low concentrations (50% CO_2) lead to prolonged induction and severe and frequent adverse effects that include nasal bleeding, excessive salivation, seizures, and even death. This combination is used for extremely short-term procedures (Murray et al, 2000; Kohler I. et al., 1999). Much better anesthetic options are available for anesthesia of longer duration today.

Halothane is a potent volatile anesthetic agent, used frequently in the past. Although it produces good surgical anesthesia and muscle relaxation, it is a strong cardiovascular and respiratory depressant; moreover, it has been shown to be mutagenic and hepatotoxic in humans after repeated and prolonged exposure (Kohn et al., 1997).

Isoflurane is generally the inhalant anesthetic agent of choice in mice and rats, based on its rapid induction and recovery with high personnel safety profile, and low sensitization of the myocardium to catecholamines. Isoflurane is also used as a sole anesthetic agent, enabling animal manipulation and injection, blood collection and minor surgical procedures. Induction and emergence "delirium," respiratory depression, a dose-dependent hypotension, immune depression, delayed growth, and cleft palate in litters whose mothers have been exposed to this anesthetic are potential side effects (Kohn et al., 1997, Mazze et al., 1985).

Sevoflurane can provide faster induction and recovery compared to isoflurane, but also causes respiratory depression and hypotension in a dose-dependent manner (Kohn et al., 1997). It also appears to be better tolerated by mice and rats during face mask and chamber induction because of its low pungency and low airway irritability. Since it is less potent than isoflurane, the doses used are usually higher than those used for isoflurane and can be individualized based on the animal response (Mantziaras et al., 2004). However, recent reports are depicting that isoflurane and sevoflurane provide an equally reliable anesthesia in laboratory mice (Nicholls et al., 2010).

Desflurane physical properties are equal to those of sevoflurane, with the exception that the very low boiling point of desflurane makes the use of a heated

Table 6: % MAC Values in Laboratory Mouse and Rat

Species	Halothane	Isoflurane	Sevoflurane	N_2O	Desflurane[a]
Mouse	0.96–1.0	1.35–1.41	2.5	150–275	6.2- 9.12
Rat	0.81–1.23	1.17–1.52	2.4–2.5	155–235	5.7-7.1

Sources: Flecknell, P.A., 2009; Fish R.E. et al., 2011.
[a] Mantziaras, G.I. et al., 2004.

Table 7: Recommended Dosages of Inhalation Anesthesia in Mice and Rats

Drugs	Route	Oxygen (L/min)	Concentration for induction (%)	Concentration for maintenance (%)
Halothane	Mask, or chamber, or intubation	0.8-1	4-5	1-2
Isoflurane	Mask, or chamber, or intubation	0.8-1	4-5	1-3
Sevoflurane	Mask, or chamber, or intubation	0.8-1	6 or Individualized, based on the response	2.3-4.6 or Individualized, based on the response
Desflurane[a]	Mask, or chamber, or intubation	0.8-1	6 or Individualized, based on the response	2.3-4.6 or Individualized, based on the response

Sources: Flecknell, P.A., 2009; Fish R.E. et al., 2011.
[a]Graham, S.G., 1994.

vaporizer necessary for controlled delivery (Hau et al., 2003). The lower solubility contributes to the rapid recovery from anesthesia compared with other volatile agents (Gong et al, 1998). On a cellular level, synaptic activity recovers more quickly in the hippocampus of rats subjected to normal anesthetic doses of desflurane, improving both anesthetic and oxygen delivery to pulmonary compromised patients (Dimalculangan et al., 2006). Like sevoflurane, the required MAC of desflurane is inversely related to the age of the anesthetized small rodent, with neonates requiring a much higher MAC than adults (Fish et al., 2008).

Nitrous oxide is a gas with good analgesic action, and minimal depressant effects on the respiratory and cardiovascular system; it is useful in animals with a body weight over 2 kg, it is a weak anesthetic and thus it is not generally used at least on its own in small laboratory animals (NRC, 1984).

POST-OPERATIVE MANAGEMENT

Post-operative anesthetic care is a critical phase of anesthesia that includes a continuation of patient support and monitoring. After the procedure, the animal should be placed in a warm and quiet environment, where lighting should reflect the day/night light cycle that is appropriate for the species (NRC, 1996b). Hypothermia is probably the single most important cause of anesthetic mortality in mice (Flecknell, 1993), therefore thermal support is critical to the successful recovery, such as a warm cage with bed or pad surface and supplied with supplemental heat as required. However, all materials should be suitable to prevent wound contamination. The overriding principle that seems to work most efficiently is to prevent heat loss rather than to treat it once it occurs (Cantwell, 2001).

Animals should be minimally handled, placed alone in recovery caging to avoid fighting or cannibalism and also non-ambulatory rodents should be separated from ambulatory animals. Dehydration can be treated by the administration of appropriate fluid therapy, initially 1 to 2 ml of warm fluids (0.9% NaCl or equivalent) per 100 grams of body weight, by subcutaneous injection. If blood loss occurred during the surgical procedure, or if the animal is slow to recover from anesthesia, additional fluids should be provided. Mortality has been shown to decrease significantly in mice receiving 0.9% sodium chloride prior to recovery from anesthesia (Flecknell, 1993, Smith et al., 1999). Five to seven days after surgery, the general condition of the animal must be monitored**; during the post-operative period,** limited weight loss is observed, but with proper analgesia and the provision of food, body weight returns to normal levels (Hoff et al., 2006).

The post-operative analgesic management should minimize both the acute postoperative pain, and the rebound hyperalgesia, and have long-term effects. Post-operatively, mice and rats should be bright, alert, and active; interacting normally with cage mates, eating and drinking. Animals that are depressed, anorectic, or lethargic should be thoroughly examined (e.g. untreated pain or infection).

CONCLUSION

Anesthesia includes more than the selection of anesthetic drugs. A comprehensive individualized anesthetic plan will minimize peri-operative morbidity and optimize peri-operative conditions. Monitoring the ability to discern normal from abnormal and expedient intervention are critical to ensure that potentially reversible problems do not become irreversible. According to the aims of the surgical research, anesthetic protocol should be tailored by the use of tranquilizers, injectable/inhalation anesthetics and analgesics. Continued improvements in anesthetic regimens for laboratory mice and rats are an important implementation of Russell and Birch's "3 R's" (Russell, 1959), both for improvement of animal health and welfare and for the quality of experimental procedures results.

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