Dear Participant,

You are invited to take part in the practical part of the course laboratory animal science for researchers-Rodents

Date: XXX at 08.00 o’clock

Place: UCCB (search for UCCB on campus map http://www.umu.se/english/about-umu/campus-maps/campus-map-large?languageId=1)

Please notify us if you not will attend.

The practical part of the course laboratory animal science for researchers-Rodents at University of Umeå is divided into two sessions. The second session can earliest be entered one month after participation in the first part:

- The first part with focus on the training of basic techniques
  - Animal handling
  - Sample and injections techniques
  - Anesthesia for minor and surgical procedures
  - Euthanasia with acceptable methods

- The second part is a follow up with focus on anaesthesia and analgesia for minor and surgical procedures. At this occasion which is going to be held at specific course date (see UCCB home page) the participant should show the acquired skills with emphasis on how to anesthetize a rodent (mouse or rat) and be able to describe:
  - How to assess anaesthetic deep
  - How to monitor anaesthesia
  - Complication of anaesthesia how to minimize the risk of complications.
  - Discuss and describe the use of analgesia in rodents.

It is highly suggested that the participant read the provided/attached document “Mice Anesthesia, Analgesia, and Care, Part I” before entering the course, especially the second follow up part of the practical course.

To get a personal license for working with rodents both sessions have to be attended and approved.

It is the responsibility of the participant to enlist to the second session by sending an e-mail to the University veterinarian Anders Thornell (anders.thornell@umu.se) asking for course access at one of the UCCB course dates.
Acceptance criteria for the practical training in basic animal techniques in mice and rats at Umeå University

Background:
According to new regulations from the Agriculture Department, January 1, 2013, requires practical training and formal approval for all persons who work with laboratory animals.

Basic techniques
The basic techniques are:
• Handling of laboratory animals
• Subcutaneous, intraperitoneal, intravenous injections
• Blood sampling through the jaw and tail vein (mouse), and the mirror at the tail vein (rat)
• Anaesthesia and analgesia
• Euthanasia - dislocation of the cervical spine, or with the help of carbon dioxide / inhalation (Isoflurane), incl. ensuring death.

Handling /Management of animals
Assessment: To pass, the student should be in a calm and safe way to take the animal out of the cage and grab it at least 15 seconds in preparation for example, an IP injection.
Furthermore, the student is able to immobilize the animal in an acceptable way from an animal welfare point of view.

Subcutaneous injection
Examination: an approval is required that participants in a peaceful and safely administer 100 ul saline solution subcutaneously on any of the proposed sites of injection without leaking fluid through the entrance hole or sticking straight through the skin.

Intraperitoneal injection
Examination: an approval is required that participants in a peaceful and safely inject anaesthetic / injection in a fully awake animal.
Blood sampling via submandibular vein (mouse) or vena saphena (rat)

Examination: an approval is required for the student to be able to lose about 20-50 μL blood from submandibular vein from a mouse and from saphneous vein in rats.

Intravenous injection into the tail vein and blood sampling

Examination: an approval is required to attend the course branch on a quiet and safe manner, inject physiological sodium chloride (50 ul mouse and 100 ul rat) into the tail vein of an anesthetized animals. Most importantly, the course participant can show that it can apply the needle into the vein, this requirement shall be met in addition, the "displacement" at the injection and / or blood drawing from the cannula.

Killing by dislocation of the cervical spine, apply Mouse

Assessment: To pass the course the student perform the operation on a killed animal with successful results.

Euthanasia by carbon dioxide or overdose of the inhalation anaesthesia

Requirements: The participant must make an oral declaration for the how the carbon dioxide box for killing is to be used. The participant must have knowledge of how the gas is turned on and off and another possible.

The student should also know the signs that death has occurred, the change in colour of skin, absence of respiration and heartbeat (palpation).

Anaesthesia and analgesia

Requirements: The participant must be able to make an oral declaration for how to use inhalation anaesthesia (isoflurane) and injection anaesthesia (see above IP-injection). Further-on be able to briefly describe the tree different groups of analgesia and give examples.

Acceptance criteria for the second follow up session of basic animal techniques in mice and rats at Umeå University

Handling /Management of animals

To pass, the student should be in a calm and safe way to take the animal out of the cage and grab it at least 15 seconds in preparation for example, an IP injection.
Furthermore, the student should be able to immobilize the animal in an acceptable way from an animal welfare point of view.

**Anaesthesia and analgesia**

Requirements:

The participant must be able to show and practically demonstrate how to anaesthetise a mouse either using inhalation anaesthesia (isoflurane) or injection anaesthesia (see above IP-injection). Further-on be able to briefly describe the three different groups of analgesia and give examples.

The participant should be able to demonstrate knowledge about peri- and post-operative measurements to minimize risks for the animal in procedure.

The participant should be able to describe how to assess that an adequate anaesthetic deep is reached (loss of reflexes, clinical parameters).

The participant should be able to describe parameters for monitoring of anaesthesia.
Mice Anesthesia, Analgesia, and Care, Part I: Anesthetic Considerations in Preclinical Research

Sara Gargiulo, Adelaide Greco, Matteo Gramanzini, Silvia Esposito, Andrea Affuso, Arturo Brunetti, and Giancarlo Vesce

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Abstract

Animal experiments are necessary for a better understanding of diseases and for developing new therapeutic strategies. The mouse (Mus musculus) is currently the
most popular laboratory animal in biomedical research. Experimental procedures on animals often require anesthesia and/or analgesia to obtain adequate immobilization and to reduce stress or pain. Mice anesthesia is challenging for several reasons including the animals’ size, metabolic rate, and the high risk of hypothermia and hypoglycemia. Moreover, anesthetic agents influence physiological parameters, further interfering with experimental results. Small animal imaging procedures are increasingly used in biomedical research both because the animals allow in vivo monitoring and because they are readily available for longitudinal and noninvasive studies as well as investigations into the evolution of diseases and the effects of new therapies. Anesthesia must adapt to the imaging technique, the procedure length, and the aim of the study. The purpose of this article is to review the existing literature on anesthetic protocols adopted in mice for molecular imaging studies and to report our experience.

Key words: analgesia; anesthesia; chemical restraint; longitudinal studies; mice; preclinical research; small animal imaging

Introduction

Animal models are of paramount importance in biomedical research to improve in vivo understanding of the physiology of living organisms and the pathophysiology of most diseases and to provide fundamental information to address new treatment strategies. Among small laboratory animals, mice (Mus musculus) are by far the most popular animal model in biomedical research because of their biological similarity to man, reduced maintenance cost, easy handling, short reproduction rate, and, more recently, their outbreak in genetic manipulation studies. In fact, genetically engineered mice make up most of the current models of human diseases.

The use of mice in research has increased dramatically as a result of striking progress in the use of biomedical tools that have broadened medical knowledge and allowed new means of relatively noninvasive body exploration and treatment. Recently developed tools such as miniaturized imaging machinery as well as micro pumps, micro dialysis, and modern stereotaxic apparatuses have opened new mice research fields. Advances in anesthetic techniques have enabled investigators to overcome the small size of this species. Skilled techniques in anesthesia and analgesia have made it possible for scientists to avoid or at least greatly lessen the animals’ stress and suffering. Because animals do not voluntarily endure human manipulation, scientists are actively working to replace animals in medical research
with the growing number of alternative methods such as ex vivo and in vitro studies, computer simulations, and artificial models. Such methods are also aimed at reducing the number of animals used for a single experiment, restricting the authorization to operate on live animals, and refining the experiments through humane and gentle techniques. Nevertheless, in spite of these efforts, in vivo studies are still a necessity for understanding diseases and for developing new therapeutic strategies. Under these circumstances, it is of paramount importance to refine animal experiments and to use highly sophisticated anesthetic procedures that include systemic and local analgesia, sedation, chemical restraint, and both trained and dedicated personnel. Because experimental procedures are stressful and potentially painful, it is compulsory to use sedation, analgesia, and sometimes general anesthesia for most animal experiments.

The individual distress and suffering of animals must be minimized not only for ethical reasons but also because of their potentially adverse effects on experimental results. Therefore, safe and effective anesthetic and analgesic management is a crucial aspect of the refinement of experimental methods, taking into account the specific influence of the chosen agents on physiological variables that are relevant for the results of a study. For these reasons, the choice of a suitable anesthetic procedure is a key factor in preclinical studies, and should comply also with the type and length of the procedure and the aim of the study.

Laboratory mice exhibit specific anatomic and physiologic peculiarities that influence the effects of anesthetic drugs. Due to their small body size, drug metabolism and excretion are extremely fast, reducing the half-life of injectable drugs and rendering the duration of anesthesia a more critical factor compared with larger species. For instance, a 1-hour procedure in a mouse requires the same metabolic cost as a 6-hour procedure in a cat. Moreover, the elevated body surface area of mice promotes heat loss and hypothermia, while their reduced glycogen reserve predisposes them to hypoglycemia. In addition, their high oxygen consumption rate reduces the survival rate for hypoxemia. In fact, irreversible central nervous system (CNS) damage occurs only a few seconds after respiratory arrest in mice (Abou-Madi 2006).

Primary factors to take into account in choosing an anesthetic technique for mice are strain, age, weight, the disease model to be investigated, and the type of experimental procedure to be used. Small animal imaging procedures are being used increasingly in biomedical research because of their potential utility in localizing physical and biochemical phenomena, and for allowing investigators to monitor in
vivo, by serial and noninvasive means, the effects of pharmacological or gene therapies. Thus, imaging procedures pose their own anesthetic requirements based on the use of particular procedural techniques and on the duration of studies. In the text below, we review the existing literature on anesthetic protocols recommended for mice, and we report our own experience in longitudinal imaging studies.

**Mice Anesthesia**

**Preanesthetic Care and Clinical Examination**

Preanesthetic care reduces the incidence of complications that can occur in the course of anesthesia by ensuring the choice of the most suitable technique and regimen. As a rule, animals purchased from external sources should be singly housed for 1 or 2 weeks both to acclimate the animals and to allow time before an experiment for animal care personnel to observe and evaluate the health of the animals. Although the physical status of small rodents is documented when they are first inspected, subsequent signs of actual disease are often discovered incidentally later, during an experiment, during anesthesia, or even after anesthesia. For this reason, it is mandatory to ascertain before an experiment the animals' behavioral patterns, body condition score, respiratory rate, food and water intake, as well as defecation, urination, absence of skin lesions, eyes or nose discharges, or perineal soiling.

Personnel attending the mice must be trained to handle the animals gently but firmly because this handling has a strong influence on the animals' physiological functions. Physical restraint and manipulation can induce corticosteroid and epinephrine release, and can stimulate cardiovascular and respiratory functions that increase glucose levels, body temperature, and anesthetic induction dosages because they increase cardiac output (Hildebrandt et al. 2008). Stress can also negatively affect the quality of some molecular imaging procedures such as restraint distress during fluorodeoxyglucose ($^{18}$F-FGD) injection, which can cause an evident uptake of radiotracer in dorsal muscles, interfering with visualization of the heart, lungs, or metastatic chest lesions. Moreover, cardiovascular response to stress can upset the echocardiograph evaluation of left ventricle systolic function.

Preanesthetic fasting in mice is generally deemed unnecessary because the animals cannot vomit. Conversely, prolonged fasting can cause hypoglycemia due to their low hepatic glycogen reserve (Rao and Verkman 2000). Some concern about
anesthetizing mice that have a full stomach is linked to limited diaphragmatic excursion and to gastric blood pooling during digestion. Furthermore, in imaging studies a full stomach may interfere with adjacent structures. However, a fasting time of about 6 hours is suggested as an effective way to ensure uniformity in some studies such as positron emission tomography with $^{18}$F-FDG (Hildebrandt et al. 2008). In addition, when optical imaging is performed, it is necessary to consider dietary composition because food components such as chlorophyll can be a source of background autofluorescence (McNally et al. 2006). Mice should always have free access to water, even shortly before general anesthesia.

Preanesthetic Considerations

Anesthesia of small laboratory animals is particularly challenging due to the following problems that relate to their small body size: hypothermia, high metabolic rate, and lack of reliable clinical signs of respiratory and cardiovascular functions. Because hypothermia is potentially lethal, preservation of body heat is an integral part of anesthetic management. Core body temperature decreases suddenly after induction and continues to diminish during the course of prolonged general anesthesia, especially in small rodents. Therefore hypothermia must be prevented by providing heat through warming pads and infrared lamps. Hypothermia can also negatively affect the quality of some molecular imaging procedures such as positron emission tomography with $^{18}$F-FDG, increasing $^{18}$F-FDG uptake by interscapular brown fat and meddling the visualization of neighbouring structures (Fueger et al. 2006).

In mice, strain, body weight, age, and sex add to the well-known anesthetic variability that exists among individuals of other species. As an example, mice less than 8 weeks of age metabolize anesthetics less efficiently than adults due to their immature liver enzymatic system and reduced homeostatic response. They also pose an increased risk of hypoglycemia, hydroelectrolytic and acid–base imbalances, and hypothermia due not only to their smaller size but also to the immaturity of their thermoregulatory centers (Paddleford 2000). Similar problems ensue in mice more than 18 months old, due to subclinical pathologies related to senescence such as hepatic, renal, or cardiac impairment.

The sex of mice influences the pharmacokinetics and metabolism of anesthetics probably due to differences in plasma corticosteroids, sexual hormones, or hepatic enzymes (Hildebrandt et al. 2008). As an example, a higher dose of ketamine is recommended for female mice compared with that for males. Obese mice present an
altered biodistribution of lipophylic agents and a high incidence of hepatic dysfunctions and are therefore at high anesthetic risk because of hypoventilation and hypoxia. However, cachectic mice present low plasma protein binding and might hide renal, hepatic, or cardiac deficiencies. Several murine models that mimic human diseases such as obesity, diabetes mellitus, myocardial ischemia, and neoplasia require specific anesthetic protocols and pose increasing difficulties in serial studies. Furthermore, genome alterations of transgenic mice can influence the effects of anesthetic agents either accidentally (Quinlan et al. 1998) or deliberately, as reported by Xie and colleagues (2000), eliminating the anesthetic potential of tribromoethanol in VPSXR mice.

Knockout mice can withstand both obesity and altered sensitivity to anesthetics, as do knockout mice for the brain neuropeptide Y1 subtype receptor that is involved in learning and memory. Such mice display both obesity and reduced sensitivity to pentobarbital and to avertin (Kushi et al. 1998; Naveilhan et al. 2001), whereas deletion of the Y2 receptor results in increased body weight and increased sensitivity to pentobarbital (Naveilhan et al. 2001). Due to the lack of an easy vascular access, we recommend giving a 1-mL subcutaneous (SC) or intraperitoneal (IP) fluid bolus to mice before anesthesia to allow for fluid loss and to expand the circulating volume.

Premedication

Preanesthetic medication by tranquillizers and analgesics is generally administered to reduce apprehension, favor stress-free induction and recovery, reduce doses and side effects of other anesthetic agents, and achieve pre-emptive analgesia. However, in mice a “single shot” anesthetic protocol is advisable to minimize the stress caused by multiple injections. Atropine (0.04 mg/kg SC, IP, or intramuscular [IM]) has been recommended before induction of anesthesia to reduce bronchial and salivary secretions and to protect the heart from vagal inhibition (Flecknell 1989). Atropine can be mixed with other hydrosoluble agents or administered 10 minutes before induction by the IM route. Nevertheless, Zuurbier and colleagues (2002) reported that the addition of atropine to ketamine/medetomidine anesthesia does not prevent alpha-2-generated bradycardia.

Anesthetic Regimen

Anesthesia in laboratory animals is a state of unconsciousness, analgesia, muscle relaxation, and a-reflexia (Kohn et al. 1997). Anesthetic regimens can be of two
kinds: injectable or inhaled, according to the nature of the administered drugs. A main indication for general anesthesia in imaging procedures is the need for a constant immobility, avoiding movement artifacts. Anesthetic depth in mice can be clinically monitored by observing the loss of the righting and palpebral reflexes, and by assessing muscular tone, response to painful stimulation, and rate and depth of respiration. An ideal anesthetic agent for mice should be easy to administer, produce a fast and adequate immobilization, have limited side effects, and be reversible and safe for animals and operators. Unfortunately such an anesthetic is not available, and the best drug selection is highly variable according to different experimental circumstances.

Induction of general anesthesia in mice can be achieved by a variety of drugs and techniques (Flecknell 1989). The most commonly used anesthetics in mice include the injectable agents avertin, pentobarbital, and ketamine, which are often combined with other agents such as acepromazine, xylazine, diazepam, several narcotic analgesics, and the inhalation agents halothane, isoflurane, and sevoflurane. Compared with injectable techniques, inhalation anesthesia provides greater safety, particularly for prolonged procedures, due to a lesser cardiovascular depression, a reduced impact on liver and kidney functions, and because it promotes rapid recovery and allows quick adjustments and easy maintenance of a steady anesthetic depth. However, inhalant agents foster respiratory depression (particularly in the presence of respiratory diseases), myocardial depression, vasodilation, and hypotension (Paddleford 2000), exhibiting weak analgesic effects. Compared with injectable agents, the modern inhalation anesthetics require complex and expensive equipment such as precision vaporizers and flowmeters, specific breathing systems, and efficient scavenging systems to prevent pollution.

In spite of some obvious advantages, injectable anesthetics also present disadvantages such as difficulty in choosing an initial dose, impossibility of removal from the patient once injected, and no chance of accurately modulating the depth of prolonged anesthesia. Furthermore, they can cause prolonged recovery, pronounced alterations of heart and respiratory rate, and risks in combination with misplaced injection as for abdominal organs by the IP route. On these grounds, reversible agents with high therapeutic index are preferable. Moreover, in line with “balanced anesthesia” principles, a combination of injectable agents should be chosen to minimize the adverse effects of each one.

Injectable Anesthesia
In mice, injectable anesthetics can best be administered via IP, IM, and IV routes. The SC route is unpredictable for anesthetic induction because of its variable and slow absorption rate. The injection volume should be carefully considered according to the available route: adequate volumes by the IP route range from 0.1 to 1mL, by the IV route from 0.05 to 0.2 mL, and by the IM route not to exceed 0.05 mL in adult mice (Flecknell 1989). One advantage of some injectable anesthetic agents is their reversibility by specific antagonists such as yohimbine and atipamezole for alpha-2 agonists, flumazenil for benzodiazepines, and naloxone for opioids.

**Barbiturates**

Barbiturates are γ-aminobutyric acid (GABA\(^\gamma\))-mimetic drugs that inhibit the release of norepinephrine and glutamate. The most popular two barbiturates are pentobarbital, a short-acting oxybarbiturate (sleep time of 60-120 minutes), and thiopental, an ultrashort-acting thiobarbiturate (sleep time of 10-20 minutes) (Table 1). Although pentobarbital can be administered by the IM route, only IV or IP administration is recommended for thiopental because of its high histotoxicity, which causes significant tissue damage (van Zutphen et al. 1993).

<table>
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<tr>
<th>Drugs</th>
<th>Dosage</th>
<th>Comments</th>
<th>References</th>
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<tbody>
<tr>
<td>Pentobarbital</td>
<td>50-90 mg/kg IP</td>
<td>Cardiorespiratory depression, hypotension; long duration</td>
<td>Flecknell (1989), Kohn et al. (1997), Hildebrand et al. (2008)</td>
</tr>
<tr>
<td>Thiopental</td>
<td>30-40 mg/kg IP</td>
<td>Cardiorespiratory depression, hypotension; short duration</td>
<td>Flecknell (1989), Hildebrand et al. (2008)</td>
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</table>
Significant differences in sleep time and loss of righting reflex between the long-sleep (LS) and short-sleep (SS) selected lines of mice have been well-established after pentobarbital administration (Bennett 2000; Christensen et al. 1996). In studies of the effects of 60 mg/kg of pentobarbital in 23 inbred strains, investigators reported the following: DBA mice showed the longest sleeping time, and NZW the shortest; C57Bl/6 mice slept significantly longer than BALB/c; and ob/ob mice showed shorter pentobarbital sleeping times than +/ob ones (Kohn et al 1997). Due to the restricted therapeutic index of barbiturates, their use is often confined to nonsurvival procedures or to record some neurophysiologic signals such as visual or auditory evoked responses (University of California San Francisco, Institutional Animal Care and Use Committee). Their main adverse effects are respiratory and cardiovascular depression. In some European countries, barbiturates are controlled substances, therefore their detention and use are strictly regulated.

**Tribromoethanol (TBE)**

Commercially named Avertin, TBE is a popular injectable anesthetic for mice that offers the advantage of not being a controlled substance. It should be administered at a dosage of 240 mg/kg IP to provide good muscular relaxation and short-lasting anesthesia (15-30 minutes). TBE causes moderate cardiopulmonary depression and hyperglycemia. The effects of TBE are somewhat unpredictable in mice less than 16 days old, in obesity or diabetes mouse models (db/db o ob/ob), and in strains with genetic predisposition to hyperglycemia such as C57Bl/6J. Its effects are also variable among individuals, different strains, and stock of solution used (Zeller et al. 1997).

Since 1980, several studies have reported that TBE can cause necrosis of abdominal skeletal muscular fibers, and at high dosages and after reiterated injections, it can induce peritonitis, abdominal adhesions, and intestinal ileus (Zeller et al. 1997). TBE decays in the presence of heat or light, producing toxic by-products that are known to be both nephrotoxic and hepatotoxic. In fact, administration of degraded TBE solutions has been associated with delayed death up to 24 hours after surgery (Table 2). On these grounds, Swiss and Dutch animal ethics committees strongly disapprove of the use of TBE, while in the United States the use of TBE is limited to terminal procedures and is banned for embryo-transfer procedures or serial studies (Arras et al. 2001; Meyer and Fish 2005).
Table 2: Recommended dosages for tribromoethanol

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<th>Drugs</th>
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<th>Comments</th>
<th>References</th>
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<tr>
<td>Tribromoethanol</td>
<td>240 mg/kg IP</td>
<td>Peritonitis, intestinal ileus, peritoneal inflammation, adhesions, mortality after the second administration</td>
<td>Zeller et al. (1997)</td>
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<td>Flecknell (1989)</td>
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<td>Arras et al. (2001)</td>
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<td>Meyer and Fish (2005)</td>
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**Dissociative Anesthetics**

Dissociative anesthetics produce catalepsy, analgesia, amnesia, and immobilization characterized by CNS activation and sympathetic stimulation (Paddleford 2000). Dissociative anesthetics include ketamine and tiletamine, both of which have a wide margin of safety and a pronounced analgesic effect that prevents spinal sensitization (wind-up) by inhibiting N-methyl-D-aspartate receptors (Table 3). They can induce light respiratory depression while preserving cardiovascular function. Their peculiar anesthetic effects reflected in the term *dissociative* are consistent with increased muscular tone and rigidity, persistence of some cranial nerve reflexes, and electroencephalogram activation patterns. Dissociative anesthesia produces a sympathetic stimulation that increases the plasmatic levels of norepinephrine and could interfere with studies involving the sympathetic nervous system. They can increase intracranial blood flow and pressure although in mice, unlike other species, they show no proconvulsant activity. The use of the S (+) isomer of ketamine has been reported to produce a shorter sleeping time and fewer side effects (ataxia, tail flicking, hyperactivity) compared with the commonly used racemic mixture R (-) ketamine. Ketamine is often combined with other anesthetic agents such as phenothiazines, α2 agonists, and benzodiazepines to improve the quality of
anesthesia while reducing its side effects (Kiliç and Henke 2004). Inasmuch as dissociative anesthetics allow open eyes, personnel should protect the animals’ corneas with an eye ointment to prevent corneal drying and damage.

Ketamine is a controlled substance in some European countries, therefore its use is limited by the same strict rules enforced for opioids, barbiturates, and other drugs of abuse. Tiletamine is a parent compound that is about 10 times more potent than ketamine, and it is commercially available in combination with the benzodiazepine zolazepam in products called Zoletil® or Telazol®. The combination tiletamine-zolazepam by itself provides profound dissociative anesthesia without muscle rigidity and proconvulsant effects. In combination with xylazine, its anesthetic power is enhanced, fitting the needs of surgical anesthesia. Unfortunately Zoletil® is not a suitable anesthetic agent for mice due to its long-lasting effects, delayed recovery, and severe cardiorespiratory depression.

Table 3: Recommended dosages for dissociative anesthetics

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<th>Drugs</th>
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<tbody>
<tr>
<td>Ketamine</td>
<td>80-100mg/kg IP</td>
<td>Only sedation</td>
<td>Xu et al. (2007)</td>
</tr>
<tr>
<td>Tiletamine + zolazepam</td>
<td>40/80 mg/kg IP</td>
<td>Only sedation</td>
<td>Flecknell (1989)</td>
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</table>

**Alpha-2 Adrenergic Agonists**

The alpha-2 adrenergic agonists are a class of tranquilizers that include molecules with different selectivity for alpha adrenergic receptors. These drugs produce species-dependent dose-related CNS depression, and are powerful sedatives, hypnotics, and analgesics. Among the advantages offered by these molecules is the availability of receptor-specific antagonists such as yoimbine or atipamezole. Peculiar side effects in combination with the administration of alpha-2 agonists include hypertension/hypotension, increased peripheral vascular resistance, decreased cardiac output, increased central venous pressure, respiratory depression, hyperglycemia, glycosuria and enhanced diuresis. Reflex bradicardya and second
degree atrioventricular heart block may occur as a consequence of hypertension and cannot be prevented by administering anticholinergics. The alpha-2 agonists used in mice include xylazine, medetomidine and dexmedetomidine.

The combination of ketamine and xylazine is still the most widely used ketamine combination in mice, providing good immobilization with some degree of analgesia. Several different dosage combinations of the ketamine/xylazine mixture have been reported for mice in the medical literature (Table 4), varying from 65/4 to 100/10 mg/kg. The large variability of the recommended dosages depends on differences related to strain, sex, age, and type of experimental procedure. As an example, echocardiography can be performed with lower doses compared with invasive and painful procedures, which require higher dosages. Medetomidine is a newer compound that is about 10 times more powerful than xylazine and that exhibits a high selectivity for alpha-2 receptors. It provides the specific antagonist “Atipamezole.” In combination with ketamine, medetomidine prolongs mice anesthesia time (about 50 minutes), and recovery time is proportional to sleeping time. Moreover, the compound produces greater bradicardia and bradypnea compared with equipotent doses of xylazine (personal observation). Recently, Wells and colleagues (2009) reported that in male mice, anesthesia following 0.5 mg/kg of medetomidine and 50 mg/kg of ketamine created a potential risk of “obstructive uropathy” due to the formation of a seminal coagulum, whereas such a complication was not observed with a dose of 100 mg/kg of ketamine and 10 mg/kg of xylazine. The complication has been observed in our laboratory as well. The ketamine-medetomidine mixture is less commonly used than the ketamine-xylazine mixture, which is reserved for painful or short-term procedures that require good immobilization (e.g., dual energy x-ray absorptiometry [DEXA] or computed tomography [CT]). Dosages reported in the literature vary between 40 and 80 mg/kg for ketamine and 0.25 to 1 mg/kg for medetomidine.

Dexmedetomidine is the newest of the alpha-2 agonist compounds that produces enhanced sedative and analgesic effects. It is the pharmacologically active dextro-isomer of medetomidine, which possesses a higher affinity for α2 receptors. Dexmedetomidine also is reversed by Atipamezole. Although not supported by some reports in the literature, empirical doses of dexmedetomidine of 0.5 to 1 mg/kg have been reported for mice anesthesia in combination with 50 to 75 mg/kg IP of ketamine (www.utsouthwestern.edu/).
<table>
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<th>Drugs</th>
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<th>References</th>
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<tbody>
<tr>
<td>Ketamine + xylazine</td>
<td>100/10 mg/kg IP</td>
<td>α2 agonist reversible with atipamezole 1mg/kg IP; to prolong anesthesia can be administered ¼ – ½ of the dosage used of ketamine</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td></td>
<td>65/4 mg/kg IP</td>
<td></td>
<td>Buitrago et al. (2008)</td>
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<tr>
<td></td>
<td>100/5 mg/kg IP</td>
<td></td>
<td>Chari et al. (2001)</td>
</tr>
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<td></td>
<td>100/1.25 mg/kg IP</td>
<td></td>
<td>Roth et al. (2001)</td>
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<td>Janseen et al. (2004)</td>
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<td>Schaefer et al. (2005)</td>
</tr>
<tr>
<td>Ketamine + medetomidine</td>
<td>75/1 mg/kg IP</td>
<td>α2 agonist reversible with atipamezole 1mg/kg IP; moderate respiratory depression</td>
<td>Voipio et al. (1988)</td>
</tr>
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<td></td>
<td>female 75/1 mg/kg IP</td>
<td></td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td></td>
<td>male 50/1 mg/kg IP</td>
<td></td>
<td>Taylor et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cruz et al. (1998)</td>
</tr>
<tr>
<td>S-Ketamine +</td>
<td>75 mg/kg +</td>
<td>α2 agonist reversible with atipamezole 1mg/kg IP; action more short and awakening more fast than</td>
<td>Kilic and Henke</td>
</tr>
</tbody>
</table>
medetomidine | 0.25 mg/kg | racemic ketamine | (2004)
---|---|---|---
racemic ketamine + medetomidine | 50 mg/kg + 0.25 mg/kg |
Ketamine + dexmedetomidine | 50-75/0.5-1 mg/kg IP | α2 agonist reversible with atipamezole 1mg/kg IP | moderate respiratory depression | UT Southwestern

**Phenothiazine Tranquillizers**

Several molecules of the phenothiazine class of tranquilizers are used for mice restraint and anesthesia. Acepromazine, which is among the most powerful and widely used compounds of this class of drugs in animals, produces minimal depression on the respiratory system and antiemetic, antihistaminic, and antiarrhythmogenic effects. Related compounds are promazine, chlorpromazine, and propionylpromazine.

The phenothiazine tranquilizers produce no analgesic effects, therefore they should not be used for painful procedures unless analgesia is provided by other means. Their main undesirable effects are cardiovascular depression and hypotension due to their alpha adrenergic blocking action, which limits their use in pediatric or geriatric mice. Furthermore, because they decrease seizure threshold, they are not recommended in subjects with CNS lesions. In mice, acepromazine is used to potentiate and prolong ketamine and xylazine anesthesia (Arras et al. 2001) (Table 5).

| Table 5: Recommended dosages for combinations with ketamine and phenothiazine |
|---|---|---|---|
| Drugs | Dosage | Comments | References |
|ketamine + acepromazine | 100/5 mg/kg IP | Sedation | Flecknell (1989) |
|ketamine + xylazine + acepromazine | 100/10/3 mg/kg IP | Surgical anesthesia | Arras et al. (2001) |
Benzodiazepines are graded as hypnotics, central muscle relaxants, and “minor tranquilizers” because of their limited sedative action. They include the lipo-soluble main compound diazepam, the hydro-soluble midazolam, zolazepam, and flunitrazepam, and many other molecules that produce mild CNS depression through their agonist activity at GABA-a-type receptors. Benzodiazepines can be reversed by the GABA receptor antagonist Flumazenil. They exert minor cardiovascular and respiratory depressant effects, and their dosage is limited by their ceiling effect. Among laboratory animals, benzodiazepines display significant species variations in that they cause minimal sedation in some species but marked sedation in rabbits and rodents (Flecknell 1989).

Midazolam has shorter onset and duration compared with diazepam. Because they both cause minimal cardiopulmonary depression, they are recommended for old animals and diseased patients including cardiovascular and metabolically compromised patients. They are very popular and are compatible with the majority of anesthetic agents (Table 6).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine + diazepam</td>
<td>100/5 mg/kg IP</td>
<td>Immobilization</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td>Ketamine + midazolam</td>
<td>100/5 mg/kg IP</td>
<td>Immobilization</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td></td>
<td>100/3 mg/kg IP</td>
<td></td>
<td>Schaefer et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>50/3 mg/kg IP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Zolazepam is commercially available only in combination with the dissociative agent Tiletamine (see above) as an anesthetic compound called Zoletil® or Telazol®. This compound is recommended mainly for anesthetizing rats and guinea pigs, and its use in mice can produce severe cardiorespiratory depression (Flecknell 1989; Gardner et al. 1995).

### Opioids

A wide range of opioid analgesics is available for use in animals with different analgesic potency, duration, and effects on body systems (Table 7). Opioids are usually classified as agonists, partial agonists, mixed agonist-antagonists, and antagonists. Agonists such as morphine, meperidine, and fentanyl as well as partial agonists such as pentazocine and buprenorphine are excellent analgesics with different power and duration. Pure agonists can cause some undesirable side effects (e.g., respiratory depression, sedation, bradycardia, and peripheral vasodilation induced by histamine release, especially at higher doses) such as those that might be administered when using opioids as a part of a balanced anesthetic regimen. Opioid effects can be antagonized by naloxone (Buitrago et al. 2008; Flecknell 1989).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>10 mg/kg SC</td>
<td>2-4 hourly</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Flecknell (1998)</td>
</tr>
<tr>
<td>Meperidine</td>
<td>20 mg/kg SC</td>
<td>2-3 hourly</td>
<td>Flecknell (1989)</td>
</tr>
</tbody>
</table>
Fentanyl 0.06 mg/kg SC Short-acting synthetic opiate (30 minutes); widely used during surgical procedures. Flecknell (1989)

Buprenorphine 2 mg/kg SC 12 hourly Flecknell (1989)

Naloxone 0.01-0.05 mg/kg IV, IM Flecknell (1989)

Neuroleptoanalgesia

Neuroleptoanalgesia is carried out by combining a narcotic analgesic with a tranquilizer to produce a state of deep sedation with profound analgesia sufficient to perform short painful procedures. According to De Castro and Mundeleer (1959), some neuroleptoanalgesic compounds are marketed as premixed combinations. For example, Leptofen®, Innovar®, and Innovar Vet® combine at different concentrations but with a fixed ratio of 50:1. The butyrophenone Droperidol combines with the opioid Fentanyl, or the compound Hypnorm®, with the same ratio of 10 mg/mL of Fluanison with 0.2 mg/mL of Fentanyl. Currently the term neuroleptoanalgesia is used for many different combinations of any opioid with several kinds of tranquilizers (Table 8). The use of Innovar in mice is limited because it can produce tissue necrosis at the injection site (Flecknell 1989). Fentanyl (0.05 mg/kg) can also be combined with midazolam (5 mg/kg) and with medetomidine (0.5 mg/kg) to perform surgery in mice, the advantage of this combination being that at any time each or all of the agents can be reversed by naloxone, flumazenil, and atipamezole, respectively (Thal and Plesnila 2007). The main side effects related to the use of opioids are respiratory depression and bradycardia.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl</td>
<td>0.06 mg/kg SC</td>
<td>Short-acting synthetic opiate (30 minutes); widely used during surgical procedures.</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>2 mg/kg SC</td>
<td>12 hourly</td>
<td>Flecknell (1989)</td>
</tr>
<tr>
<td>Naloxone</td>
<td>0.01-0.05 mg/kg IV, IM</td>
<td></td>
<td>Flecknell (1989)</td>
</tr>
</tbody>
</table>

Table 8: Recommended dosages for neuroleptoanalgesics
Muscle Relaxants

Neuromuscular blocking drugs produce paralysis of skeletal muscles and are used to aid stable mechanical ventilation by blocking respiratory movements, or to provide more suitable conditions for surgery. In rats, the use of pancuronium (2 mg/kg), a blocking agent that acts by competing with acetylcholine for receptor sites at the neuromuscular junction, has been reported. Its action can be reversed by neostigmine, which blocks the activity of enzymes that break down acetylcholine (Flecknell 1989). Administration of pancuronium (1 mg/kg) is also reported in mice (Walker et al. 1999).

Inhalation Anesthesia

Inhalation anesthetics offers a wide margin of safety and allows the maintenance of a constant plane of anesthesia compared with injectable ones. Absorption and elimination of inhalation anesthetics occur through the lungs and allow rapid induction and recovery.

Inhalation anesthetics are administered by anesthesia machines and delivered via a breathing system such as a simple Ayre’s T piece up to a complex automatic ventilator. The basic anesthesia machine consists of a source of oxygen, a flowmeter, a precision vaporizer, a breathing circuit, and a scavenging system. In small animals, inhalation anesthesia can be easily induced by placing the animal in an “induction chamber” and maintaining the desired depth with a face mask. The non-rebreathing “Bain circuit” is commonly used in rodent anesthesia. To perform thoracic surgery, to improve gas exchanges, or to control breathing motion during imaging of the chest, artificial ventilation is needed. The anesthetic potency of
inhalation agents is indicated by their minimum alveolar concentration value, which, like toxic dose 50 (TD\textsubscript{50}), measures the alveolar concentration needed to abolish the response to a standardized painful stimulus in 50% of a population. The lower the minimum alveolar concentration value, the more potent is the anesthetic. The concentration of an inhalation agent for anesthetic induction and maintenance is usually expressed as the percent value of inspired gas mixture (Kohn et al. 1997). Currently the most popular inhalation anesthetics for laboratory animals include nitrous oxide and the halogenated compounds halothane, isoflurane, and sevoflurane (Table 9).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>MAC (%)</th>
<th>Concentration for Induction (%)</th>
<th>Concentration for Maintenance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>0.95</td>
<td>4-5% + 0.8-1 L/min</td>
<td>1-2% + 0.8-1 L/min</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.38</td>
<td>4-5% + 0.8-1 L/min</td>
<td>1-3% + 0.8-1 L/min</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>Individualized based on the mouse response + 0.8-1 L/min</td>
<td>Individualized based on the mouse response + 0.8-1 L/min</td>
<td></td>
</tr>
</tbody>
</table>

**Nitrous Oxide**

Nitrous oxide is a compressed gas that displays good analgesic qualities with minimal depressant effects on respiratory and cardiovascular functions. However, it has very low anesthetic potency, which can be used to speed up induction and to reduce the required concentration of other agents, thereby reducing the degree of cardiovascular and respiratory depression at a given depth of anesthesia. It is usually administered 60 to 75% in a mixture with oxygen. After nitrous oxide inhalation anesthesia, 100% oxygen must be delivered for a few more minutes because the anesthetic agent leaving peripheral tissues floods alveolar spaces causing transient dilution hypoxia in the immediate postanesthetic period (Flecknell 1989).

**Halothane**
Halothane is a potent volatile anesthetic agent that produces good surgical anesthesia and muscle relaxation. Unfortunately, it is also a potent cardiovascular and respiratory depressant; it sensitizes the heart to catecholamines and can produce immune depression after repeated exposition. About 20% of inhaled halothane is metabolized by the liver (Paddleford 2000). After repeated and prolonged expositions, halothane has been shown to be mutagenic and hepatotoxic in humans (Kohn et al. 1997).

**Isoflurane**

Isoflurane is presently the animal inhalation anesthetic agent of choice for both short and lengthy procedures due to its short induction and recovery time and the reliability of its effects. It does not sensitize the myocardium to catecholamines, and it spares cardiac output more than other volatile agents. Indeed, isoflurane can cause a more severe respiratory depression compared with halothane and a dose-dependent hypotension. Isoflurane is also used for very short procedures because it enables mice manipulation and injection, blood collection, and minor surgical procedures. Side effects of isoflurane include induction and emergency “delirium,” immune depression, delayed growth, and cleft palate in litters whose mothers have been exposed to this anesthetic (Kohn et al. 1997; Mazze et al. 1985).

**Sevoflurane**

Sevoflurane can provide an even faster anesthetic induction and recovery compared with isoflurane, and it maintains heart rate at constant values. Like isoflurane, it can induce respiratory depression and hypotension in a dose-dependent manner. Its high cost does limit the use of sevoflurane in laboratory animals (Kohn et al. 1997).

**Anesthetic Monitoring and Physical Management**

During sedation and anesthesia, it is imperative to carefully monitor and support mice body temperature, heart and respiratory rates, mucous membranes, and the degree of CNS depression. After induction, animal care personnel should position the animal on a heated platform or use a heating lamp to maintain body temperature above 95 to 99°F. They should measure core temperature by an esophageal or rectal probe, monitoring tissue oxygenation by pulse-oxymetry, and hearth rate and rhythm by electrocardiogram. Personnel should gently tape down the animal’s limbs and monitor the delivery of oxygen by a nose cone placed over the muzzle. Finally, they
Anesthesia is considered adequate when the animal stays still quietly, is unresponsive to external stimuli, and has constant heart and respiratory rates. In mice the absence of the palpebral reflex suggests a fair anesthetic depth.

**Analgesia and Recovery Care**

Sometimes imaging procedures entail slightly painful or invasive procedures such as intracavitary or intravascular injections, blood vessel catheterization, or endocavitary probe penetration. Under these circumstances, it is important to adopt an adequate analgesic protocol, possibly in line with “pre-emptive analgesia.” To alleviate acute postoperative pain (Table 10) after assessing its degree, appropriate analgesic medication (either opioids or nonsteroidal anti-inflammatory drugs) or local anesthesia can be used successfully in mice.

*NSAID: Non-Steroidal Anti-Inflammatory Drug

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam (NSAID)*</td>
<td>1 mg/kg SC, PO 30 min presurgery and q24h postsurgery</td>
</tr>
<tr>
<td>Carprofen (NSAID)</td>
<td>5 mg/kg SC PO q24h</td>
</tr>
<tr>
<td>Ketoprofen (NSAID)</td>
<td>2-5 mg/kg SC q12-24h</td>
</tr>
<tr>
<td>Buprenorphine (opioid)</td>
<td>0.05-0.1 mg/kg SC q12h</td>
</tr>
<tr>
<td>Tramadol (opioid)</td>
<td>10-30 mg/kg IP or 1mL 5% solution in 150 mL of water</td>
</tr>
<tr>
<td>Lidocaine (local anesthetic drugs)</td>
<td>1-4 mg/kg or 0.4 mL/kg of a 1% solution</td>
</tr>
</tbody>
</table>

Opioids are indicated in animals with moderate or severe pain. Opioids have a relatively short duration of action in small rodents due to their faster metabolic rates compared with larger species. As a result, buprenorphine, which has a prolonged duration of action in most rodents, is preferred in mice. As an alternative or an adjunct to opioid administration, the use of nonsteroidal anti-inflammatory drugs is also recommended in mice. Local anesthetics can be used to provide mice surgical analgesia as well, granting high quality pre- and postoperative analgesia by nerve blocks, central analgesia, infiltration of the surgical field, or topical application of
lidocaine or prilocaine cream (Flecknell 1989; Flecknell 1998). Ossibuprocaine eye ointment is effective for most painful eye procedures. It is critically important to prevent surgical field infections by maintaining aseptic surgical conditions in combination with the use of local or systemic antibiotics (Table 11).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>5-20 mg/kg q24h SC or 50-200 mg/L in drinking water</td>
</tr>
<tr>
<td>Thrimetroprim-sulphonamides</td>
<td>15-30 mg/kg PO, SC, IM q12h</td>
</tr>
</tbody>
</table>

The safest systemic antibiotics for mice that are not harmful to their symbiotic intestinal bacterial population include fluorochinolones and the thrimetroprim–sulphonamides combination. Gentamicine (5-10 mg/kg) can be used SC (Quesenberry and Carpenter 2004) or, in our experience, topically; however, antibiotic administration should be delayed until complete anesthetic recovery takes place because of hypotension and prolonged anesthesia due to their calcium blocking action. A warm, dry, and quiet environment with an oxygen-enriched atmosphere is recommended during recovery to reduce shivering and hypoxemia. Fluid therapy is a strong support for a fast and complete anesthetic recovery. Administering by the SC or IP routes 1.2 mL of 0.9% saline or of half-strength dextrose/saline solution for a 20-g mouse, divided in two doses daily, will prevent dehydration.

**Conclusion**

Mice anesthesia demands a deep knowledge of the physiology and pharmacology of this species. According to the aims of experimental procedure, anesthetic protocol should be tailored by the use of tranquilizers, injectable/inhalation anesthetics and analgesics. Patient monitoring and post-operative care reduce the rate of complications, improving animal welfare and experimental results.

**References**


Wells S, Trower C, Hough TA, Stewart M, Cheeseman MT. 2009. Urethral obstruction by seminal coagulum is in combination with medetomidine-ketamine
anesthesia in male mice on C57BL/6J and mixed genetic background. J AALAS 48:296-299.


RODENTS

The response to an anaesthetic drug can vary considerably and may be influenced by the strain of animal, sex, age and environmental conditions in which the animal is housed. Strain variations may also become apparent only during recovery from anaesthesia; for example, different inbred strains of mice have shown varying degree of respiratory depression immediately after isoflurane anaesthesia. When using a drug or drug combination for the first time, or when anaesthetizing a different strain of animal, it is advisable to proceed cautiously. As experience is gained, a dose rate appropriate to the particular strain can be established.

SIGNS USED TO ASSESS ANESTHETIC DEPTH

A. Muscle movements/voluntary movement especially in response to noxious stimuli Procedure: Observe animal in response to stimuli Assessment: Lifting of the head, kicking or vocalizing indicates that the animal is experiencing pain and is semi-conscious.

B. Jaw Tone Procedure: Gently open the animal’s mouth. Assessment: Assess the degree of muscle relaxation. If jaw is relaxed and easy to open, then the animal is adequately anesthetized. If there is resistance, then the animal is too light (not deeply anesthetized).

C. Swallowing reflex Procedure: Gently place traction on the tongue Assessment: Chewing and swallowing can be visualized externally, and if seen the animal is too light. When the tongue is not retracted, the animal is adequately anesthetized.

D. Withdrawal Reflex (Toe, Paw) Procedure: Extend one leg and pinch the web of skin between the toes with your fingernail to ensure adequate pressure is applied. Assessment: A positive reflex is indicated by the flexion of the limb. If the animal makes general body movements or cries out this is not a reflex response but indicates that the animal has felt the stimulus.

E. Withdrawal Reflex (Ear Pinch) Procedure: Pinch the ear. Assessment: A positive reflex occurs when the animal shakes its head or moves its whiskers forward.

F. Palpebral Reflex Procedure: Gently touch the medial canthus of the eye (inner corner). Assessment: A positive reflex occurs if the animal blinks.

G. Corneal Reflex Procedure: Gently touch the cornea/eyeball with a cotton swab or Q-tip. Assessment: A positive reflex occurs if the animal blinks.

H. Respiratory Rate (Normal 70-115 breaths/min) Procedure: Monitor respiratory rate, especially any response to a painful stimulus. Assessment: If the animal is conscious of painful stimuli and too light, the respiratory rate will increase. Animal may gasp.

I. Heart Rate (Normal 250-450 beats/min) Procedure: Special equipment is required to monitor rodent heart rate. Rate and response to noxious stimuli are monitored. Assessment: If the animal is conscious of painful stimuli the heart rate will increase.
**J. Blood Pressure (Normal 84-134/60 mm Hg)** Procedure: Special equipment is required to monitor rodent blood pressure. Resting pressure and response to noxious stimuli are monitored. Assessment: If the animal is conscious of painful stimuli the blood pressure will increase.

**K. Mucous Membrane Colour** Procedure: Examine eyes, ears, mouth/nose, paws. Press on gum, or nail beds and count # of seconds for the blanched area to return to pink. Blanched area should return to normal pink within 1-2 seconds. Assessment: Areas should be pink. If pale, blue, or gray animal is too deep and near death.

**ASSESSMENT OF ANAESTHETIC DEPTH**

**A. Too Light**

1. Animal is relaxed with loss of awareness. In response to painful stimuli such as a pin prick, animal will move and the physiological parameters will increase (heart rate, respiratory rate, and blood pressure).
2. Jaw is relaxed, and mouth is easily opened.
3. Swallowing reflexes are lost.
4. Withdrawal reflexes are present.
5. Corneal and palpebral reflexes are present.
6. Heart rate and respiratory rate are fast, and will increase in response to pain.
7. Blood pressure can be high due to stress/excitement and/or pain.

**B. Surgical Anaesthesia**

1. Muscles are relaxed and animal is unconscious.
2. Withdrawal reflexes are absent! Try at least 2 toes and the ears so that you are sure that this reflex is absent.
3. Palpebral reflex is lost, but corneal reflex will remain.
4. Respiratory rate is within the normal range (or slightly decreased) and the chest moves up and down in a slow and regular rhythm. If rate increases during surgical manipulation, the animal is not adequately anesthetized.
5. Heart rate is within the normal range. If rate increases during surgical manipulations, the animal is feeling pain, and additional anaesthetic should be given.
6. Blood pressure should be at the normal level. If it drops, the animal is going into shock. A pulse in the groin (femoral pulse) is lost at 50 mmHg, and circulation to the kidney, brain and heart are impaired at this point. On the other hand, a rise in blood pressure is indicative of pain perception and the animal should receive more anaesthetic.

**C. Too Deep**
1. Respiratory rate is very slow and well below normal range. Worry if rate becomes less than 60 breaths/minute. Animal is very close to death at this stage. Breathing may stop. Administer oxygen, stop anaesthetic (if gas), rub body, and/or give artificial respiration.

2. Heart rate is very slow and below the normal range.

3. Blood pressure is low.

4. Corneal reflexes may be absent or may persist into very deep planes of anaesthesia. This is an unreliable indicator.

5. Mucous membrane color is no longer pink. Color will be gray or blue if too deep.