Mouse Anesthesia and Analgesia

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Providing anesthesia and analgesia for mouse subjects is a common and critical practice in the laboratory setting. These practices are necessary for performing invasive procedures, achieving prolonged immobility for sensitive imaging modalities (magnetic resonance imaging for instance), and providing intra- and post-procedural pain relief. In addition to facilitating the procedures performed by the investigator, the provision of anesthesia and analgesia is crucial for the preservation of animal welfare and for humane treatment of animals used in research. Furthermore, anesthesia and analgesia are important components of animal use protocols reviewed by Institutional Animal Care and Use Committees, requiring careful consideration and planning for the particular animal model. In this article, we provide technical outlines for the investigator covering the provision of anesthesia by two routes (injectable and inhalant), guidelines for monitoring anesthesia, current techniques for recognition of pain, and considerations for administering preventative analgesia.

Keywords: mouse • anesthesia • analgesia

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INTRODUCTION

The following is a technical overview of the provision of anesthesia and analgesia for the laboratory mouse. Information is presented in an outline format for quick and easy referencing of dosage information, injectable and inhalant (gas) anesthesia protocols, and analgesic selection and administration. This outline was designed with the laboratory technician and/or researcher in mind; therefore, the following represents the working knowledge and practical considerations of anesthesia and analgesia in mice. For more in-depth coverage of concepts and detailed descriptions, please see the references cited.

Anesthesia

Definitions

1. **Anesthesia**: the reversible loss of consciousness, memory, mobility, and sensation of pain (Kohn et al., 1997).
2. **Neuroleptanalgesia**: combination of tranquilizer or sedative classes of drugs with an opioid drug class to produce sedation and analgesia. The use of one drug potentiates the other (de Castro and Mundeleer, 1959), which allows for reduction of the amount of drugs from either class, thereby minimizing side effects associated with either class.
3. **Injectable anesthesia**: the administration of anesthetic drugs via syringe and needle.
4. **Inhalant anesthesia**: the administration of anesthetic drugs via an inhalant anesthetic.

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Table 1 Pros and Cons of Injectable and Inhalant Anesthesia

Route	Pros	Cons
Injectable	Duration is good for quick procedures No special equipment required (needles, syringes) Reversible agents available Inexpensive Simple technique	Duration is not suitable for lengthy procedures More susceptible to overdosing due to volume errors Controlled substances Slow onset and recovery from anesthesia Difficult to control depth of anesthesia with single injection
Inhalant	Duration is short but lengthy procedures can be accommodated Rapid onset and recovery from anesthesia Easy to control depth of anesthesia Minimal metabolism/secondary products formed	Requires expensive and bulky equipment (anesthesia machine) Requires training of personnel for the use of inhalants Exhaled anesthetic must be scavenged and wasted (environmental pollution) Inexperienced users may have trouble controlling depth of anesthesia due to rapid changes in depth at different settings

Considerations

Injectable and inhalation anesthesia each have pros and cons. When deciding which method to select, it may be beneficial to refer to Table 1, which provides the pros and cons of these two methods.

General anesthesia preparation

1. For surgical procedures: a dedicated preparation area separate from a dedicated surgical area should be used.
2. Instruments and materials [see materials for injectable (Basic Protocol 1) and inhalant (Basic Protocol 2) anesthesia] should be gathered and cleaned appropriately beforehand.
3. Adequate lighting should be available for both preparation and surgical areas.
4. A heat source should be placed in both the preparation and surgical areas and turned on prior to induction of anesthesia. A sterile drape may be placed over heating pads in the surgical area.
5. Personal protective equipment should be donned and consist of clean laboratory coat, facemask, and clean nitrile gloves.

Analgesia

Definitions

1. **Analgesia:** the absence of pain in response to stimulation that would normally be painful (Fish et al., 2008).
2. **Preventative analgesia:** the practice of providing pain-relieving agents throughout painful procedures (Dahl and Kehlet, 2011).
3. **Multimodal analgesia:** the use of multiple classes of pain-relieving agents to address pain (e.g., a systemic opioid in conjunction with a lidocaine local block; Kehlet and Dahl, 1993).

Considerations

Pain is experienced when specialized nerve fibers (nociceptors) are activated by a painful stimulus and transmit pain sensation along peripheral nerves to be processed centrally within the brain. As such, classes of drugs are designed to interrupt this process at different stages of pain signal transduction and may act centrally (at the brain and spinal cord) or peripherally (at the site of painful stimulus). Examples of this concept are the use of the opioid class of drugs (e.g., morphine) to bind nerve receptors within the brain and spinal cord in order to inhibit the transmission of pain stimuli and the use of lidocaine to block the transmission of pain stimuli at the nerve fibers that initially sense painful stimuli (Minami and Satoh, 1995; Scholz, 2002).

INJECTABLE ANESTHESIA

BASIC PROTOCOL 1

Injectable anesthesia is accomplished by the delivery of anesthetic drugs via injection into one of several potential spaces: intraperitoneal (i.p.), subcutaneous (s.c.), intravascular (i.v.), and intramuscular (i.m.). Drug injection into any of these sites ultimately results in distribution of the drug into the bloodstream and to the central nervous system receptors to carry out their activity.

See Figure 1 for example setup.

Materials

Mouse subject(s)
Injectable anesthetic (see Table 2)
Eye lubricant
Pre-warmed balanced fluids [lactated Ringer's solution (LRS); normal saline (0.9% NaCl); Normosol R]
Tared container for weighing mice
1-cc or 3-cc syringe
23-G needles
Recovery cage (no bedding placed within, or home cage with paper towel on bedding; see Fig. 2)

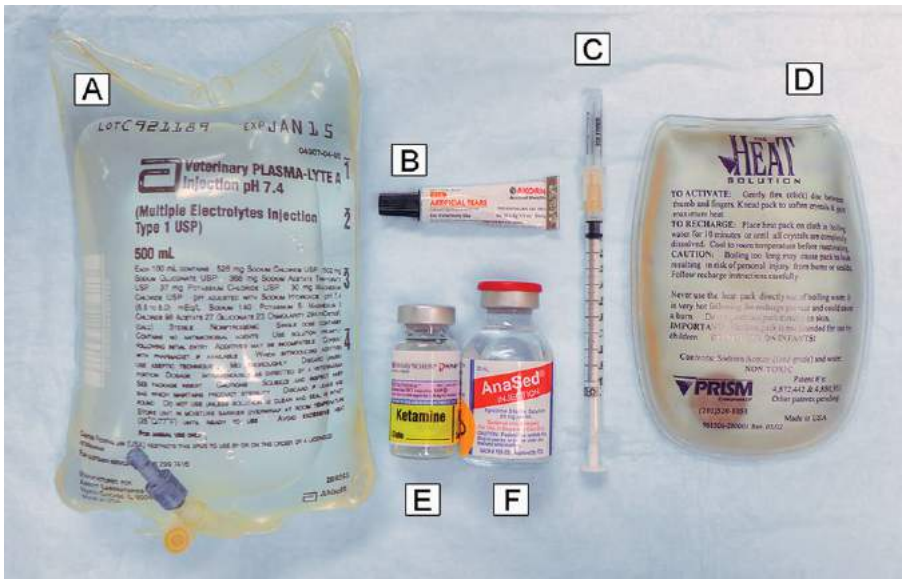

Figure 1 (**A**) Plasma-lyte A fluids. (B) Eye lubrication. (**C**) 1-cc syringe with 23-G needle. (**D**) Reusable heating pad. (**E**) ketamine (100 mg/ml), and (**F**) xylazine (20 mg/ml).

Table 2 Injectable Agents

Agent	Dosage (mg/kg)	Route	Duration (min) at surgical plane of anesthesia	Reference
Pentobarbital[a]	Pentobarbital 40-70	i.p.	10-60	(Flecknell, 1993; Gardner et al., 1995)
Ketamine/xylazine[b]	Ketamine 80-100 Xylazine 3-10	i.p., s.c.	15-20	(Chaves et al., 2001; Fish et al., 2008; Xu et al., 2007)
Ketamine/ dexmedetomidine[c]	Ketamine 50-80 Dexmedetomidine 0.5-1	i.p., s.c.	45	(Baker et al., 2011; Burnside et al., 2013)
Ketamine/xylazine[d]/ cepromazine	Ketamine 100 Xylazine 2.5 Acepromazine 2.5	i.p., s.c,	40-60	(Arras et al., 2001; Fish et al., 2008)
Propofol	Propofol 12-26	i.v.	5-10	(Fish et al., 2008)

[a]Pentobarbital is not recommended for survival surgeries.
[b]Xylazine may be reversed with atipamezole, 0.1-1 mg/kg, given i.p./s.c./i.v.; reversal at 20 min or later with this combination.
[c]Dexmedetomidine may be reversed with atipamezole, 0.1-1 mg/kg, given i.p./s.c./i.v.; reversal at 40 min or later with this combination.
[d]Xylazine may be reversed with atipamezole, 0.1-1 mg/kg, given i.p./s.c./i.v.; reversal at 40 min or later with this combination.

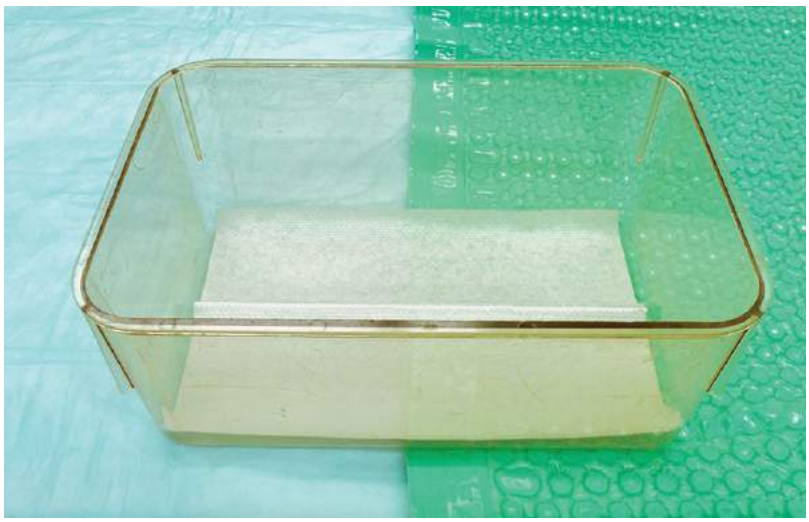

Figure 2 Recovery cage setup: a clean paper towel is placed in the bottom of the cage and the cage is placed halfway atop a heating pad (green recirculating hot water heating pad shown above).

Heating pad (hot water recirculating), reusable gel pack, or heat lamp

1. Using a tared container on the laboratory scale, remove the mouse from its home cage and determine and record its weight. Return the animal to its home cage and use its weight to calculate the dose of injectable anesthetic to be administered using the dosages listed in Table 2.

Example: A 25-g mouse will receive 25 g × 1 kg/1000 g × 80 mg/kg ketamine = 2 mg and 25 g × 1 kg/1000 g × 8 mg/kg xylazine = 0.2 mg.

2. Use the 1- or 3-cc syringe and 23-G needle to withdraw the calculated drug volumes.

   Drugs should be mixed right before use into a single syringe.

3. Manually restrain the mouse and administer the drug via the suggested route in Table 2. Return the animal to its home cage and monitor continuously for loss of consciousness.

   Loss of consciousness is evidenced by recumbency and loss of righting reflex, as well as change in respiration rate and character.

4. Once the animal is anesthetized, apply eye lubricant to the surface of the eye and place the animal on a heating pad.

   A surgical anesthetic plane is reached when the animal no longer responds to a toe pinch (paw withdrawal reflex).

5. Before performing the surgical procedure, it is best practice to administer an analgesic (described further in the Analgesia section in the introduction to this unit). It is also recommended to provide subcutaneous fluids prior to the first incision if a body cavity will be exposed, or if the duration of surgery is greater than 20 min.

   Administer prewarmed fluids (LRS, 0.9% NaCl, Normosol R) at 0.5 to 2 ml/100 g/hr i.p. or s.c.

6. Occasionally, re-dosing is necessary. If changes in respiratory pattern (rapid breathing) or gross intentional movements are observed, one-half of the original ketamine dose, or a one-third to one-half dose of the original drug combination, may be given. Suspend surgical procedure until loss of toe pinch withdrawal is observed.

   Repeated dosing may result in a prolonged recovery.

7. When the surgical procedure is complete, place the mouse in a recovery cage. The recovery cage should have a heat source available to one half of the cage (easily accomplished by placing the cage halfway on to a heating pad) and should be devoid of bedding (which the animal may inhale and aspirate) and cagemates (which may injure or smother the animal). See Fig. 2 for an example of an appropriately set up recovery cage. These conditions allow the animal to recover more smoothly.

   Anesthetic recovery is associated with risks. By providing heat and a suitable environment, these risks are diminished and increase the odds of a successful recovery.

8. Once the animal has regained its righting reflex and is able to stand, recovery is complete and the animal may be returned to its home cage.

INHALANT ANESTHESIA

BASIC PROTOCOL 2

Inhalant anesthesia is accomplished by the delivery of a volatile anesthetic by way of oxygen as a carrier gas. This is contingent upon delivering an amount of volatile compound that causes anesthesia, and this amount coincides with the concentration of drug delivered to the lung alveoli. The amount of volatile agent required to inhibit movement in 50% of recipients is termed the minimal alveolar concentration (MAC), and multiples of the MAC are used to achieve anesthesia. The volatile agent is vaporized within the anesthetic machine and carried by 100% oxygen; thus, the animal receives both anesthetic and oxygen during inhalant delivery.

Table 3 Inhalant Anesthesia Concentrations

Agent	Stage	MAC[a]	Vaporizer setting (%)	Oxygen setting (liters/min)
Isoflurane	Induction		3-5	1-3
	Maintenance	1.4 (Tranquilli et al., 2007)	1-3	0.5-1
Sevoflurane	Induction		3-8	1-3
	Maintenance	2.7 (Tranquilli et al., 2007)	2.5-5	0.5-1

[a]Minimal alveolar concentration.

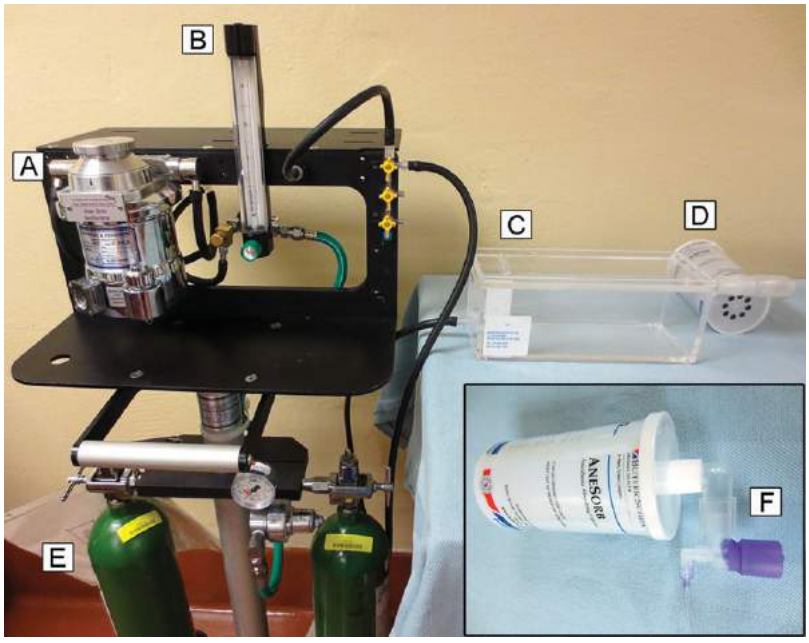

Figure 3 (**A**) Vaporizer (isoflurane or sevoflurane). (**B**) Oxygen flow meter. (**C**) Induction chamber. (**D**) Activated charcoal canister. (**E**) Oxygen tank. (**F**) Face mask with fresh gas flow, elbow unit, and activated charcoal canister.

A higher multiple of MAC is used to initially induce the animal to become anesthetized; the animal is then maintained under anesthesia at a lower multiple of MAC. See Table 3 for recommended concentrations of several common inhalants for induction and maintenance.

Delivery of inhalant anesthetics in larger animals is typically accomplished by intubation with an endotracheal tube; this may be performed in mice as well, but is technically challenging, and the use of a facemask usually meets researchers' needs.

Materials

Mouse subject(s)
Ocular lubricant
Prewarmed balanced fluids (Plasma-lyte A, LRS, Normosol-R)

Anesthetic machine (J.A. Baulch & Associates; see Fig. 3) equipped with:

Volatile anesthetic
Activated charcoal scavenger

Table 4 Anesthetic Monitoring Parameters

Monitoring Parameter	Awake	Light anesthesia	Surgical anesthesia	Deep anesthesia
Spontaneous movement	Present	Present	Absent	Absent
Jaw tone	Present	Present	Absent	Absent
Withdrawal reflex (toe pinch)	Present	Present	Absent	Absent
Mucous membrane color	Pink	Pink	Pink	Pale/blue/gray
Respiratory rate	80-230 bpm (Danneman et al., 2012)	>70 bpm (Ewald et al., 2011)	55-65 bpm (Ewald et al., 2011)	<50 bpm (Ewald et al., 2011)
Respiratory character	Rapid, regular	Rapid, superficial	Rapid, shallow	Gasping
Heart rate[a]	500-600 bpm (Danneman et al., 2012)	>450 bpm (Ewald et al., 2011)	300-450 bpm (Ewald et al., 2011)	<300 bpm (Ewald et al., 2011)

[a]Values obtained using pulse oximeter.

Oxygen supply (mobile or central)

Induction box (chamber; see Fig. 3)
Facemask (see Fig. 3)
Heat source: heating pad (hot water recirculating) or heat lamp
Recovery cage (no bedding placed within, or home cage with paper towel on bedding)

Inhalant anesthesia preparation

1. Weigh the activated charcoal canister on a laboratory scale, verify that the weight does not exceed the suggested expiration weight (e.g., 50 g) as indicated by the manufacturer, and record the weight and date.
2. Ensure the induction chamber is properly assembled (see Fig. 3) with fresh gas flow (oxygen) from the anesthetic machine entering the chamber from one side and a hose leading to the charcoal canister on the opposite side.
3. Assemble the facemask and connect to the anesthetic machine (see Fig. 3).
4. Place a heat source at the induction chamber (hot water recirculating pad underneath the induction chamber, or heated lamp angled at chamber).
5. Verify that the volatile agent level is sufficient for quantity of animals and duration of procedures. Also, examine the oxygen tank pressure (if using mobile oxygen source) and ensure tank is full.

Anesthetizing the mouse

6. Using a tared container on the laboratory scale, remove the mouse from its home cage and determine and record its weight. Use the weight to calculate volumes of analgesic and fluids.
7. Turn oxygen flow on, set to the **induction** settings as described in Table 3, and allow induction chamber to fill with oxygen prior to placing animal in induction box.

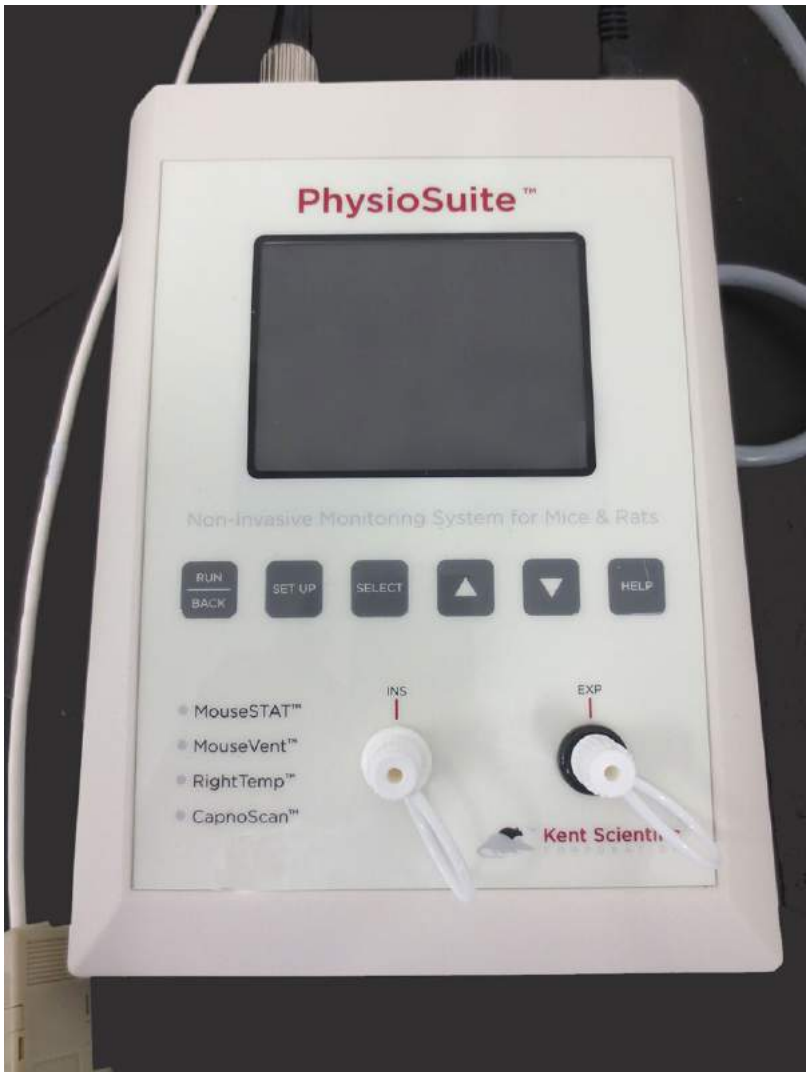

Figure 4 The PhysioSuite (Kent Scientific) is an example of a commercially available tool for mice that provides pulse oximetry, heart rate, ventilator control, capnography (end tidal CO_2), and body temperature.

8. Place the mouse in the induction box and turn the vaporizer to the **induction** settings as described in Table 3 for the appropriate volatile agent.
9. Continuously monitor the animal during the induction process: the animal should lose its righting reflex, and its breathing pattern should change (slower, deeper respiration). Gently tilt the box if unsure if animal is unconscious, to see if it reacts and tries to right itself; once the animal is unable to do so, it is unconscious and may be transferred to the facemask for maintenance of anesthesia.
10. Apply ocular lubricant to the surface of the eye, and then place the animal's muzzle into the facemask and upon the heating pad.
11. Change the vaporizer and oxygen settings to the **maintenance** settings as described in Table 3.
12. *Recommended:* Before performing the surgical procedure administer an analgesic (described further below in the **Analgesia** section).

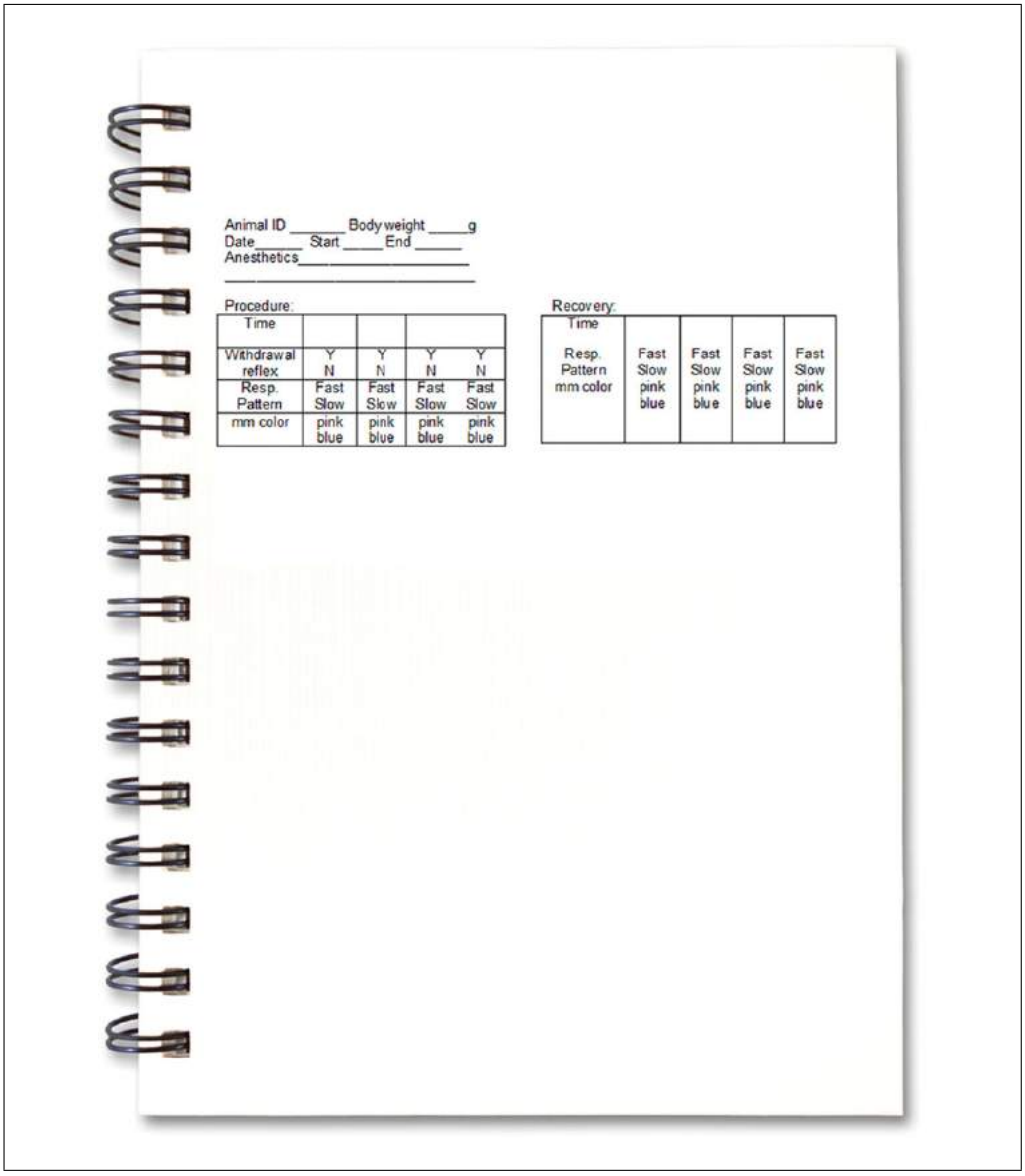

Figure 5 Anesthetic monitoring should be performed continuously while the animal is anesthetized, and parameters should be recorded every 15 min on a form such as this one.

It is also recommended to provide subcutaneous fluids prior to the first incision as well if a body cavity will be exposed or if the duration of surgery is greater than 15 to 20 min.

Prewarmed fluids (LRS, 0.9% NaCl, Normosol R), 0.5 to 2 ml/ 100 g/hr, may be given i.p. or s.c.

13. Continuously monitor the animal for signs of diminishing anesthetic plane—change in respiratory pattern to rapid, shallow breathing, gross intentional movements, reaction to toe pinch (paw withdrawal reflex), and reaction to surgical stimulation (see Table 4). If these should appear, stop any surgical manipulation and increase the vaporizer setting by 0.25% increments (i.e., from 1% to 1.25%) until the animal returns to a deeper plane of anesthesia. Titrate the vaporizer setting as needed to maintain the animal at the optimum plane (no toe pinch response, even, steady respiratory pattern, pink mucous membranes, and skin tone).

Inhalant agents may cause arrhythmia, hypotension, hypothermia, reduced respiratory rate and effort, and bradycardia in a dose-dependent manner.

Figure 6 The mouse grimace scale (MGS) is a useful metric by which to assess pain; however, it may require some practice to reliably identify the following facial expression changes: 1. Orbital tightening, evidenced by a squinted appearance of the eyes; 2. nose bulge, caused by contraction of skin and muscles of the nose resulting in a bulge over the bridge of the nose; 3. cheek bulge, contraction of the cheek muscles present as a bulged surface of the cheek; 4. ear position, with increased pain, the ears lay closer to the head; 5. whisker orientation change, in which whiskers may appear to stand on end and orient either against the face or towards the nose. In the MGS, intensity of each feature is coded on a three-point scale. For a more detailed description, see the original manuscript by Langford et al. (2010). Reprinted with permission from Langford et al., (2010).

Monitoring the anesthetic depth of the laboratory mouse can be accomplished in many ways depending on the tools available to the researcher. At a minimum, respiratory rate and character, mucous membrane, skin color, response to toe pinch (paw withdrawal reflex) and surgical stimulation, and spontaneous movement may be used to assess the anesthetic depth (Flecknell, 2009). Additional tools for monitoring anesthesia include pulse oximetry (see Fig. 4 for an example of a pulse oximeter), capnography, and body temperature. By evaluating these parameters, the rodent user may adjust the depth of anesthesia by increasing or decreasing the inhalant vaporizer setting. During a procedure, the mouse's vital signs should be monitored and recorded at least every 15 min on a monitoring form such as that shown in Figure 5.

Allow recovery

14. When the surgical procedure is complete, place the mouse in a recovery cage.

The recovery cage should have a heat source available to one half of the cage (easily accomplished by placing the cage halfway on to a heating pad) and should be devoid of bedding (which the animal may inhale and aspirate) and cage mates (which may injure or smother the animal); see Figure 2 for an example. These conditions allow the animal to recover more smoothly.

Anesthetic recovery is associated with risk. By providing heat and a suitable environment, these risks are diminished and increase the odds of a successful recovery.

15. Once the animal has regained its righting reflex and is able to stand, recovery is complete; return the animal to its home cage.

ASSESSING PAIN

BASIC PROTOCOL 3

1. Pain may be identified using the following clinical signs:

   Piloerection: fur standing on end rather than laying flat (Mayer, 2007)
   Reduced grooming: unkempt coat, rather than laying flat (Mayer, 2007)
   Decreased spontaneous activity (Goecke et al., 2005)
   Hunched posture (Mayer, 2007)
   Squinting (Langford et al., 2010).

   These signs often require familiarizing oneself with the animal's appearance prior to carrying out painful procedures.

2. Refer to the Mouse Grimace Scale, which provides a reliable and quantifiable means to evaluate pain in the mouse using facial features (Langford et al., 2010).

   Figure 6 demonstrates the visible changes to the mouse facial features in response to pain.

Table 5 Common Procedures, Expected Pain, and Analgesic Choice

	Minimal to mild pain	Mild to moderate pain	Moderate to severe pain
Procedure	Catheter implantation	Embryo transfer	Major laparotomy/organ incision
	Ear notching	Hypophysectomy	Thoracotomy
	Multiple antigen injections	Minor laparotomy incision	Heterotopic organ transplantation
	Ocular procedures	Orchidectomy	Vertebral procedures
	Orbital sinus venotomy	Thymectomy	Burn procedures
	Superficial lymphadenectomy	Thyroidectomy	Trauma models
	Superficial tumor implantation		Orthopedic procedures
	Tail clipping		
	Vascular access port implantation		
	Vasectomy		
Analgesic	Lidocaine or bupivicaine	Lidocaine or bupivicaine + carprofen	Lidocaine or bupivicaine + buprenorphine
	Carprofen	Lidocaine or bupivicaine + butorphanol	Lidocaine or bupivicaine + morphine
	Meloxicam	Lidocaine or bupivicaine + buprenorphine	Buprenorphine + carprofen
	Butorphanol		

Table 6 Dose, Route, and Frequency of Analgesic Administration

Drug	Dose	Route	Frequency
Lidocaine, 2% (Flecknell, 2009)	Dilute to 0.5% and no more than 10 mg/kg	Intradermal s.c.	Once, prior to incision
Bupivicaine, 0.2-0.5% (Flecknell, 2009)	No more than 2 mg/kg	Intradermal s.c.	Once, prior to incision
Butorphanol (Fox et al., 2002)	1-5 mg/kg	s.c.	Every 4 hr as needed
Carprofen (Carpenter, 2005; Fish et al., 2008)	2.5-5 mg/kg	s.c.	Once, prior to procedure (mild)
			Once daily × 3 days (moderate, severe)
Meloxicam (Carpenter, 2005)	1-2 mg/kg	s.c.	Once, prior to procedure (mild)
			Once daily × 3 days (moderate, severe)
Buprenorphine (Carpenter, 2005; Quesenberry and Carpenter, 2011; Roughan and Flecknell, 2002)	0.01-0.2 mg/kg	s.c.	Once, prior to procedure (mild)
			Every 6-12 hr × 3 days (moderate, severe)
Oxymorphone (Quesenberry and Carpenter, 2011)	0.2-0.5 mg/kg	i.p., s.c.	Every 4-6 hr (severe)

Selection of analgesic drug

The selection of analgesic should be based on the amount of pain that is expected to be elicited by the procedure to be performed. Table 5 lists common procedures, the expected pain level caused by the procedures, and the analgesic of choice for a given pain level. Table 6 indicates the dose, route, and frequency of administration for common analgesics.

Conflict of Interest

The authors have declared no conflicts of interest for this article.

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