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Anesthesia Induction and Maintenance

URL: https://www.jove.com/science-education/10263

Overview

Source: Kay Stewart, RVT, RLATG, CMAR; Valerie A. Schroeder, RVT, RLATG. University of Notre Dame, IN

The *Guide for the Care and Use of Laboratory Animals* ("The Guide") states that pain assessment and alleviation are integral components of the veterinary care of laboratory animals.[1] The definition of anesthesia is the loss of feeling or sensation. It is a dynamic event involving changes in anesthetic depth with respect to an animal's metabolism, surgical stimulation, or variations in the external environment.

Principles

Precise and constant monitoring of anesthesia is required to safely maintain the depth needed for a procedure. Parameters to be monitored include heart rate, respiratory rate, body temperature, and blood oxygen levels. For mice and rats, none of these parameters are easily monitored due to these animals' small body sizes. Because the heart rate in rodents is so rapid, the stethoscope normally used for auscultation is inadequate for capturing an accurate heart rate. The stethoscope can only be used to detect the presence or absence of a heartbeat. The normal heart rate for a mouse is 328-780 beats per minute, while the regular rate for a rat is 250-600 beats per minute. Respiratory rates in rodents are also elevated above what can be accurately counted using visual methods or during auscultation. The normal respiratory rate for a mouse is 90-220 breaths per minute, and for the rat this value is 66-144 breathes per minute. To accurately ascertain a heart rate and respiratory rate, specialized electronic monitoring equipment is required. Sensors are either surgically implanted into the animal, or placed externally and interact with the monitoring platform onto which the animal is placed.[3,4]

The most common cause of anesthesia-related deaths in rodents is due to hypothermia. Rodents have a high surface area to body mass ratio. Additionally, an anesthetized animal loses the ability to shiver to maintain body temperature. Thus, body temperature monitoring and supplemented heat, such as a heating pad, are essential during survival surgical procedures. The normal body temperature for a mouse is 96.6-99.7°F (35.8-37.4°C)[5] and for a rat is 96.6-99.5°F (35.9-37.5°C).[5] Most thermometers were designed for larger animals and modeled after those used for humans. Mercury thermometers have been largely replaced with digital and electronic versions. Although the digital and electronic thermometers have been documented as accurate when used rectally, orally, and in the ear, their size is inappropriate for small rodents. Rectal probes designed specifically for mice and rats are commercially available, and their use is encouraged.

Blood oxygenation levels are used to evaluate adequate oxygen uptake from the lungs resulting in the appropriate concentration of oxygen in a rodent's arterial blood. Monitoring oxygen uptake also indirectly monitors respiration and ventilation, as it reveals if there is adequate inspiration of oxygen and expiration of waste gases. The heart rate is also implicated in the oxygenation of the blood, as a decrease in the heart rate will result in a reduction in oxygen levels, which could cause inadequate perfusion of blood.[6]

The goal of the anesthetist is to adequately immobilize and alleviate all pain sensations for an animal with the lowest dose or concentration of anesthesia. Properly assessing the depth of anesthesia is required to achieve this goal. There are four stages of anesthesia and four planes within the surgical stage of anesthesia. During stage one, the animal becomes disoriented. In stage two, there is an excitatory phase with an irregular respiration rate, including breath holding in some mouse and rat strains. The righting reflex-that is the ability to roll back over when placed in a dorsal position-is also lost.

Stage three is the surgical stage of anesthesia. In Plane I, the palpebral and swallowing reflexes are absent. Laryngeal and corneal reflexes are lost in Plane II. With Planes I and II, there are no amnesia or analgesic effects; thus, the animal must reach Plane III prior to the beginning of a surgical procedure. Plane III creates paralysis of the intercostal muscles that results in diaphragmatic respiration. Although initially in Plane III there is only partial analgesia, it progresses to complete amnesia and analgesia as the anesthesia level deepens. It is at this level that the animal is fully anesthetized for a surgical procedure. At Plane IV, the animal has been overdosed and can segue quickly into Stage IV.

As the anesthesia level further deepens, there are complications that can result in the death of the animal. In Stage IV there is complete paralysis of both the intercostal muscles and the diaphragm, which causes severe apnea. This results in respiratory arrest, medullary paralysis, vasomotor collapse, and finally death. The pupils dilate, remaining fixed in dilation while the muscles relax.

Procedure

The proper choice of anesthetics for surgery and other potentially painful procedures must be determined by a veterinarian. This is based on numerous aspects, including the extent and duration of the procedure, the species and strain, the age, and the physiological status of the animal.

Anesthetics are available as inhalants or injectables. Surgical anesthesia can be accomplished using a combination of injectable and inhalant anesthetics.[2]

1. Inhalant anesthesia induction

Inhalant anesthesia includes isoflurane, sevoflurane, and desflurane, with isoflurane being used most commonly. These anesthetics are used more often because, with them, it is easier to control the depth of anesthesia. Induction of anesthesia using inhalation anesthetics can be accomplished with a bell jar or an induction chamber that is fitted to a precision vaporizer.

1. Bell jar
    1. Equipment: A bell jar or desiccation jar with a ceramic or plastic perforated platform, a cotton ball, a liquid anesthetic (isoflurane, sevoflurane, or desflurane), and a fume hood vented to the outdoors.
    2. Preparation
        1. Use the bell jar under a hood, and not on the bench, to avoid exposure of personnel to anesthetic gases.
        2. Assemble the bell jar with the platform in the bottom, creating a space between the bottom of the jar and the platform. This is necessary to prevent the animal from coming into contact with liquid anesthetic.
        3. Wearing nitrile gloves or other impervious gloves, saturate the cotton ball with anesthetic.
        4. Place the cotton ball under the platform in the bell jar.
        5. Secure the lid to the bell jar to prevent escape of the anesthetic vapor.
    3. Induction
        1. Place the animal into the bell jar by sliding the lid to one side.
        2. Once the animal is in the jar, it will be necessary to observe its activity and respiration to determine the depth of anesthesia.
        3. The animal is exposed to the anesthetic, to effect.
        4. Once the animal is fully anesthetized, slide the lid to one side, leaving a space sufficient to allow the introduction of a hand. Grasp the tail, scruff, or body of the anesthetized animal, and gently remove it from the bell jar.

2. Precision vaporizers
   Precision vaporizers can be used with either an induction chamber or a facemask. The anesthetic machine should be inspected prior to each procedure. The proper anesthetic should be added if levels are low. The scavenging system should be checked to ensure that the waste gases are fully removed. For passive waste gas anesthesia systems, the scavenging canister should be weighed to determine if it is still effective. Generally, an increase in weight of 50 grams above the starting weight is the point at which the canister is spent.
    1. Equipment
        1. An induction chamber, a precision vaporizer, a waste gas scavenging unit (either passive or active), and a liquid anesthetic (isoflurane, sevoflurane, or desflurane, as determined by the type of vaporizer in use).
    2. Preparation
        1. Assemble the induction chamber such that the input is from the vaporizer and the output is to the waste gas scavenging system.
    3. Induction
        1. Place the animal into the induction chamber. Some chambers have a sliding lid, and others have a hinged lid that latches.
        2. Once the animal is in the chamber, the oxygen flow is started and the precision vaporizer is turned on at an induction level of 3-4 for isoflurane. Lower anesthetic delivery levels will result in a longer induction time.
        3. The animal is exposed to the anesthetic, to effect.
        4. Once the animal is fully anesthetized, flush the chamber with oxygen before removing the animal to prevent personnel exposure to anesthetic gases. If the induction chamber is placed in a fume hood, flushing with oxygen is not necessary to purge the anesthetic from the chamber prior to opening.
        5. Grasp the tail, scruff, or body of the anesthetized animal, and gently remove it from the chamber.

3. Face mask
    1. Equipment includes a rodent nose cone or mask, a precision vaporizer, a waste gas scavenging unit (either passive or active), and a liquid anesthetic (isoflurane, sevoflurane, or desflurane, as determined by the type of vaporizer in use).
    2. Preparation
        1. Assemble the nose cone or mask such that the input is from the vaporizer, and the output is to the waste gas scavenging system.
        2. Rodent anesthetic machines often have a toggle to switch the anesthetic vapor delivery from the induction chamber to the rodent nose cone or mask. Make sure this is properly set for nose cone anesthetic delivery.
    3. Induction
        1. As the anesthetic gases have an unpleasant smell, many animals will object to being masked for induction. The preferred method is to use the induction box followed by maintenance with the nose cone. Once the animal's nose or face is securely in the mask, the oxygen flow is started and the precision vaporizer is turned on at an induction level of 5 for isoflurane. Lower anesthetic delivery levels will result in a longer induction time and an increase in struggling by the animal.
        2. It is imperative that the animal is monitored for respiration, as too firm a grasp during induction can result in asphyxiation.
        3. As soon as the animal begins to relax, the nose or face can be adjusted in the nose cone or mask, and the anesthetic delivery is reduced to a maintenance level of 1.5-0.5 for isoflurane once complete relaxation is achieved.

2. Induction of anesthesia using injectable anesthetics

Injectable anesthetics are primarily a mixture of ketamine and sedatives or muscle relaxers.

The common combinations are: 1) Rodent Cocktail, which consists of ketamine (100 mg/ml), xylazine (20 mg/ml), acepromazine (10 mg/ml), and sterile saline (0.9% NaCl); 2) ketamine/xylazine 2:1, which consists of ketamine (100 mg/ml), xylazine (20 mg/ml), and sterile saline (0.9% NaCl); and 3) ketamine/xylazine Mouse Mix, which consists of ketamine (100 mg/ml), xylazine (20 mg/ml), and sterile saline (0.9% NaCl). When using ketamine/xylazine combo, boosting should only be done with ketamine only, not xylazine, due to the half-lives of these drugs.

The combination of ketamine with sedatives and/or muscle relaxants needs to be prepared as a stock solution from which individual doses can be drawn. The agents must be precisely measured and diluted with sterile saline to ensure that proper doses are administered to the animals. Because ketamine is a controlled substance, the amount used from the bottles must be noted on a "Controlled Drug Log," and the mixtures must have individual "Controlled Substance Logs." When preparing mixtures, add the ketamine slowly to the bottle, as it tends to foam if injected with force. A sterile stoppered 20 ml bottle is used for the mixture. The bottles must be properly labeled with the name of the compounds, the date mixed, the expiration date, the ketamine lot number (as it is a controlled substance), and the suggested dosage. The expiration date may be determined by the date of the ingredient soonest to expire (depends on the rules/guidelines of the facility/state). For accurate recordkeeping of ketamine, both the empty bottle and the filled bottle must be weighed. Then, the weights must be recorded on the label of the mixture and on the individual Controlled Substance Log sheet that is prepared for each bottle. Store ketamine mixtures in a dark, temperature-controlled area to maintain potency.

1. Preparation of Rodent Cocktail
    1. Equipment for preparation of the anesthetic solution includes 3 cc syringes, a 12 cc syringe, 22 g x 1" needles, 1.8 cc of ketamine injectable 100 mg/ml, 1.8 cc of xylazine injectable 20 mg/ml, 0.6 cc of acepromazine injectable 10 mg/ml, 15.8 cc of sterile saline for injection, and an anesthetic label.
    2. Draw up 15.8 cc of sterile saline, and inject it into the bottle. Use the 12 cc syringe and a 3 cc syringe, and eliminate air bubbles for accurate measurements.
    3. Add the ketamine, xylazine, and acepromazine to the bottle.

2. Induction of Rodent Cocktail
    1. In general, Rodent Cocktail is used for mouse anesthesia. Due to variation in response to Rodent Cocktail in adult rats, it is best used in rats under 5 weeks of age. Rodent Cocktail may be used in conjunction with inhalation anesthesia, especially in rats.
    2. Rodent Cocktail is generally given to mice according to weight, using the following calculation: (BW x 10) - 50 = microliters Rodent Cocktail to be given. This will vary based on strain, age, and health status.
    3. This solution is only given intraperitoneally. If injected intramuscularly, there can be a severe reaction in the tissue.

3. Preparation of ketamine/xylazine 2:1
    1. Equipment for the preparation of the anesthetic solution includes 3 cc syringes, 22 g x 1" needles, 10 cc of ketamine injectable 100 mg/ml, 5 cc of xylazine injectable 20 mg/ml, a sterile stoppered 20 ml bottle, and an anesthetic label.
    2. Add the ketamine and xylazine to the bottle.

4. Induction
    1. Ketamine/xylazine 2:1 is used for anesthesia for rats, and may be used in conjunction with inhalation anesthesia.
    2. Ketamine/xylazine 2:1 is generally given starting at a dose of 0.3 cc and increased by 0.02 cc with subsequent anesthetic events. This will vary based on strain, age, and health status.
    3. Ketamine/xylazine 2:1 is only given intramuscularly.

5. Preparation of ketamine/xylazine Mouse Mix
    1. Ketamine/xylazine Mouse Mix equipment required for the preparation of the anesthetic solution includes 3 cc syringes, 22 g x 1" needles, 3.6 cc of ketamine injectable 100 mg/ml, 0.4 cc of xylazine injectable 20 mg/ml, 16 cc sterile saline (0.9% NaCl), a sterile stoppered 20 ml bottle, and an anesthetic label.
    2. Add the ketamine and xylazine to the bottle, making a 9:1 solution of ketamine to xylazine.
    3. Add the saline to the bottle, resulting in a 1:4 mixture of ketamine/xylazine 9:1 to saline.

6. Induction of ketamine/xylazine Mouse Mix
    1. Ketamine/xylazine Mouse Mix may also be used in conjunction with inhalation anesthesia.
    2. Ketamine/xylazine Mouse Mix is generally given to mice according to weight, using the following calculation: (BW x 10) - 50 = microliters ketamine/xylazine Mouse Mix to be given. This will vary based on strain, age, and health status.
    3. Ketamine/xylazine Mouse Mix can be given intraperitoneally.

3. Anesthesia Assessment

Anesthetic depth can be assessed by testing the response to various stimuli. Voluntary movement will result from physical stimuli of the body. See Table 1 for a list of physical methods utilized for anesthetic depth assessment.

Method	Procedure	Response
Toe pinch	Extend the leg and isolate the webbing between the toes. This area is firmly pinched using either the fingernails or atraumatic forceps.	A positive reflex is indicated by the retraction of the leg or withdrawing of the foot. The animal is not at a surgical plane of anesthesia if there is leg or body movement, vocalization, or marked increase in respirations.
Tail pinch	The tail tip is pinched using either the fingers or atraumatic forceps.	A positive reaction is indicated by twitching or movement of the tail. The animal is not at a surgical plane of anesthesia if there is movement of the tail, vocalization, or marked increase in respirations.
Ear pinch	Using the fingers or atraumatic forceps, pinch the tip of the pinna.	A positive reaction is shaking the head or the movement of the whiskers forward. If there is movement of the head, whiskers, vocalization, or marked increase in respirations, the animal is not at a surgical plane of anesthesia.
Palpebral reflex	Using a fingertip, touch the medial canthus (inner corner) of the eye.	A positive reflex is indicated by a blink in response to touching the eyelids. If there is movement of the eyelids, whiskers, or marked increase in respirations, the animal is not at a surgical plane of anesthesia.
Corneal reflex	Using a cotton-tipped applicator, gently touch the cornea (eyeball).	A positive response is indicated by a blink. If there is movement of the eyelids, whiskers, or marked increase in respirations, the animal is not at a sufficiently deep plane of surgical anesthesia.

Table 1. Physical stimuli methods for assessing anesthetic depth.[2]

Physiological indicators such as heart rate, respiratory rate, blood pressure, mucous membrane color, and capillary refill time should also be used. While general observations can be useful to detect changes in the respiratory rate of the animals, to utilize the heart rate, or blood pressure for depth assessment, specialized equipment is required. If an electrocardiograph is available, the rate and the strength of the heartbeats can be measured. For measuring the blood pressure, there are a variety of devices that are fitted over the tail or even over the entire body. Physical stimuli as described in Table 1 will cause an increase in all three of these parameters.

The color of the mucous membranes, eyes, ears, mouth, nose, anus, and-to a lesser extent-the paws and tail are observed for changes. The areas should be pink, indicating adequate respiration and cardiac function. When the animal moves to Stage IV anesthesia, the respirations cease, resulting in cyanosis-indicated by a blue or gray color-to the mucous membranes and surrounding skin.

Capillary refill time is defined as the amount of time taken for color to return to an external capillary bed after it has been blanched by the application of pressure over the area. An applicator stick or a finger is pressed on the gums, pinna, or nail beds of the anesthetized animals. The number of seconds that it takes for the blanched area to return to a pink color should not be more than 1-2 seconds. An extended refill time suggests a reduction in heart rate or strength of cardiac contractions, indicating the animal may be too deeply anesthetized and near death.

It is important to utilize several different parameters to assess anesthetic depth. Using the same toe or ear for repeated pinches will desensitize the area, and the response will be repressed and not give an accurate assessment of anesthetic depth. Use alternate sites for toe and ear pinch assessments. Anesthetic depth should be reassessed every 10-30 minutes throughout the surgery.[2]

Studies have shown that there are cardiorespiratory changes in an anesthetized animal. While anesthetized with injectable drugs, the animals experience a stable respiratory rate; however, they demonstrate variability in cardiac output. The response to injectable anesthetics has been reported to vary greatly between different strains, thus it is difficult to standardize the dosage.[7] Inhalant agents tend to decrease the respiratory rate but have a lesser impact on the cardiovascular system. As the dosage of inhalant anesthesia is easily adjusted throughout the duration of the procedure, it is often the preferred method.

Summary

The proper use of anesthetics for surgery, or other potentially painful procedures, is crucial not only for the animal's wellbeing, but also for the integrity of the scientific data collected during the procedure. There are many variables that factor into choosing the appropriate anesthetic regiment. The depth of anesthesia must be closely monitored, as each individual animal can respond differently to the drug. With the use of the proper anesthetic and careful monitoring, painful procedures can be accomplished with no pain and minimal physiological changes in the animal.

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