

RAT BIOMETHODOLOGY

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PURPOSE OF THIS DOCUMENT

This is a handout that accompanies a hands-on rat biomethodology workshop in the Laboratory Animal Resources Center (LARC) and other institutions.

OBJECTIVES

- A. Instruct participants in methods of safe, humane handling and restraint.
- B. Instruct participants in substance administration to include {intramuscular (IM), intraperitoneal (IP), subcutaneous (SC), and intravenous (IV)} as well as the technique of gavage.
- C. Instruct participants in techniques associated with the collection of blood samples.
- D. Instruct participants in the areas of sedation, anesthesia, and analgesia.
- E. Instruct participants in methods of euthanasia.

BASIC INFORMATION ABOUT WORKING WITH RATS

- A. Wear a minimum of a clean laboratory coat and gloves. The use of surgical masks or respirators may assist in reducing allergen exposure.
- B. Keep records of each procedure performed on each rat or group of rats.
- C. If bitten:
- Don't punish the rat for its natural response! Calmly return the animal to its cage.
 - Wash the wound thoroughly with an antiseptic soap and water.
 - Cover the wound with a bandage.
 - Notify your immediate supervisor of the bite so that procedures appropriate to the injury can be followed.
- D. Rat psychology:
- Rats are basically docile, curious animals and usually develop closer bonds with humans than mice do.
 - Rats respond positively to quiet, gentle handling. They are normally not aggressive (except for some strains/stocks, e.g. Fisher 344), but if frightened or distressed can inflict painful bites.
 - Like any animal, rats are creatures of habit. Everyday events do not tend to stress or excite rats. However, out of the ordinary events such as being picked up, handled, and restrained are stressful and can result in the rats being fractious. Conditioning the rats to handling and restraint will prevent the rats from associating being handled with "negative" things (like being stuck with a needle) and often makes the animals much easier with which to work.
 - Work quietly among the animals, and try to avoid performing procedures in the animal housing room. This will minimize the excitement of the rats from smells and noises, and will allow you to easily perform your tasks on a more tractable, less stressed animal.

GENERAL INFORMATION

General Biology

The laboratory rat (*Rattus norvegicus*) is a mammal of the order Rodentia. The laboratory rat was the first animal in which the primary reason for domestication was for use in scientific endeavors. Rats are one of the most common (second to mice) laboratory animal used for research.

Rats have several unique biological characteristics. The acute hearing of rats makes them sensitive to ultrasounds and high pitched sounds. Rats have poor vision; they are unable to detect color and are blind to long-wave (red) light. The rat's tail is the principal organ for heat exchange.

Biological Data

The following table provides general biological data for many rats, however, for strained specific characteristics go to

http://www.criver.com/flex_content_area/documents/rm_rm_n_techbul_spring_99.pdf and <http://www.taconic.com/wmspage.cfm?parm1=893>.

Adult body weight: male	450-520 gm
Adult body weight: female	250-320 gm
Body surface area (cm ²)	10.5 (wt. in grams) ^{2/3}
Life span	2-3.5 years
Food intake – growing	8.0-20.0 g/rat/day
Food intake – fully grown	15-20 g/rat/day
Food intake – Nursing or pregnant	Up to 65 g/rat/day
Water consumption	10-12 ml/100 g/day
Breeding onset: male	65-110 days
Breeding onset: female	65-110 days
Gestation Period	21-23 days
Body Temperature	35.9-37.5 C
Heart rate	250-450 beats per minute
Respiratory rate	70-115 per minute
Tidal Volume	0.6–2.0 ml

Behavior

Rats are nocturnal and thus are active primarily during the night at which time they feed. During the daytime, rats tend to rest and sleep. Handling animals during the night can be more difficult due to this increase activity. The diurnal rhythm can be changed by a 12-hour shift in the light cycle. It takes approximately two weeks for rats to adjust to this shift.

Rats tend to get along well with other rats. However, please realize that introducing two rats of the same sex to each other after weaning age can result in fighting and, potentially, serious injuries. Similarly, rats of the same sex that have been housed together may fight if separated and later re-introduced. Male and female rats tend to accept each other. If one is to introduce post-weaning rats that are strangers, one should take the following precautions: Introduce the rats together into a clean cage so that neither rat has established the cage as their home territory. Supervise the rats closely over the next hour or so to see how they do. Be prepared with another cage to separate the rats if needed. The Laboratory Animal Resources Center can assist by providing enrichment items for the cage that may serve as a distraction.

The *Guide for the Care and Use of Animals* indicates that animals that form social groups (such as rats) should be housed with conspecifics (group housed). Rats can be single housed only for clinical reasons or if scientifically justified (and approved in the IACUC protocol).

Basic Husbandry

Most rats are housed in shoe box cages composed of a plastic (polycarbonate or polysulfone) material with a stainless steel wire bar lid used to hold the water bottle and feed. Bedding is placed directly into the shoe box cage allowing the absorption of urine. The type of cage in use will generally hold up to three large adult rats. When removing the lid from this type of cage, it is important to remove the water bottle to prevent spillage (unless automated watering is used). If the cage is to be transported, the bottle should be turned sipper tube up to prevent spillage during transport. However, you should remember to turn the bottle back over to allow access to water after transport.

The animal care staff change the cages on a fixed schedule (frequency depends upon the type of housing, bedding and research needs), thereby providing the animals a clean cage with new bedding, food, and water. Water bottles and feed hoppers are checked daily by caretakers to insure the provision of food and water and to monitor for health or other problems. Some rats are housed on wire mesh bottom cages if scientifically justified and approved in the IACUC protocol. This type of housing is not preferred and is used only when dictated by experimental design.

Pelleted natural ingredient diets are used to feed all rodents and are composed primarily of cereal grains supplemented with additional protein, vitamins and minerals. The water provided to the rats is treated to make it as pure as possible. For rats housed under sterile conditions, the water is autoclaved.

Two levels of barrier housing of rats are available. One level involves the housing of rats in a cage as described above. However, in addition, the cage is fitted with a filter top (microisolater top). This filter top allows pathogen exclusion and containment. LARC also maintains ventilated cage racks which provide HEPA filtered supply and exhaust air to each individual cage. This type of housing also provides for pathogen exclusion and containment. Depending upon the needs of the investigator, a change-out hood can be placed in the animal room to allow for pathogen containment and exclusion when the filter top is removed for cage change-outs or animal manipulations. Also, arrangements can be made to have the entire cage and its contents autoclaved to allow for a sterile environment in which the rats can live. Please contact LARC to discuss the details of these housing options.

A health surveillance program is in place utilizing sentinel animals to detect the presence of rodent pathogens. Rodent pathogens often do not produce clinical signs in affected animals but their presence serves as an unwanted research variable.

Identification

Cage cards are utilized to identify the strain of rat, sex, number, principal investigator, and IACUC protocol #. Cage cards should not be removed from the cage to avoid misidentification of the animals. Temporary identification of individual rats can be accomplished by pen marks on the tail, hair clipping or dyeing the fur. Pen marks will only last a few days whereas hair clipping may last up to 14 days. Ear punch identification and ear tags can be utilized but may be

obliterated by fighting between individuals. Microchips and tattoos have also been used for identification.

Handling

When handling rats it is advisable to wear clean gloves to prevent the development of allergies due to direct contact with animal allergens. Rats typically become accustomed to repeated handling. In a naive animal, the temperament of the animal can be determined by placing the hand into the cage to allow exploration by the animal prior to touching. Initial gentle stroking of the animal followed by gradual grasping the animal will prevent startling the animal and initiating an aggressive response.

Rats are normally lifted by grasping the whole body with the palm over the back, with the fingers and the thumb behind the forearms. This extends the rat's forelimbs so that they may be controlled. Holding with one hand is usually adequate for control, but the tail, rear legs or lower part of body may be held by the other hand for close control, treatment, or examination. The use of both hands is often necessary for rats weighing over 350 grams. Young rats may be handled like mice (see Mouse Biomethodology handout). One should exercise caution when lifting rats by the tail as they may strip the skin from the tail (de-gloving injury). This is particularly likely for heavy rats (>450 grams). When picking up a rat by the tail, be sure to grasp the base of the tail with the thumb and forefinger. For transporting short distances it is helpful to support the rat with your arm or hand while holding the tail.

Rats may bite and certain strains/stocks are more aggressive than others (e.g., F344 rats tend to be more aggressive than Sprague-Dawley), so care and experience are essential to safe handling. Various commercial restraint devices are available for use with rats (see below).

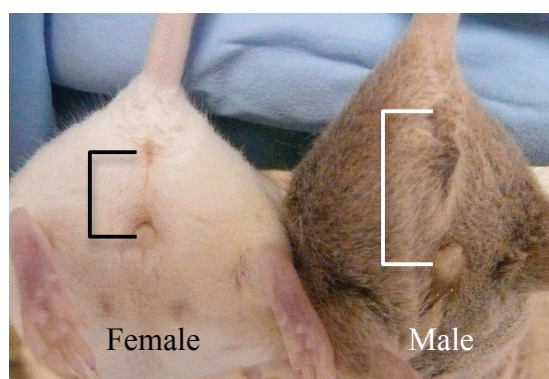
Restraint Devices

Numerous types of restraint devices are commercially available to restrain rats. Quality devices prevent the animal from turning around yet allow easy access to strategic parts of the animal. Devices should also be easy to clean and provide adequate ventilation.

Sexing

Male and female rats can be differentiated by observing the distance from the anus and genital papilla (called the anogenital distance), which is greater in males. This difference is also present in neonatal rats.

In addition, one can usually determine gender by looking for the presence of testicles. However, one must realize that rodents have the ability to retract their testicles into the abdominal cavity (thus the apparent absence of testicles does not necessarily mean the rat is a female).



HANDLING AND RESTRAINT EXERCISES

A. Rat Restraint Technique I - For removal from caging and transport

Materials: Disposable gloves

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away especially if it is an aggressive rat. DO NOT suspend the rat by the tail or the upper body for a prolonged time period because of the stress on the animal. Support the body weight quickly.
2. Lift the rat out of cage and place in new caging or on a firm surface or hands.
3. If the rat is docile, you may just grasp the rat straight out of the cage with your hand.
4. With firm but gentle pressure, grasp the rat around the thorax with the thumb and fingers under each of the front legs. Alternatively, one or two fingers can be in front of the foreleg.



B. Rat Restraint Technique II - For technical manipulation

Materials: Disposable gloves

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With firm but gentle pressure, grasp the rat around the thorax with the thumb and fingers under one front leg and under the chin.
3. Alternatively, slide the thumb across the ventral thorax and underneath both forelegs.
4. The rat is now ready for technical manipulations.
5. The base of the tail may be held or the rear quarters supported by the other hand for additional control.

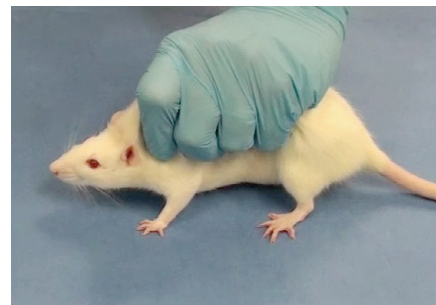


C. Rat Restraint Technique III- For technical manipulation

Materials: Disposable gloves

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With the other hand, place the palm of the hand on the rat's back. With the thumb and fingers gather the loose skin along the dorsum including that along the dorsal neck. The thumb and index finger are utilized to stabilize the head while the other three fingers stabilize the trunk.
3. Be careful not to grasp too tight as this may compromised breathing.

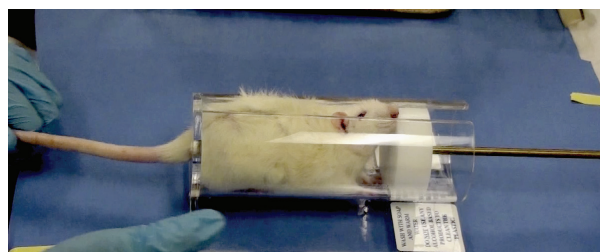


D. Rat Restraint Technique IV - For technical manipulation using mechanical restraint

Materials: Disposable gloves, Plexiglas restraint box

Procedures:

1. With the non-dominant hand, grab the base of the tail with the thumb and index finger to keep the rat from running away.
2. With firm but gentle pressure, grasp the rat around the thorax with the thumb and fingers under each of the front legs. Alternatively, one or two fingers can be in front of the foreleg.
3. Place the rat's head into the opening of the restraint box.
4. Release hold on the body, while maintaining grasp on the tail.
5. Place securing block in appropriate slot for necessary restraint.

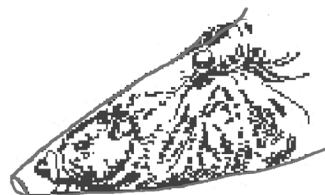


E. Rat Restraint Technique V - For technical manipulation using DecapiCones®

Materials: Disposable gloves, DecapiCones®

Procedures:

1. Grab the base of the tail with the thumb and index finger.
2. With the head first, place animal in DecapiCone.
3. Gentle restraint is done by wrapping DecapiCone around snug around the animal.
4. The animal is now ready for technical manipulations such as IP injections or decapitation. Note that decapitation of conscious rodents can only be performed when scientifically justified and approved in the IACUC protocol.



F. Rat Restraint – Other techniques



Dorsal restraint with a towel provides a safe restraining technique for overly aggressive rats

Towel over the back of the rat provides a safe, quick and easy approach for IP and IM injections



A claw technique is accomplished by restraining the head between the index and middle fingers while cupping the back of the rat with palm of the hand

INJECTION TECHNIQUES AND BLOOD WITHDRAWAL EXERCISES

Always use sterile syringes and needles for all procedures. To insure aseptic techniques and sharp needles, the one time use of disposable supplies is strongly recommended. When administering injections, select the smallest gauge needle possible to minimize tissue trauma and injection discomfort. Before injecting the solution, always check for correct placement of the needle by slightly pulling back the plunger of the syringe to create a vacuum. This is known as aspiration. The signs to look for will vary with the injection site. If blood or other fluids are aspirated, placement may be incorrect.

Due to the small muscle mass of many rodents, an intramuscular injection may cause discomfort and local tissue irritation, especially if too large a volume of a solution or a solution with an acidic or alkaline pH is administered. An understanding of anatomy and careful technique are necessary to avoid the ischiatic (sciatic) nerve in the hind leg, just behind the femur. Injection into or close to the nerve may lead to unnecessary discomfort, temporary lameness, or permanent paralysis of the leg. As a result of nerve damage, an animal may chew off the affected extremity.

If too much blood is withdrawn too rapidly, or too frequently without replacement, one may induce hypovolemic shock and/or anemia. As a general guide, up to 10% of the circulating blood volume can be taken on a single occasion from normal healthy rats with minimal adverse effect (rat blood volume = 70 ml/kg body weight). This volume may be repeated after 2-3 weeks. For repeat bleeds at shorter intervals, a maximum of 1% of an animal's circulating blood volume can be removed every 24 hours. However, care should be taken in these calculations as the percentage of circulating blood will be about 15% lower in obese and older animals.

INJECTIONS EXERCISES

BASIC PROCEDURE

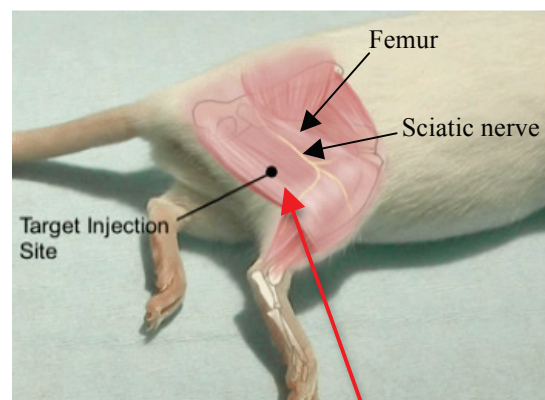
- Wipe the top of the drug bottle with alcohol before withdrawing the dose.
- Slowly withdraw the dose and tap the air bubbles out of the syringe. Air bubbles injected intravenously or intraarterially can potentially cause air emboli and associated problems.
- Always check route of administration for the particular drug to be injected.

A. Intramuscular (IM) Injection

Materials: Disposable gloves, syringe (1 ml), hypodermic needle (22-30 ga), injection article, isopropyl alcohol, gauze

Procedures:

★Maximum injection volume = 0.3 ml in an adult rat



Direction of injection

1. Fill syringe with appropriate amount of article to be administered.
2. Restrain rat.
3. Prep area with alcohol swab.
4. Insert needle into caudal hind leg muscles.
5. Aspirate syringe to insure proper placement. Any sign of blood in the syringe indicates improper placement-reposition.
6. Administer article in a steady, fluid motion. DO NOT administer rapidly because of tissue trauma.



Direction of injection

B. Subcutaneous (SC) Injection

Materials: Disposable gloves, syringe (1-10 ml), hypodermic needle (22-30 ga), injection article, isopropyl alcohol, gauze

Procedures:

★Maximum injection volume = 5 – 10 ml in an adult rat

1. Fill syringe with appropriate amount of article to be administered.
2. Restrain rat.
3. Prep area with alcohol swab.
4. Tent the skin and insert the needle at the base of the tent.
5. Aspirate syringe to insure proper placement. Any sign of blood indicates improper placement; also, a lack of negative pressure in the syringe indicates the needle has punctured out through the opposite side of the skin. Remove syringe and reposition.
6. Administer article in a steady, fluid motion.



C. Intraperitoneal (IP) Injection

Material: Disposable gloves, syringe (10 ml), hypodermic needle (22-30 ga), injection article, isopropyl alcohol, gauze

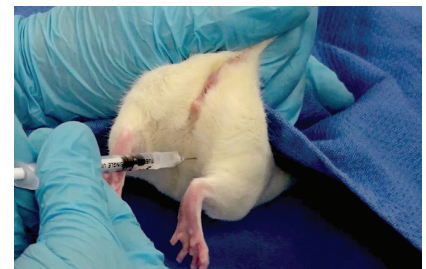
Procedures:

★Maximum injection volume = 5-10 ml in an adult rat

1. Fill syringe with appropriate amount of article to be administered.

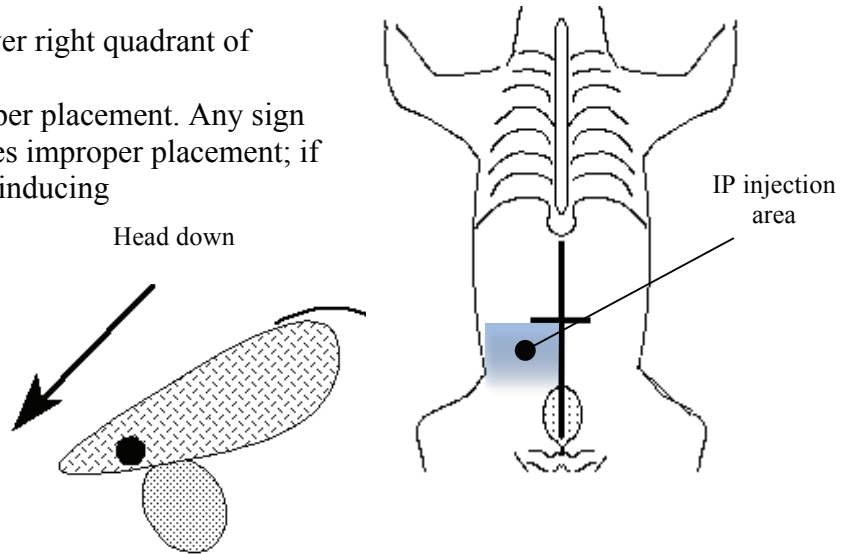


45° Approach



Rear Approach with dorsal Towel Hold

2. Restrain rat by tilting body at a 45° angle with the head down or dorsal towel hold. This will position the intestines cranial to the injection site.
3. Prep area with alcohol swab.
4. Insert needle into the rat's lower right quadrant of abdomen at a 30° angle.
5. Aspirate syringe to insure proper placement. Any sign of blood or other fluid indicates improper placement; if these are seen then to prevent inducing peritonitis, remove syringe, discard, and use new syringe, needle, and article.
6. Administer article in a steady, fluid motion.



D. Intradermal (ID) Injection

Materials: Anesthetic, disposable gloves, syringe (1 ml), hypodermic needle (25-30 ga), gauze, clippers, #40 blade, isopropyl alcohol

Procedures:

★Maximum injection volume = 0.05-0.1 ml depending on thickness of skin.

1. Intradermal injection MUST be done UNDER ANESTHESIA!
2. Clip hair on injection area and prep with alcohol swab.
3. Insert needle between layers of skin on the back at about 20° angle.
4. Aspirate syringe to insure proper placement. Any sign of blood or other fluid indicates improper placement-reposition.
5. Administer article slowly. Successful injection results in a small circular skin welt.



E. Intravenous (IV) Injection Utilizing Lateral Tail Veins

Note: Injection into the lateral tail vein in rats can be difficult due to the thickness and toughness of the skin over the area of the vein. The secret of successful injection of the tail vein is to dilate the veins. This has been accomplished in various ways such as the following: Placing the tail in warm water (47 °C for about 1 minute (do not exceed 47 °C as this can result in thermal injury to the tail); warming gauze in the warm water bath as stated above and then wrap the tail on the gauze for about 1 minute; placing the animal in an incubator (37° C) for 10 – 15 minutes; or wrapping the tail in an electric heating pad that is warm (not hot) to the touch. In addition one can place a tourniquet around the base of the tail to facilitate visualization of the vein. See illustration on tourniquet preparation later in this handout. A rubber band and mosquito hemostat also make suitable tourniquets.

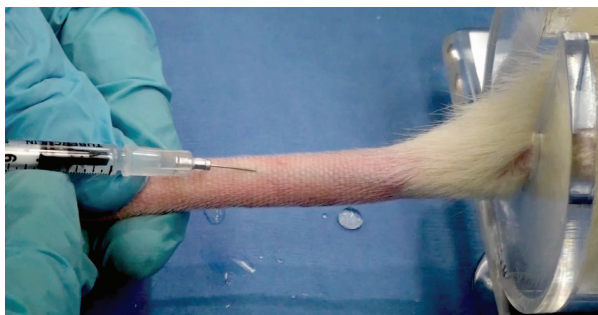
The veins can be seen when the tip of the tail is lifted and rotated slightly in either direction. The tip of the needle can be followed visually as it penetrates the vein. Trial injection verifies proper needle placement. Also, accurate placement can be confirmed when the vessel is visually flushed when the compound is administered. The formation of a bleb at the site indicates improper placement of the needle. A second attempt can be performed by removing the needle and trying a site on the same vessel in a more proximal (closer to the animal's body) location on the tail. Practice is essential.

Materials: Disposable gloves, plexiglas restraint box, syringe (1 ml), hypodermic needle (25-30 ga), injection article, isopropyl alcohol, gauze, method to dilate the tail vein (see below)

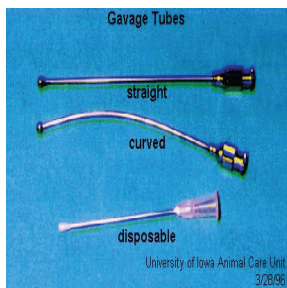
Procedures:

★Maximum injection volume = 0.5 ml in an adult rat.

1. Restrain rat.
2. Prep tail with alcohol swab.
3. Needle placement should be no closer to the body than half the length of the tail.
4. With tail under tension, insert needle into skin approximately parallel with the vein.
5. Insure proper placement by inserting needle at least 3 mm into lumen of vein.
6. Administer article in a slow fluid motion to avoid rupture of vessel.
7. Upon completion, insure good hemostasis before returning to cage.



GAVAGE EXERCISES



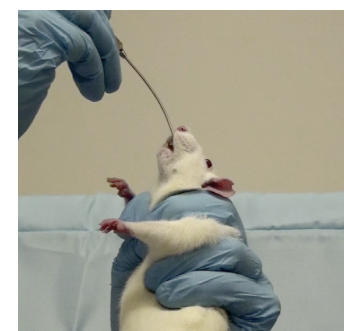
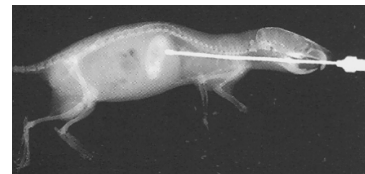
Materials: Disposable gloves, gavage tubes, syringes (1-10 ml), injection article

Procedures:

★ Maximum injection volume = 10 ml/kg considered best practice & 40 ml/kg as possible max vol. Large dose volumes (40 ml/kg) have been shown to overload the stomach capacity, pass immediately into the small

bowel (Hejgaard *et al* 1999) and may also reflux into the esophagus.

1. Measure the distance from the tip of nose to the last rib. This is the length the needle should be inserted.
2. Fill syringe with appropriate amount of article to be dosed.
3. Restrain rat (Refer to Restraint Technique II).
4. Place tip of needle in the rear of the rat's mouth to induce swallowing, while gently pressing the shaft of the needle against the roof of the mouth to extend the neck and align the mouth and esophagus.
5. Slide tip down back of mouth, moving tip forward in one fluid motion.
6. Take your time, any resistance felt indicates improper placement. Needle should slide down into esophagus easily. A violent reaction (coughing, gasping) usually follows accidental introduction of the tube into the larynx or trachea.
7. Once the needle is properly placed, administer the article.



Recommended Standard Gavage Tube Sizes for Rats

Wt. range (grams)	Gauge	Length (inches)	Ball Diameter (mm)	Shape
50-75	20	1, 1 ½	2 ¼	Straight
75-120	18	1, 1 ½	2 ¼	Straight, Curved
100-200	18	2, 3	2 ¼	Curved
	16	2	3	Straight, Curved
150-300	16	3, 4	3	Curved
200-350	14	3	4	Curved
	13	3	4	Straight

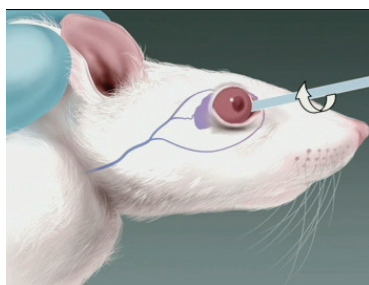
BLOOD COLLECTION EXERCISES

A. Blood Withdrawal Utilizing Orbital Venous Plexus*

*Note: This technique has been largely replaced with less invasive blood collection techniques such as from the lateral saphenous vein.

Materials: Anesthetic (systemic and local), Disposable gloves, Hematocrit tubes, Collection vessel, Gauze

Procedures:



1. Anesthetize rat. After the rat is anesthetized, place a drop of the proparacaine or tetracaine (local anesthetic) in the eye from which the sample is to be collected. The local anesthetic takes effect in about 30 seconds and lasts for about 5-10 minutes.
2. Place hematocrit tube at the medial canthus of the eye and insert behind the eye.
3. Rotate tube on its axis on back of orbit until you feel a “pop” and blood flows. Please note that this is a finesse procedure and does not require force.
4. Instill sterile eye ointment when



finished.

5. Upon completion, insure good hemostasis by holding eyelids closed.

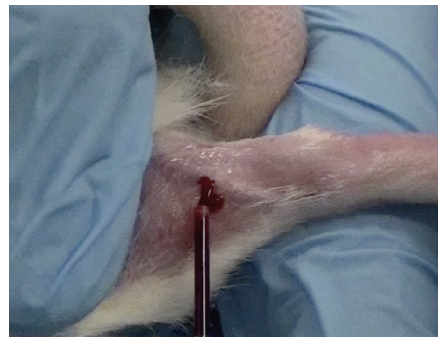
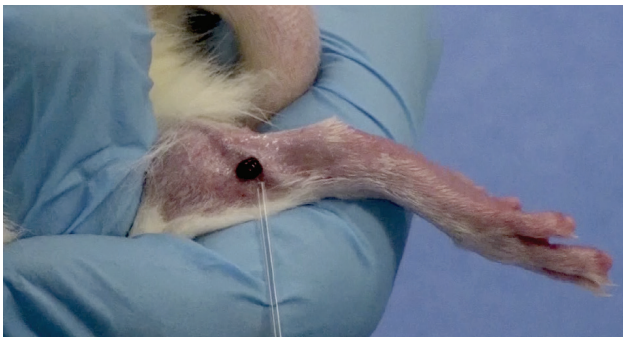
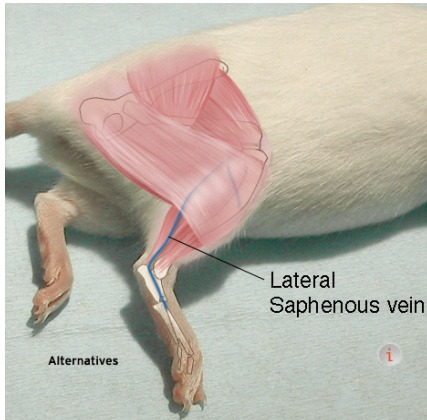
C. Blood Withdrawal Utilizing the Lateral Saphenous Vein

Materials: +/- Anesthetic, Disposable gloves, Hypodermic needle (20-22 gauge), Gauze, Electric clippers, #40 blade, Hematocrit tube or Microvette, petrolatum-based lubricant (can be applied to the puncture site to make blood bead for easier collection)

Procedures:

1. Restrain or anesthetize rat.
2. Clip hair from lateral aspect of lower leg (between the ankle and knee joints). **When clipping the leg, be sure to use small clippers like you will use in the lab. Large clippers can easily induce trauma by cutting the leg.**

3. Prep with alcohol swab.
4. Wipe dry with clean gauze.
5. Apply a thin film of petrolatum-based lubricant (such as KY Jelly) over puncture site.
6. Constrict lateral saphenous vein above knee joint between the thumb & index finger.
7. Puncture the vein with a needle. Collect the blood via a hematocrit tube or Microvette.
8. Upon completion, insure good hemostasis by applying gentle pressure to the collection site until blood flow stops.

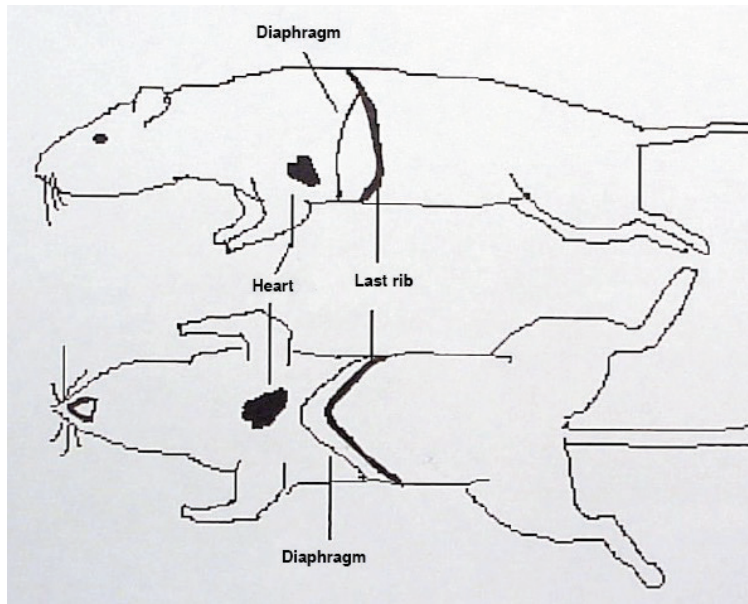


D. Intracardiac (IC) Puncture (Terminal Collection)

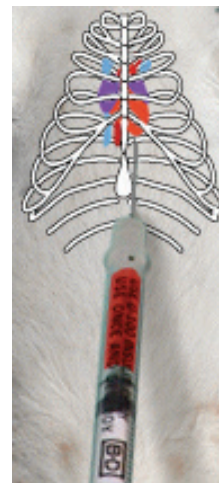
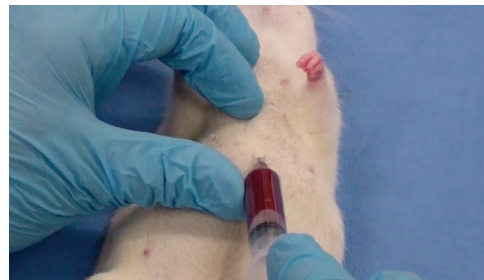
Materials: Anesthetic, Disposable gloves, Syringe (3 - 5 ml), Hypodermic needle (21-23 ga), Isopropyl alcohol, Gauze

Procedures:

1. Intracardiac puncture **MUST** be done **UNDER ANESTHESIA**.
2. Prep area with alcohol swab.
3. Insert needle at base of sternum on a 20 degree angle under or just left to the xyphoid process.
4. Aspirate syringe slowly.



5. Alternatively, the procedure may be performed with the rat lying on its right side. In this case the needle is inserted just behind the forelimb where the heartbeat is easily felt. The location of the heart is approximately under the elbow when leg is placed on a normal anatomic position.
6. **This procedure must be followed by euthanasia as it is only permissible as a terminal procedure unless it is scientifically justified and approved in the IACUC protocol.**



E. Axillary Cutdown (Terminal Collection)

Materials: Anesthetic, Disposable gloves, Syringe (1-3ml), Isopropyl alcohol, Gauze, Scissors

Procedures:

1. Anesthetize rat.
2. With the rat in dorsal recumbency (lying on its back), prep axillary (armpit) area with alcohol swab.
3. Cut axillary region with scissors or a scalpel blade to expose the subclavian artery and vein which are deep in the armpit.
4. Cut the subclavian artery and vein with the scissors or a scalpel blade.
5. Collect the blood sample with the syringe (no needle) as the blood pools in the axillary region. **This is a terminal procedure.**

TOURNIQUET PREPARATION FOR BLOOD COLLECTION

The following figure displays a practical method of creating a tourniquet for blood collection from the lateral saphenous, dorsal metatarsal, tail vein, or to prepare the tail for injection into the tail vein.

A rubber band is placed and secured with a hemostat proximal to the site of blood collection or injection.



ANESTHESIA AND ANALGESIA

METHODS OF ANESTHETIC DELIVERY/EQUIPMENT (OVERVIEW)

There are generally two methods of anesthetic delivery to rodents, parenteral and inhalation.

- A. Parenteral anesthesia involves the injectable routes of administration.
- B. Inhalation anesthesia involves the delivery of volatile anesthetic agents to the patient via the respiratory tract.

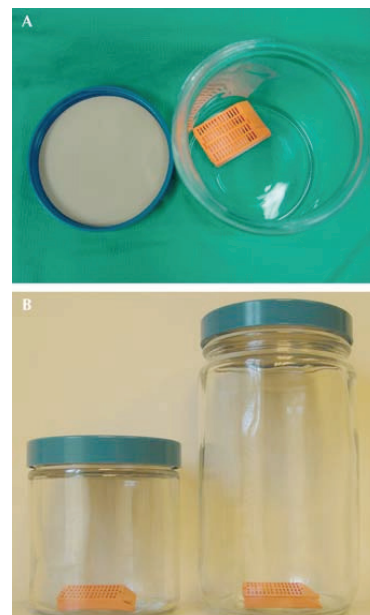
METHODS OF DELIVERY OF INHALANT AGENTS TO RODENTS

The best method for the delivery of volatile agents to rodents involves the use of a precision vaporizer and an anesthesia chamber alone or in combination with a face mask appropriately sized for rodents. **LARC has the equipment to safely and effectively administer inhalant anesthetics (isoflurane) to rodents using a precision vaporizer. Please contact LARC for details regarding use of this equipment.** The rodent is placed within the chamber for induction. Once anesthetized, the animal is removed from the chamber with anesthesia maintained by delivery through a face mask. Both chamber and mask delivery incorporate the use of a precision vaporizer for precise control of the concentration of anesthetic gas delivered to the patient. Because oxygen flow is required to volatilize the liquid anesthetic placed within the vaporizer, oxygen is also delivered to the patient and helps to maintain the blood oxygen saturation. Because fairly high fresh gas flows are required for either chamber or mask delivery, adequate scavenging of waste anesthetic gases is necessary to avoid exposure to personnel. In general, isoflurane anesthesia is superior to injectable anesthesia. Animals are more quickly induced and recovered, and close to 100% of the gas is eliminated through the lungs without being metabolized, (<1% of isoflurane is metabolized). This allows for greater control of the anesthetic depth and tends to minimize experimental variables.

Precision vaporizers must be recertified at the manufacturer's recommended interval. In the absence of a manufacturer's recommendation, certification must be performed on an annual basis.

If gas is delivered without a precision vaporizer (called the "Open Drop" method), the following guidelines should be considered:

For induction, a concentration of 2-5% concentration of isoflurane gas is normally adequate. To use anesthetic gas accurately, the induction chamber volume must be known. After determining the chamber volume (it is recommended to record this permanently somewhere easily retrievable), add 0.1-0.2 ml of gas (in liquid form from the bottle) for each liter



of chamber capacity. This can be done by applying the gas in liquid phase from its bottle to a cotton ball below the false floor of the container. For small containers, a piece of cotton can be enclosed in a histology tissue cassette and the agent may be poured or applied onto the cotton in the cassette. Use of 0.2 ml liquid agent per 1000 ml chamber volume will give about a 4% concentration of gas. In the experience of the veterinary staff at Emory University, using nine naïve ICR mice (5 males & 4 females; 2 months of age) introduced to the chamber sequentially after the introduction of isoflurane (0.2 ml/L chamber volume), recumbency was obtained in 57 +/- 21 seconds. However, for rapid and effective induction, the agent had to be replenished in the chamber approximately every 3 mice. Gas delivered by this method must be done under a chemical fume hood or type IIB Biosafety cabinets that are vented to the outside.

Volume of liquid agent/ 1000 ml chamber volume	Approximate concentration of isoflurane or halothane
0.05 ml	1%
0.1 ml	2%
0.2 ml	4%
0.3 ml	6%

ANESTHETIC MONITORING OF RODENTS

Parameters that can be used to assess the depth of anesthesia in rodents include:

- ☐ recumbency and loss of purposeful movements
- ☐ muscle relaxation
- ☐ lack of vocalization
- ☐ loss of response to aversive stimulation (e.g. pinching the toes)

In most instances, cardiovascular and respiratory assessments are limited to observations of chest wall movement to determine respiratory rate and palpation of the apical pulse through the chest wall.

Because the ratio of body surface area to body mass is greater in rodents than in larger species, thermal support is critical to the successful recovery of rodents from anesthesia. Body heat may be dissipated from the tail, soles of the feet and ears with a resultant profound decline in the core and surface body temperature. This hypothermia may, in turn, lead to a decline in both anesthetic metabolism and any urinary excretion of the anesthetic agent.

SUPPORTIVE CARE OF ANESTHETIZED RODENTS

Methods to minimize heat loss to the environment during anesthesia of rodents include increasing the ambient temperature of the operating room; placement of a thermal blanket (e.g.

recirculating warm water blanket) or drape between the animal and the stainless steel operating table; use of heat lamps (carefully placed!); minimization of organ exposure from body cavities during surgery; recovery of the animal on a warming blanket or within a temperature-supported cage; administration of warmed subcutaneous or intraperitoneal fluids before, during or after the anesthetic episode; housing on bedding during recovery to provide thermal insulation; and recovery with cage mates to permit animals to huddle together and thus provide thermoregulation. Do not place an unconscious rat in a cage with an awake rat as the alert rat will tend to mutilate the anesthetized rat.

Rodents have high energy requirements due to their small size and high metabolic rate, yet they have minimal fat reservoirs which can be mobilized to supply needed energy (more so in mice). Nutritional support is critical upon recovery to avoid hypoglycemia. Nutritional support can be provided by simply providing a high-quality pelleted rodent diet as soon as the animal has recovered sufficiently to ambulate and eat (remember - rodents do not vomit so pre-anesthetic fasting is not typically performed).

Fluid deficits can be corrected by subcutaneous or intraperitoneal injection of warmed saline, Lactated Ringers solution or replacement fluids (e.g., Normosol®). Recommended fluid replacement for rats is 25 ml/kg (~5 ml for a 200 g rat) SC or IP.

Because rodents are frequently anesthetized with injectable agents that inhibit blinking (e.g., ketamine), ocular lubrication is important to protect against corneal ulceration.

Rat Anesthetics, Anesthetic Cocktails and Sedatives

Drug	Dose	Route	Indications & Comments
Ketamine	80-90	mg/kg IP, IM	Surgical anesthesia
Xylazine	8-10		
Ketamine	75	mg/kg IP	Moderate surgical anesthesia. Not for major surgery
Medetomidine	0.5		
Ketamine	31.25	mg/kg SC	Simple to moderate surgical procedures, e.g. laparotomy, certain sterotaxic procedures
Xylazine	6.25		
Acetylpromazine	1.25		
Ketamine	37.5	mg/kg SC	Extensive surgical procedures
Xylazine	7.5		
Acetylpromazine	1.5		
Pentobarbital	40-50	mg/kg IP	Surgical anesthesia. For perfusion use 80-120 mg/kg
Yohimbine	2.10	mg/kg IP	Xylazine antagonist for reversal of ketamine/xylazine anesthesia

Atipamezole	1.00	mg/kg	IP, SC	Medetomidine antagonist for reversal of ketamine/medetomidine anesthesia
Acetylpromazine	1-2	mg/kg	IM	Sedation
Diazepam	2-4	mg/kg	IM, IP	Sedation
Xylazine	1-3	mg/kg	IM	Sedation

IM = intramuscular

IP = intraperitoneal

SQ or SC = subcutaneous

IV = intravenous

PO = orally

	<u>Induction</u>	<u>Maintenance</u>
Isoflurane (Aerrane®, Isoflo®)	Up to 5%	1 -3%

When using isoflurane in a calibrated vaporizer, an oxygen flow rate of 1 liter/minute should be used in conjunction with a non-rebreathing system.

Analgesia Considerations in Rats

For *Guidelines for Evaluating and Treating Postoperative Pain in Mice and Rats* (GDLARC001) please visit the LARC website (<http://vpr.utsa.edu/larc.php>).

Commonly Used Systemic Analgesics in Rats

		Class
Buprenorphine*	0.01-0.05 mg/kg SC, IV q 8-12 h	Opioid
Buprenorphine	0.1-0.25 mg/kg PO	Opioid
Butorphanol	1-2 mg/kg SC q 2-4 h	Opioid
Morphine	2-5 mg/kg SC q 4 h	Opioid
Ketoprofen	5 mg/kg SC q 24 h	NSAID**
Carprofen	5 mg/kg SC q 24 h	NSAID
Flunixin	1.1-2.5 mg/kg SC, IM q 12 h	NSAID
Meloxicam	1-2 mg/kg SC, PO q 12 h	NSAID

* Buprenorphine is the only opioid with long duration effect in rodents.

** NSAID: Non-steroidal anti-inflammatory drug.

NSAID dosing caution:

1. Ensure that animals are adequately hydrated (skin pinch test, or serum Total Protein test) before administering an NSAID to avoid renal damage.

2. NSAIDs must be used with caution beyond 3 days as it may have deleterious effect on the gastrointestinal mucosa. This may be especially true when using ketoprofen and flunixin.

Opioid dosing caution:

1. Opioid agents enhance sedative and respiratory depressive effects of anesthetics.
2. For rodents anesthetized without respiratory support (intubation, ventilation and oxygen supplementation), you may wish consider opioid administration until the end of surgery.
3. In this case, an NSAID may be the preemptive analgesic of choice
4. If ventilatory support can be provided and an opioid is used as a preemptive analgesic agent, expect to reduce the dose of anesthetic agent (e.g. pentobarbital, isoflurane) by 30-50%.

Oral dosing caution:

1. Animals should be acclimated to oral medications before the surgery. When added to the drinking water, rodents will initially refuse to drink until they become adjusted to the flavor, which could be disastrous postoperatively.
2. When used in drinking water, analgesics should generally be administered 5-7 days prior to the anticipated pain insult.
3. Consideration to the use of analgesics in drinking water must take into account that postoperatively, animals may decrease fluid intake and may therefore not receive the intended analgesic dose.

Local anesthesia/analgesia

Preoperative (preferred) or intraoperative infiltration of the incision site and underlying tissues with 1-2% lidocaine/0.25-0.5% bupivacaine (50/50 mix by volume) is an effective local anesthesia cocktail to block surgical pain from the offset and for up to 6 hours. Lidocaine provides immediate pain control, while bupivacaine provides longer pain control.

Local Anesthetic	Onset	Duration	Do not exceed (toxic dose)
Lidocaine (xylocaine)	1-3 minutes	20-40 minutes	10 mg/kg
Bupivacaine	~20 minutes	4-6 hours	6 mg/kg

Adjuvants: Adding epinephrine (1:50:000 to 1:200,000) to plain solutions of local anesthetics just before administration shortens the onset time and prolongs the duration of action. A 1:200,000 dilution is obtained by adding 0.1 ml of 1:1000 epinephrine (with a tuberculin syringe) to 20 ml of local anesthetic. **Epinephrine should not be used for peripheral nerve blocks in areas with poor collateral circulation e.g., digits, tails. Use caution if patient has cardiac problems.**

EUTHANASIA

Proper euthanasia technique includes a follow-up exam to confirm death or the absence of a heartbeat. Monitoring respiration is not considered sufficient since with some euthanasia techniques heartbeat may be maintained after visible respiration has ceased.

The need to minimize fear and apprehension must be considered in determining the method of euthanasia. Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, whenever possible, animals should not be exposed to euthanasia of others.

The euthanasia methods listed in table below are consistent with the American Veterinary Medical Association (AVMA) Panel on Euthanasia, 2007.

Method of Euthanasia	Comments
Carbon dioxide*	Acceptable method
Pentobarbital overdose (≥ 130 mg/kg IV or IP)	Acceptable method
Inhalant Anesthetic overdose	Acceptable method
Exsanguinations in anesthetized animal	Acceptable method
Decapitation in anesthetized animal	Acceptable method
Cervical dislocation in <u>anesthetized</u> animal	Acceptable method
Decapitation in <u>awake</u> animal	Acceptable only with scientific justification in writing on the IACUC protocol and subsequent IACUC approval
Cervical dislocation in <u>awake</u> animal	Acceptable only with scientific justification in writing on the IACUC protocol and subsequent IACUC approval

*Carbon dioxide (CO₂), when used properly, is classified by the 2007 Report of the American Veterinary Medical Association Panel on Euthanasia as a safe method of euthanasia for many small laboratory animals. CO₂ has many advantages including: (1) rapid depressant, analgesic, and anesthetic effects; (2) easy availability in compressed gas cylinders; and (3) inexpensive, nonflammable, nonexplosive, and poses minimal hazard to personnel when used with properly designed equipment.

Although CO₂ is generally considered an acceptable form of euthanasia for small laboratory animals when properly administered, its acceptability is predicated on the following:

The euthanasia chamber should not be prefilled with CO₂, since high concentrations (>70%) can cause nasal irritation, discomfort, and excitability. Rather, the animals should first be placed into the chamber, followed by the addition of CO₂ at a low flow rate (e.g. a rate sufficient to displace approximately 20% of the chamber volume per minute) to complete the process. In general the low flow rate can be assessed if a very low hissing sound is heard as the chamber is filled with CO₂. Rapid gas flows should be avoided since excessive noises (“winds”) can develop and induce excitement and distress in the animals. In addition, exposure to high CO₂ concentrations

is painful as the CO₂ mixes with moisture in the nasal passages to form carbonic acid. Gas flow should be maintained for at least 2 minutes after apparent clinical death (e.g. at least one minute after the animal has quit breathing). Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times, and followed by a physical method such as cervical dislocation (in <200-gram rats), bilateral thoracotomy, exsanguination or decapitation.

According to the 2007 Report of the AVMA Panel on Euthanasia, ‘Compressed CO₂ gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. CO₂ generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g. antacids) is unacceptable.’ Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

Because of the heavy muscle and bone mass in the neck region, cervical dislocation should not be done in > 200 gram rats.

Neonates: Since the time period for euthanasia is substantially prolonged in neonatal animals due to their inherent resistance to hypoxia, CO₂ narcosis is generally not recommended. Other methods (see table 2) should be used. The IACUC policy on euthanasia (<http://vpr.utsa.edu/oric/iacuc/programpolicies.php>) provides further instruction on the euthanasia of the rodent fetus and newborn.