



UNIVERSITY OF WASHINGTON
ANIMAL USE TRAINING PROGRAM

Animal Use Training Session Rat Lab Handout*



*This document is updated on an annual basis. In the interest of your research please contact the AUTS program to ensure you have a current handout.

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ANIMAL USE TRAINING SESSION: RAT

1. Introduction:

This handout is designed as a supplement to the information provided in the Rat Animal Use Training Session. Remember that in order to perform any of the techniques described in this handout all personnel and the experimental procedures being done must be on an approved Institutional Animal Care and Use Committee (IACUC) protocol. Where noted, some of the techniques in this handout require certification by a qualified individual designated by the Attending Veterinarian (AV). Please be aware, depending on where your rats are housed and what you will be doing to them, there may be other classes required of you (e.g., the Laws and Regulations Course, SPF Course, or any applicable Facility Orientations, etc).

1.1 Course Objectives:

- Performance of gender determinations.
- Proper handling and restraint of rats for routine procedures (e.g., physical examination, administration of medication, and venipuncture).
- Observation of rats for recognition of normal and abnormal physical and behavioral changes (e.g., anorexia, poor grooming, diarrhea, porphyrin staining, etc.) and how to report abnormal findings to appropriate veterinary service personnel.
- Demonstration of identification methods.
- Demonstration of methods of venipuncture and exsanguination (to include techniques, sites, restraint, volumes, and frequency). Individuals wishing to be certified to perform retro-orbital bleed technique must demonstrate competency at performing this method of blood collection.
- Demonstration of the sites and techniques for administration of medication by the following routes:
 - Oral
 - Subcutaneous
 - Intraperitoneal
 - Intravenous
- Discussion of appropriate methods of anesthesia (to include agent, route, and monitoring).
- Discussion of aseptic and sterile technique requirements for survival surgical procedures.
- Discussion of appropriate methods of euthanasia.

1.2 Why Rats are Used in Research:

- Mammal of small, uniform size
- Relatively short life span (2.5 years)
- Proficient reproductive capabilities
- Ease of maintenance
- Resistant to many bacterial and viral diseases
- Few inherited diseases
- Few neurological disorders
- Very trainable
- Baseline data available
- Brain mapped for stereotaxic procedures
- Several disease models available

1.3 Strains of Rats and Genetics:

- Random or outbred stock – Animals derived from matings of unrelated individuals; genetically heterogeneous.
 - Wistar
 - Sprague-Dawley
 - Long-Evans
- Inbred strains – A strain is regarded as inbred when it has been mated brother x sister for 20 or more consecutive generations.
 - Fischer 344
 - Brown Norway
 - Brattleboro Diabetes Insipidus
 - Gunn Jaundice (Hereditary Hyperbilirubinemia)
 - Nude T-cell deficiency
 - Obese SHR Hyperproteinemia and hypertension
 - Zucker Autosomal recessive obesity
 - GEPR CNS seizure-prone
- Rat Strain Database online: <http://rgd.mcw.edu/>

1.4 Physiological Data:

Body temperature.....	35.9-37.5°C (96.6-99.5°F)
Heart beats/min.....	250-600
Breathing rate/min.....	66-114
Weight, adult male.....	300-400 grams
Weight, adult female.....	250-300 grams
Birth weight.....	5-6 grams
Sexual maturity.....	40-65 days
Estrous cycle frequency.....	4-5 days
Duration of estrus.....	13-15 hours
Time of ovulation.....	2-3 hours after estrus onset (spontaneous)
Gestation period.....	20-22 days
Litter size.....	8-14 pups
Eyes open.....	10-12 days
Weaning age.....	21 days
Breeding life.....	8 months
Mating.....	pair (monogamous) or harem (polygamous); one male, 2 females
Life span.....	2.5-3 years
Water consumption.....	24-60 ml/day
Food consumption.....	15-30 g/day (Rats require hard foods to wear down teeth)

1.5 Rat Anatomy:

http://www.biologycorner.com/worksheets/rat_intro.html

1.6 Organ System Norms:

- Cardiovascular
 - Arterial blood pressure
 - Mean systolic 116 mm Hg
 - Mean diastolic..... 90 mm Hg
 - Heart rate 250-600 beats/min
 - Cardiac output..... 50 ml/min
 - Blood volume 5.43 ml/100 gm body wt
- Respiratory
 - Respiratory rate..... 66-114/min
 - Tidal volume 1.5 ml
 - Alveolar surface area (400 gm rat)..... 7.5 m²
- Renal
 - Urine volume/24 hr 5.5 ml/100 body wt
 - Na⁺ excretion/24 hr 1.63 mEq/100 gm body wt
 - K⁺ excretion/24 hr 0.83 mEq/100 gm body wt
 - Urine osmolarity 1658 mOsm/kg H₂O
 - Urine pH..... 7.3-8.5
 - Urine specific gravity 1.04-1.07

1.7 Intercurrent Disease:

- Important to purchase good quality rats from reputable vendors as intercurrent disease or latent viruses could have an effect on research. A list of approved animal vendors can be found on the Animal Purchasing website: <https://depts.washington.edu/compmed/animal>.
- Vendors will supply health surveillance information.
- Infectious agents that cause clinical illness or deaths are detrimental to research programs (e.g., data retrieved from a sick rat is not likely to be valid).
- Sub-clinical, persistent infections can have profound effects on biological responses.
- Many rodent viruses have been isolated from transplantable neoplasms and may modify tumor growth kinetics. The influence of viral infections on the biology of chemical carcinogenesis is not well understood, but is potentially substantial. Contact Rodent Health Monitoring (rhmp@uw.edu) for information on having tissues and cell lines tested.

1.8 Viral and Bacterial Infections in Rats:

- The majority of rodent viral infections are sub-clinical in nature, but can have profound effects on research results.
- Acute - Characterized by short incubation, short course of active infection that may be clinically apparent or asymptomatic, followed by a brief period of virus excretion and death or recovery of the host.
- Persistent - Characterized by a long course, can be chronic or latent. May be clinical or asymptomatic, but virus is commonly excreted or detectable in host tissues through the course of infection.

Note: The pathogenicity of many infections, such as Sialodacryoadenitis Virus (SDAV) can also be enhanced by immunosuppression, stress, or concomitant infection by another agent.

1.9 Movement of Infectious Agents:

- Infectious agents (pathogens) can travel from room to room via personnel and equipment. Following Specific Pathogen Free (SPF) procedures can dramatically limit pathogen transfer.
 - SPF garments (booties, gloves, bonnets, gowns) should be worn only in the room in which they are supplied. Do not exit the animal room wearing these items.
 - Coordinate any movement of animals with the Facility Supervisor and the Rodent Health Monitoring Program (RHMP).

1.10 Quality Assurance Program:

- Faculty member responsible at UW: Dr. Susan Dowling (206-221-3933 or sdowling@uw.edu).
- All rats received at UW must be ordered through Department of Comparative Medicine Animal Purchasing, which ensures some control of the pathogens coming into the facility. Quality assurance data are obtained from each vendor and rats are periodically screened for rodent viruses.
- In-house colonies are screened quarterly for common rodent pathogens and parasites.

1.11 Purchasing Rats:

- Animal Purchasing (email: animals@uw.edu, phone: 206-543-0640, web: <https://depts.washington.edu/compmed/animal>) can supply information on rat vendors. Major vendors have web addresses and can supply information about their stock and health surveillance of their colonies. Harlan, Taconic, and Charles River generally supply specific pathogen free (SPF) rats.
 - Harlan - <http://www.harlan.com>
 - Charles River - <http://www.criver.com>
 - Taconic - <http://www.taconic.com>
 - DCM Shared Rodent Service - <https://catalysttools.washington.edu/qopost/board/jenick0z/8509/>
- Bringing Rats into a University Facility - All rats must be ordered through Animal Purchasing and cleared with Rodent Health Monitoring. Rats coming from any non-approved source (e.g., vendor, institution, etc.) must go through a monitored quarantine.
- Rodent classification:
 - Germfree (or gnotobiotic) - Cesarean-derived (currently none at UW).
 - Specific Pathogen-Free (SPF) - Free of specific pathogens known to cause disease in some strains of rats.
 - All Centralized Comparative Medicine rodent facilities are SPF housing (K-wing, T-wing, Foege, Brotman, HR&T).
 - Conventional - Non-SPF housing harbor certain rat pathogens but may not cause overt disease.
 - There are no conventional housing facilities available in the Health Sciences Complex, however, there are some Decentralized facilities that can support conventional animals.

2. Laboratory Outline:

2.1 Health Monitoring:

- It is important to be familiar with the characteristics of a healthy rat and normal rodent behavior in order to recognize an unhealthy animal. Normal rats should be active, have a smooth and shiny haircoat, bright clear eyes, and good body mass.
- Signs of disease or discomfort: huddles in corner, hunched posture, scruffy haircoat (due to dehydration or lack of grooming), feels cool to the touch, avoids cagemates, diarrhea (i.e., sticky feces), pale appearance to eyes, nasal/ocular discharge, porphyrin staining (red or brown staining around eyes or nose), coughing, sneezing, chattering, vocalizations, decreased appetite, weight loss, respiration

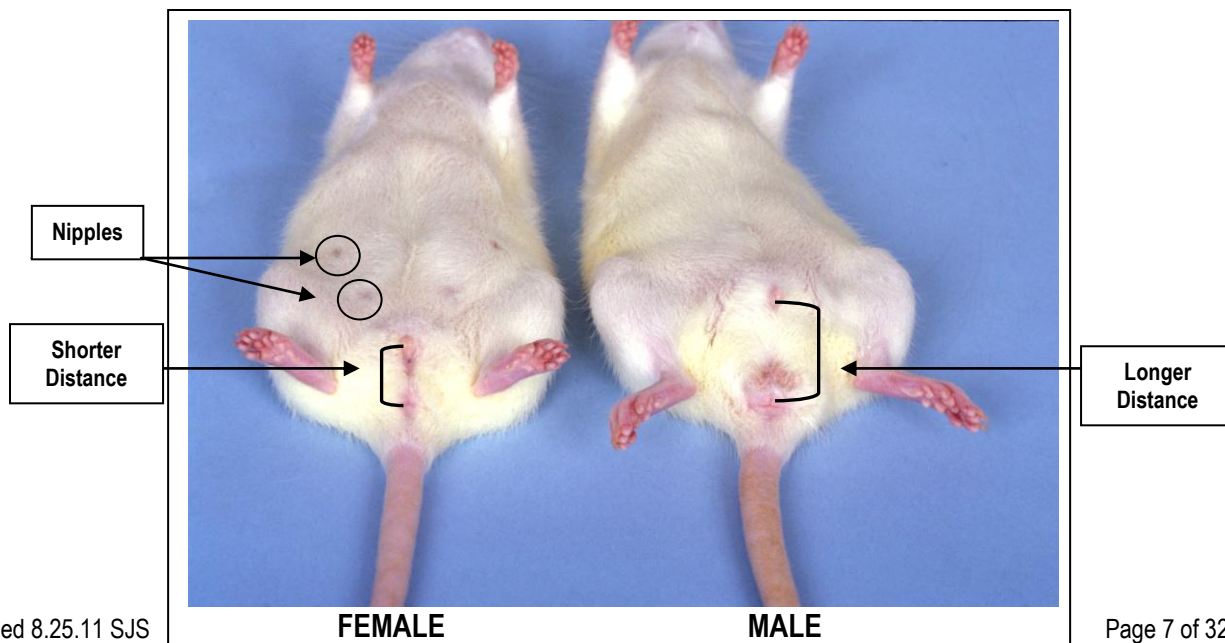
increased or labored.

- If severe signs are seen or when more than one of these signs is noted, waiting for possible improvement without immediate medical intervention or euthanasia often results in needless suffering and spontaneous death.
- Submit a Sick Animal Report to Veterinary Services: A laboratory animal veterinarian or veterinary technician will assist you in determining if noted illness is experimentally induced or spontaneous disease. IF RATS ARE DYING IN YOUR COLONY, IT COULD BE AN INFECTIOUS AGENT. CONTACT VETERINARY SERVICES.
- Use caution when handling sick rats as the stress can be sufficient to cause them to expire.



2.2 Gender Determination:

- Compare anogenital distance of males versus females (distance between anus and genital papilla is ~ 2 times longer in males). Testicles are visible in relaxed mature males, but can be retracted into body cavity. Only females have nipples.
- Perform when weaned (approximately 3 weeks of age). Accuracy may be difficult if younger than 3 weeks (determine through comparison).
- Rats are sexually mature at 40-65 days. Improper gender determination can result in unwanted pregnancies.



2.3 Restraint and Handling:

Rats are intelligent and respond well to gentle handling. When handling, include "petting" sessions so your rats associate you with pleasant activities. Rats can bite, but rarely do. Use of bite protection gloves is not usually needed. Note that rats can bite through thin leather gloves. Gloves heavy enough to prevent bites greatly reduce dexterity, increasing the chance of inflicting pain and interfering with grip on the animal. Chain mail gloves catch and tear rat toenails, and their use is discouraged.

NOTE: If bitten, it is inappropriate to throw or drop the rat. Be ready to overcome this response by reviewing/rehearsing the proper steps to take when a rat bites. When bitten, place hand on cage top - rat will release bite; return rat safely to cage. Wash bite wound with soap and water. Tetanus immunization should be up to date (within the past 5 years). The Employee Health Clinic is located at Hall Health Center (206-685-1026), please contact them if the wound is severe. Report all accidents/incidents to Environmental Health and Safety using the Online Accident Reporting System (OARS) at: <http://www.ehs.washington.edu/ohsoars/index.shtm>

- **Tail pick-up:**

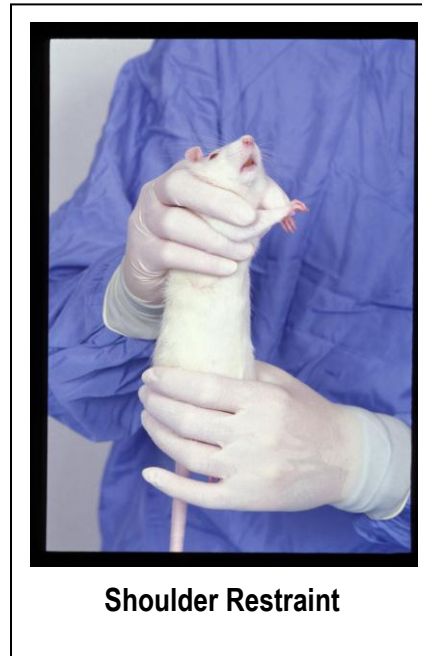
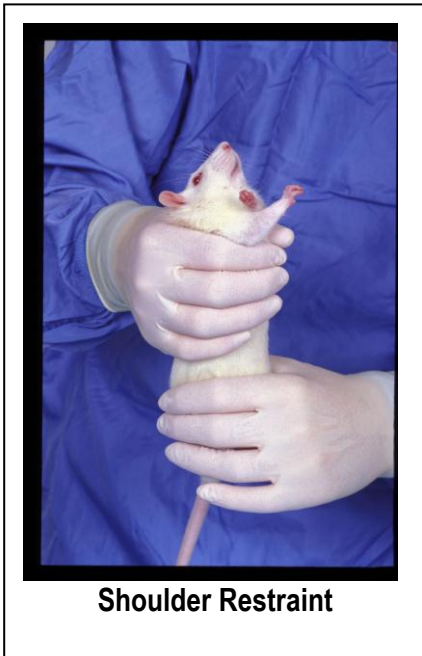
1. Grasp at base of tail close to the body with your hand, lift and support body (do not dangle).
2. Place animal on solid surface (e.g., arm or counter-top) while still gripping tail, then proceed with shoulder restraint.
3. A terry cloth towel provides a soft, secure hiding place for nervous rats, and makes restraint simple.
4. Stockinette tubes may calm some rats (see Vendor List).



Tail pick-up

- **Shoulder Restraint:**

1. Approach dorsally, encircle thorax just behind front legs with thumb and forefinger.
2. Extend forelimbs by applying gentle pressure behind elbows and then cross forepaws; restrain rear legs with opposite hand.
3. Allow comfortable breathing; observe for cyanosis (turning blue). If rat becomes agitated and vocalizes, you may be compromising its breathing by holding it too tightly.



- **Restraint tubes (Broome restraints):**

Designed for easy access to the tail. Rats will usually enter these tubes readily (see Vendor List).



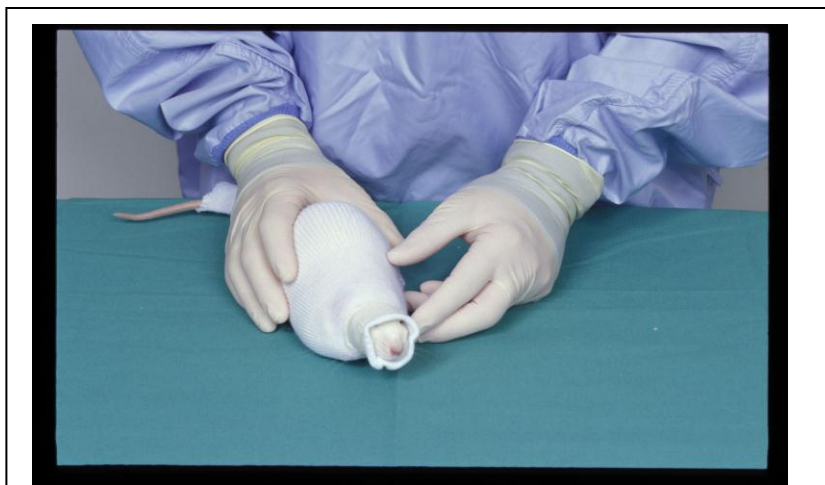
- **DecapiCone® Restraint:**

Designed for use with a guillotine, but also provides good access to tail and limbs. Caution, rats can overheat in these bags (see Vendor List).



DecapiCone® Restraint

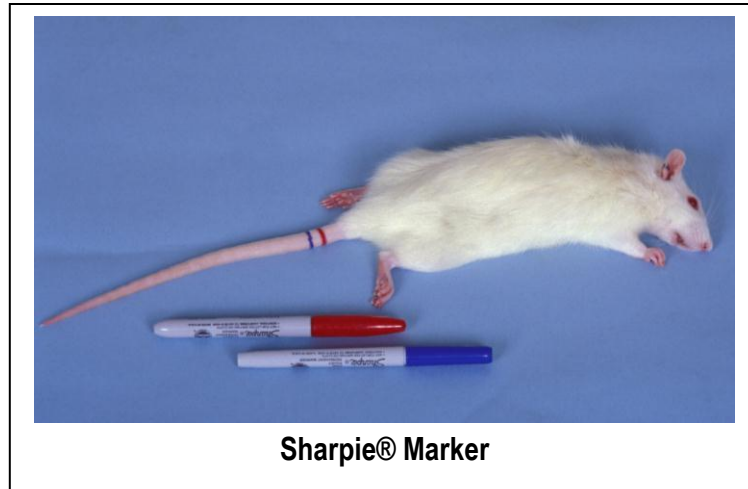
- Other restraint devices such as towels, cotton stockinet, and gloves may be used but extreme care must be exercised to ensure the comfort and safety of the animal (see Vendor List).



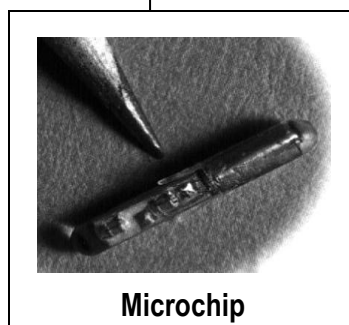
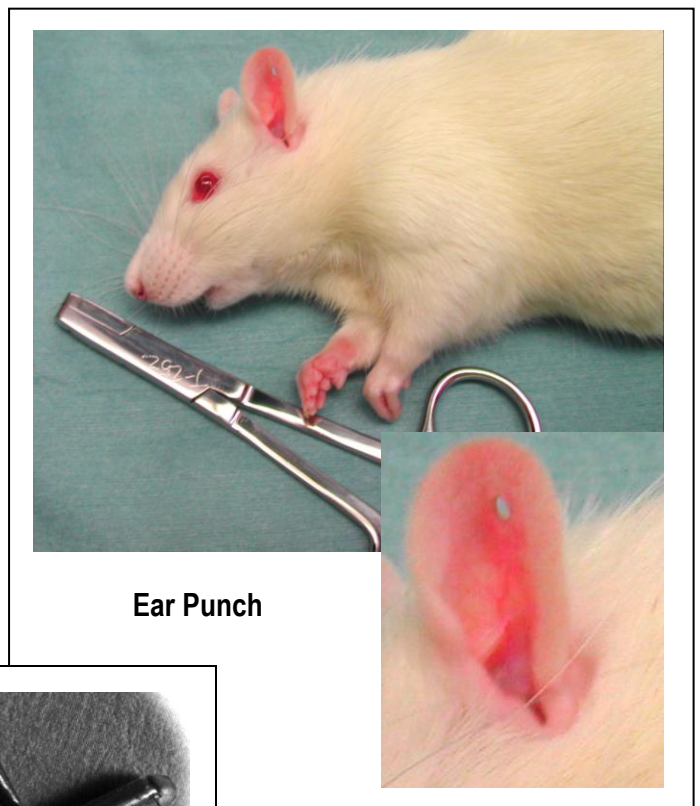
Stockinet Restraint

2.4 Methods of Identification:

- Temporary - Tail marking with “Sharpie”® marking pen (will be removed during normal grooming, usually within 24-48 hours).



- Permanent - Ear punch (see Appendix A), ear tag, implanted microchip, tattooing (see Vendor List).
NOTE: amputation of toes requires strong justification and approval from IACUC.



2.5 Injection Techniques:

A 25 gauge (g) or smaller needle is recommended. (Larger numbers indicate smaller gauge needles.)

2.5.1 Subcutaneous:

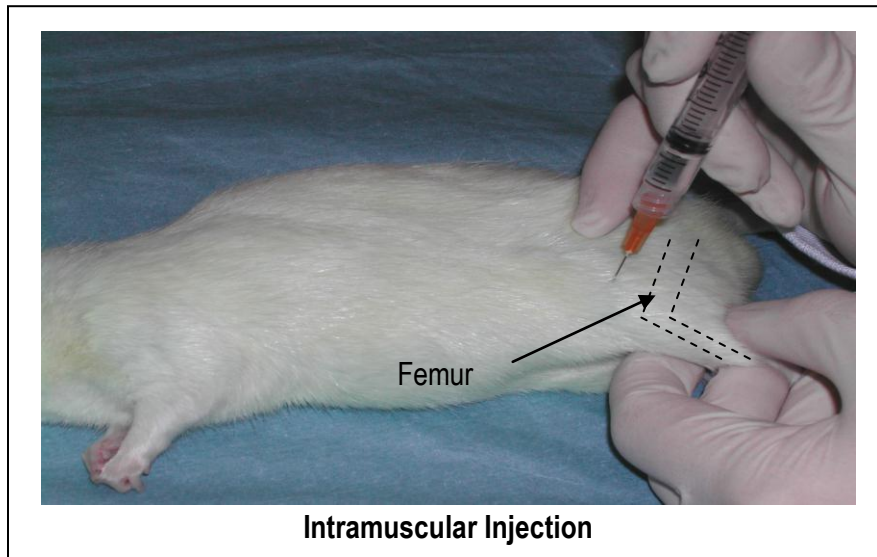
Site: Dorsum or inguinal region.
Volume: Recommended maximum 1-2 ml per site.
Restraint: Wrap in a towel or use DecapiCone® with hole for injection.



Subcutaneous Injection

2.5.2 Intramuscular:

- Site: Use quadriceps muscle located on the dorsal surface of femur. Avoid hamstring on ventral surface of femur as there is a risk of sciatic nerve injury.
- Volume: Recommended maximum 0.1-0.2 ml.
- Restraint: Anesthesia or sedation is preferred. Conscious animals may be restrained by an assistant (difficult to access and hold quadriceps still while also restraining rat). Skill and practice are needed for the technique.



2.5.3 Intraperitoneal:

- Site: Lower right or left quadrant of abdomen.
- Volume: Usually no more than 2 mls, depending on size of rat.
- Restraint: Manual restraint, wrap in towel, or secure upper half of body in a restraint tube.



2.5.4 Intravenous:

- Site: Lateral tail veins or metatarsal veins.
Volume: Recommended maximum 0.5 ml. Improve visibility of vessels (vasodilation) by placing tail in warm water.
Restraint: Sedation may be needed, or use a restraint tube or cone.



Intravenous Injection

2.5.5 Oral Gavage: Strongly recommend gavage only be performed on **awake** animals.

- Site: Placement of liquid directly in stomach via a tube through the mouth and esophagus (see Vendor List). In order to avoid inadvertently placing the liquid into the respiratory system, it is critical that the gavage needle be both the correct length (distance between the corner of the mouth and last rib, usually 20 g x 3" gavage needle is used) and be advanced gently without resistance.
Volume: Maximum 1-2% body weight (e.g., 2-4 ml per 200 gm rat).
Restraint: Hold rat upright against your lateral midsection, immobilize its rear legs with your wrist and forearm. An assistant may restrain the animal. Extend the neck of the rat to allow passage of the needle into the esophagus.



Oral Gavage

2.6 Blood Collection Techniques:

Site selection is dependent upon the volume required and whether collection is to be repeated or terminal (exsanguination). Serial/repeated sample collection: saphenous vein (hind leg) and tail vessels.

2.6.1 Lateral Saphenous Vein:

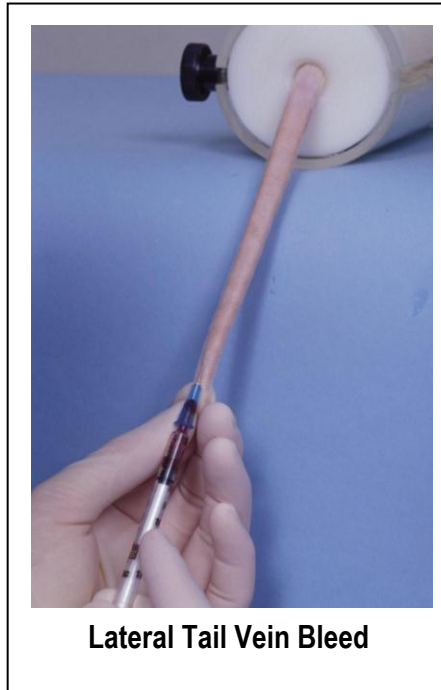
- Anesthetize rat or restrain in towel.
- Apply a lubricant ointment such petroleum jelly or ophthalmic ointment to lateral aspect of tarsus to slick the hair.
- Gently but firmly grasp skin of thigh to extend the leg and raise the vessel.
- Puncture vessel with a 25 g or 23 g needle.
- Collect blood in a small tube or a syringe.
- Apply light pressure to site for approximately 1-2 minutes to stop bleeding and decrease hematoma.
- Average volume yield from vein = 0.5-1 ml per site.



Lateral Saphenous Vein Bleed

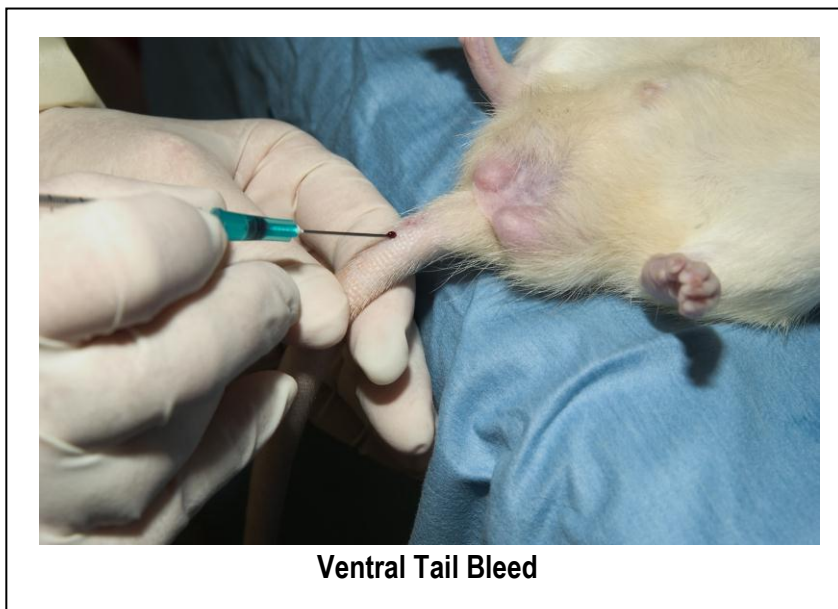
2.6.2 Lateral Tail Vein (see Appendix B):

- Start distally and work proximally.
- Use 25 g needle or butterfly with 1 ml TB syringe.
- Sedation is not always necessary, but is recommended.
- Apply light pressure to site for approximately 1-2 minutes to stop bleeding and decrease hematoma.
- Average volume yield from vein = 0.5-1 ml per site



2.6.3 Ventral Tail Artery (see Appendix B):

- Start at the proximal portion of the tail.
- Your approach angle should be 45°-90°.
- Use 25 g needle or butterfly with 1 ml TB syringe.
- Sedation is recommended.
- Apply light pressure to site for approximately 1-2 minutes to stop bleeding and decrease hematoma.
- Average volume yield from vein = 0.5-1 ml per site.



2.6.4 Tail Vessel Lancing and Tail Tip Amputation:

Lancing the tail vein or artery with a blade/needle or amputating the tip of the tail to collect blood is discouraged. If a group needs to obtain blood with this technique, scientific justification is required and will need IACUC approval.

2.6.5 Tail Prick:

Tools: 3-4mm lancet or 23g needle, gauze, collection tube/glucose strip.

Site: 2-3mm from tip of tail.

Volume: 2-3 drops.

Restraint: Holding the tail while animal explores the cage or wire top.

Key Concepts:

- Hold the tail while the animal is allowed to explore the cage or wire top. It helps if they can grip this wire top.
- Hold the tail below the prick site. About 10mm from the tip of the tail.
- Prick site is about 3-5mm from tip of tail.
- Approach the prick site with the lancet or needle perpendicular to the tail.
- Quickly and gently insert about 2-3mm of the needle/lancet into the tail and immediately pull out and a drop of blood will form.
- You can obtain slightly more blood by gently milking the tail.

2.6.6 Retro-Orbital Bleeds

- Anesthesia is required.
- Certification is required.
- Provides venous blood.
- Pipette location begins in medial canthus of eye and glides to dorsal aspect of orbit.
- After sample is obtained, risk of suborbital hematoma may be minimized by using gauze sponge to apply gentle pressure over closed eyelids for 1-2 minutes.
- Apply ophthalmic ointment after collection.
- Average volume yield = 0.5 ml.

NOTE: Certification to perform orbital bleeding technique on anesthetized rats is required by IACUC. Such certification may be obtained in a Rat AUTS class or through the training instructor or a qualified individual designated by the Attending Veterinarian. Contact the training coordinator to schedule this certification (206) 221-7709.



2.6.7 Terminal Sample Collection (Exsanguination):

- Anesthesia is required. Animal must be in a surgical plane of anesthesia.
- For cardiac puncture, palpate or auscult strongest beat on left thoracic wall near flexed elbow.
- Enter thorax between ribs at point described, using 20-25 g needle and 10-20 cc syringe.
- Needle must be advanced firmly and rapidly.
- Avoid probing or repeat attempts in thorax as severe hemorrhage may occur.
- Expected volume is 3% of body weight (e.g., 300 gram rat = 9 ml).



Cardiac Puncture

2.7 Blood Collection Volumes:

- 1-3-6% Rule for blood collection
 - 1% of body weight = maximum volume per collection every 2 weeks
 - 3% of body weight = amount expected at exsanguination
 - 6% of body weight = approximate total blood volume
- Example: 300 gram rat
 - 1% = 3.0 ml every 2 weeks
 - 3% = 9.0 ml at exsanguination
 - 6% = 18 ml total body volume

2.8 Anesthesia:

Be extremely careful when preparing anesthetics for rats as small errors can result in death of the animal. WEIGH rats before dosing. Before using anesthesia in rats, always check your IACUC protocol for approved agents.

2.8.1 Anesthetics for Rats:

- KETAMINE/XYLAZINE – Tranquilizer/dissociative agent. Useful drug combination due to ease of administration and relative safety. Will provide approximately 15-20 minutes of surgical level anesthesia. Ketamine/Xylazine can be used for any surgical procedure, as long as the animal is in a surgical plane of anesthesia. Apply sterile ophthalmic ointment to keep corneas from drying. Use front foot toe pinch to assess level of anesthesia. Do not use hind foot toe pinch because under Ketamine/Xylazine the hind foot reflex is not suppressed as it is under other anesthetics.

Note: Ketamine is a controlled substance and requires a DEA license.

Prepare: 5.0 ml Ketamine (100 mg/ml) \
1.6 ml Xylazine (20 mg/ml)
Beyond-use date is 28 days after mixing

Dosage: Draw-up 0.9 ml x body weight in kg
Then dilute to 2.0 ml with sterile saline
This results in a dose of 68.2mg/kg Ketamine and 4.4mg/kg Xylazine

Administer: Intraperitoneal (IP)

- PENTOBARBITAL (NEMBUTAL®) - Barbiturate. Provides surgical anesthesia. Rats of different ages and strains differ markedly in their responses to single injections of barbiturates. Apply sterile ophthalmic ointment to keep corneas from drying. Work with this barbiturate to establish best and safest dosage for rats being used. Dose may vary with age and strain and weight of the rat. Pentobarbital also has a narrow margin of safety and the difference between the surgical anesthesia dose and an overdose may be very small. Commercial solutions of Pentobarbital (Nembutal®) must be diluted (sterile saline) before they can be administered.

Note: Pentobarbital is a controlled substance and requires a DEA license.

Dosage: 30-50 mg/kg body weight
Administer: Intraperitoneal (IP)

- ISOFLURANE or SEVOFLURANE
 - Can only be used in a calibrated vaporizer.
 - Delivers lethal concentrations when used in an “open,” non-calibrated method (bell-jar or nose cone).
 - The rat should not be allowed to come in direct contact with the liquid form of the inhalant agents because they are drying and irritating to the mucous membranes.
 - Must be used in a fume hood, or the vaporizing system must be fitted with scavenging devices.
 - Induction and recovery is rapid.
 - Be sure to prevent hypothermia.
 - Apply sterile ophthalmic ointment to the eyes to prevent corneas from drying out.
 - These agents do not provide any post-operative analgesia, therefore, the use of analgesics pre-operatively is recommended for painful procedures.

2.8.2 Anesthesia Assessment:

- Pain reflex tests – Ear, toe, and tail pinch. Withdrawal of foot or flicking of ear/tail indicate insufficient plane of anesthesia for surgical procedure (except with Ketamine/Xylazine where hind foot toe pinch will still cause reflex regardless of anesthetic depth).
- Palpebral reflex - Slight movement or closing of eyelids when eyelashes or the medial canthus of the eye are touched indicates insufficient plane of anesthesia for surgical procedure.
- Respiratory/Cardiac rates - Increased rates associated with painful stimulus indicate insufficient plane of anesthesia for surgical procedures.

Note: In order to properly assess the anesthetic depth of a rat you should use a combination of all of the above reflexes.

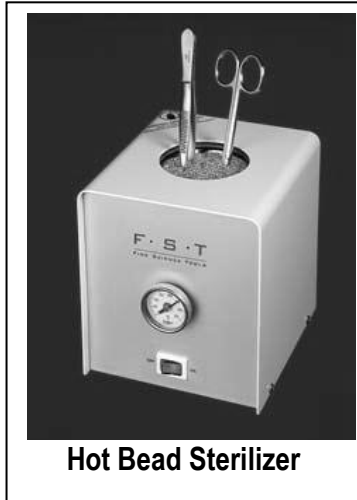
2.9 Pain Assessment and Post Operative Care:

Remember: Approach pain assessment with the view that if a procedure causes pain or discomfort in humans, then it will do the same in animals. None of the anesthetics recommended for use in rats provide significant analgesia. Therefore pre- and post-operative analgesia is recommended for all the above agents. Consult with Veterinary Services (206) 543-6257 for recommended analgesia doses and frequencies.

- Signs of pain/discomfort may include: Ruffled coat, hunched posture, pale eyes, porphyrin (red tear) staining, decreased activity. Pain is particularly difficult to assess in small rodents, and signs of pain or discomfort are often difficult to distinguish from disease in rodents. Many times signs are evidenced during the night-time (dark cycle) when we do not observe the animals. Veterinary medical staff should be consulted to determine appropriate intervention methods (e.g., analgesia, medical treatment, or euthanasia).
- Postoperative Care: Postoperative warming and monitoring is essential (warming light a safe distance from cage or circulating water heating pad). Take care not to overheat the rat. Personnel must monitor all animals until they return to consciousness and begin crawling around. Place anesthetized rat on paper towel or a similar substrate (not on bedding such as corncob or shavings). Recover rats individually (not in a cage with awake rats). Allow animals an area to move away from the heat source.
- A video demonstrating signs of pain in rats is available on CD (Pain Assessment in the Rat) and can be checked out from the training office (T-252).

2.10 Surgical Requirements:

- Non-Survival Surgery (animals not allowed to recover from anesthesia) may be performed in a suitable laboratory and aseptic (sterile) technique is not required. Per the Guide for the Care and Use of Laboratory Animals, “at a minimum, the surgical site should be clipped, the surgeon should wear sterile gloves, and the instruments and surrounding area should be clean.”
- Survival Surgery on rodents may be performed in a laboratory that is easily sanitized and not used for any other purposes during the time of surgery. To prevent infections, STERILE INSTRUMENTS AND SURGICAL GLOVES MUST BE USED. ASEPTIC PROCEDURES MUST BE FOLLOWED, INCLUDING THE PREPARATION OF THE INCISION SITE (i.e., shaving and scrubbing with an appropriate disinfectant such as Betadine®).
- A new set of sterile surgical instruments must be used if survival surgeries are done on multiple animals. Initial sterilization of instruments must be by an approved method such as autoclaving or gas sterilization. Hot bead sterilizers can be used to re-sterilize instrument tips between animals.



- Surgery must be performed by trained, experienced personnel. All personnel must be listed on the IACUC approved protocol.
- When planning to do a surgical procedure for the first time, obtain advice from a laboratory animal veterinarian or veterinary technician in Veterinary Services (206-543-6257).

2.11 Euthanasia:

Remember: Animals communicate with sounds and smells humans cannot perceive. Do not expose animals to the death of others. Be sensitive to rodents, other animals, and people in the vicinity. Per the AVMA Guidelines on Euthanasia (<http://www.avma.org/resources/euthanasia.pdf>), physical forms of euthanasia such as cervical dislocation and decapitation require scientific justification and approval by IACUC.

- Anesthetic overdose (inhalant or injectable) - Calculate injectable agent dosage at 4-5 times surgical anesthetic dosage, use a commercial euthanasia solution, or expose to fumes of inhalant anesthetic by placing rat in a small bell jar inside a fume hood (avoid any direct contact of rat to anesthetic agent), and continue exposure to fumes for 3-5 minutes after respiration has ceased. Neonatal rats (under 10-14 days) can be euthanized using an injectable agent or inhalant anesthetics as stated above.
- Carbon Dioxide (CO₂)
 - General Information
 - Always use the timer to ensure adequate length of exposure.
 - Place a dead animal tag on the bag with 1) P.I.'s name, and 2) Euthanasia date
 - Do NOT euthanize in animal rooms or in front of living animals.
 - Do NOT leave the room with the CO₂ running.
 - Do NOT overcrowd the cages. All animals must have floor space.
 - Do NOT mix males from different cages or incompatible females together.
 - Do NOT expose animals to a cage that is "pre-filled" with CO₂
 - Do NOT expose animals to a CO₂ flow rate higher than noted on the CO₂ tank. Over exposure can burn the mucous membrane.
 - When removing dead rats from the cage, run your fingers through the bedding to be sure there is no one left behind in the cage.
 - Neonatal rodents will NOT die with CO₂ alone (i.e. animals that are 14 days of age or

- younger).
- You will need to supply your own sharp scissors for decapitation.
 - If you have questions regarding euthanasia, please contact Veterinary Services within your facility, the Facility Supervisor, the IACUC office or the Facility Director.
- Euthanizing Neonates/Pups
 - Age: 0-14 days old – CO2 exposure followed by decapitation.
 1. Turn CO2 on at the proper low flow rate posted next to source.
 2. Remove cage top.
 3. Put CO2 lid on cage.
 4. Maintain gas flow for at least 10 minutes for rats. Use the timer.
 5. Turn off source.
 6. Pups should not be moving or breathing when removed from the cage.
 7. Remove animals from CO2 cage and decapitate with sharp scissors designated for decapitation.
 8. Dispose of remains in plastic bag with dead animal tag, and then into designated refrigerator/freezer.
 - Age: 0-6 days old – hypothermia followed by decapitation.
 1. Place neonates onto a surface so they DO NOT come in direct contact with ice. For example, can be on several layers of paper towel or a petri dish.
 2. Once animals have stopped moving, you can check to see if animals are anesthetized by firmly pinching a toe. If they do not respond to a toe pinch, they are anesthetized.
 3. Once anesthetized, you can decapitate them with sharp scissors designated for decapitation.
 4. Place animals in a dead animal bag and place in designated refrigerator/freezer.
 - Euthanizing Young Adults & Adults (over 14 days)
 1. Turn CO2 on at the proper low flow rate posted next to tank.
 2. Remove cage top.
 3. Put CO2 lid on cage.
 4. Maintain gas flow for at least 10 minutes for rats. Use the timer.
 5. Turn off tank.
 6. They should not be moving or breathing when removed from the cage.
 7. Remove dead animals from CO2 cage.
 8. The University of Washington IACUC requires a secondary method of euthanasia to confirm death when euthanizing with CO2, and this method must be approved in your IACUC protocol. Please refer to IACUC policy “CO₂ Euthanasia in Rodent Species”:
http://depts.washington.edu/iacuc/policies/co2euth_rodent.html.
 - ✓ Cervical dislocation by UW certified individuals and only on rats under 200g.
 - ✓ Exsanguination (remove blood from heart).
 - ✓ Thoracotomy (open up the chest to collapse the lungs).
 - ✓ Decapitation.
 - ✓ Anesthetic overdose.
 - ✓ Placed in a bag full of CO2 for disposal.
 9. Dispose of remains in plastic bag with dead animal tag and place bag into designated

refrigerator.

- Cervical dislocation – May only be performed in rats under 200 grams body weight. As per the AVMA Guidelines on Euthanasia, cervical dislocation is a humane technique for euthanasia of rats weighing < 200 grams when performed by individuals with a demonstrated high degree of technical proficiency. Must be performed rapidly to achieve separation at the cervical (neck) region and NOT the back. Always check to be sure no heart beat is palpable before discarding the carcass. Certification of competency is required. This technique should not be used in neonates (rats under 10-14 days of age). For euthanasia of neonates refer to decapitation or anesthetic overdose.
- Decapitation –
 - ADULT RATS - Should be performed using a clean, sharp guillotine of the appropriate size for the animal being euthanized. Rat may be placed in a DecapiCone® prior to decapitation (See Appendix C: Decapitation Notes). Certification is required if the animal is to be decapitated while awake. Anesthetized decapitation does not require certification.
 - NEONATES - Decapitation of neonates (rats whose eyes have not yet opened) must be performed using clean, sharp surgical scissors. Ideally neonates should be anesthetized using CO₂ prior to decapitation; this reduces movement of the rat and makes decapitation easier. If you are unable to use CO₂, anesthesia can be achieved by inducing hypothermia. For information on how to induce hypothermia please contact the Animal Use Training Program. Certification of competency is not required for decapitation of neonates.

ONLINE RESOURCES

AVMA Guidelines on Euthanasia	http://www.avma.org/resources/euthanasia.pdf
Rat Anatomy	http://www.biologycorner.com/worksheets/rat_intro.html
UW Purchasing Web Page	http://depts.washington.edu/compmed/animal/index.html
DCM Shared Rodent Service- <u>free</u>	https://catalysttools.washington.edu/gopost/board/jenick0z/8509/
UW IACUC Web Page	http://depts.washington.edu/iacuc
UW Environmental Health & Safety	http://www.ehs.washington.edu
UW Training Web Page	http://depts.washington.edu/auts
UW Instrument Sharpening	http://depts.washington.edu/hsasf/sidsvc/machine.html
Ear Punch Numbering System	http://www.medicine.virginia.edu/research/cores/transgenic/all-mice-considered/ears-page

CONTACT INFORMATION

Department of Comparative Medicine

Veterinary Services

6th Floor and T-wing	543-6257
E-mail	vs6floor@u.washington.edu
K-wing	616-8716
E-mail	vskwing@u.washington.edu
Harborview	897-5065
E-mail	vshrt@u.washington.edu
Mercer	897-1508
E-mail	vsmerc@u.washington.edu
Foegen	221-4803
E-mail	vsfoegen@u.washington.edu
Central Animal Surgery	543-6150
Rodent Health Monitoring Program	221-3933
Pathology Services	685-3040
Institutional Animal Care & Use Committee (IACUC) Protocol Review	
General Questions	543-9678
Protocol Review Questions	543-9678 or 543-3818
IACUC Web Page	http://depts.washington.edu/iacuc
IACUC Manager	543-9678
Animal Use Training Program Coordinator	221-7709
Training E-mail	auts@u.washington.edu
Training Web Page	http://depts.washington.edu/auts/
Facilities Manager	543-0641
Animal Purchasing	543-0640
Purchasing E-mail	animals@u.washington.edu
Purchasing Web Page	http://depts.washington.edu/compmed/animal/index.html

Drug Services

Ordering

Website	https://eres.lib.washington.edu/eres/coursepage.aspx?cid=1805&page=docs
Voicemail	598-6058
Fax	598-3808
Email	drugsvcs@u.washington.edu

Environmental Health and Safety

Occupational Health Nurse Consultant	221-3025
Occupational Health Clinic – Hall Health	685-1026
Environmental Health & Safety Web Page	http://www.ehs.washington.edu

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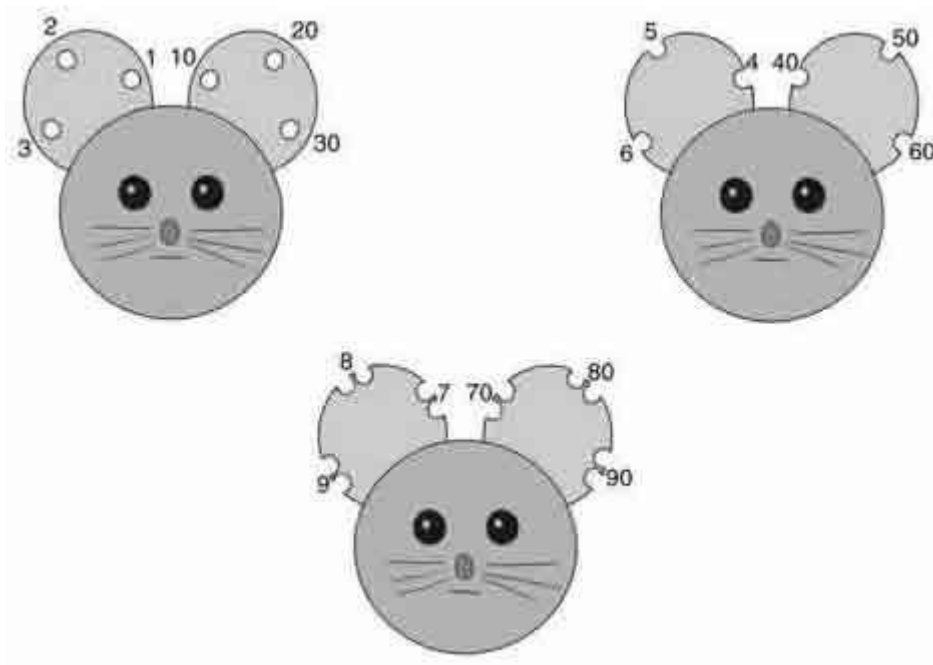
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- 2000 Report of the AVMA Panel on Euthanasia, Journal of the American Veterinary Medical Association, Vol. 218, No.5, March 1, 2001 pp. 669-696, <http://www.avma.org/resources/euthanasia.pdf>.
- Smith, A.C. and Swindle, M.M., eds., Research Animal Anesthesia, Analgesia and Surgery, Scientists Center for Animal Welfare, 1994.

VENDOR LIST

PRODUCT	VENDOR	PHONE	WEB SITE
anesthesia vaporizers and equipment	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
anesthesia vaporizers and equipment	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
anesthesia vaporizers and equipment	Summit Medical	800-877-8989	
autoclave bags and pouches	Fisher Scientific	800-766-7000	www.fishersci.com
drug storage cabinets	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
drugs and anesthetics	UWMC Drug Services	206-598-6058	
ear punches & ear tagging supplies	National Band and Tag Co.	606-261-2035	www.nationalband.com
ear punches& ear tagging supplies	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
ear punches	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
guillotine	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
			www.depts.washington.edu
guillotine & scissor sharpening	UW Scientific Instruments	206-543-5580	/hsasf/sidsvc/
heating apparatus	Physitemp Instruments Inc.	800-452-8510	www.physitemp.com
heating pad (recirculating warm water)	Gaymar Industries	800-828-7341	www.gaymar.com
heating pad (chemical)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
hot bead sterilizer	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
hot bead sterilizer	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
hot bead sterilizer	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
hot bead sterilizer	Inotech Biosystems International, Inc.	800-635-4070	www.inotechintl.com
hot bead sterilizer	Fine Science Tools	800-521-2109	www.finescience.com
micro-chip identification	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
micro-chip identification	Bio Medic Data Systems, Inc	800-526-2637	www.bmds.com
needle re-capper	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
oral gavage needles	Webster Veterinary Supply	800-225-7911	www.jawebster.com
oral gavage needles	Instech Laboratories, Inc..	800-443-4227	www.instechlabs.com
plastic rodent restraint	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
plastic rodent restraint	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
plastic rodent restraint	VWR Scientific Products	800-932-5000	www.vwrsp.com
plastic rodent restraint	Plas Labs, Inc.	800-866-7527	www.plas-labs.com
plastic rodent restraint (heated)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
plastic rodent restraint (heated)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
stockinet	Webster Veterinary Supply	800-225-7911	www.jawebster.com
surgeon supplies (glove, gown, etc.)	Fisher Scientific	800-766-7000	www.fishersci.com
surgeon supplies (glove, gown, etc.)	VWR Scientific Products	800-932-5000	www.vwrsp.com
surgeon supplies (glove, gown, etc.)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
surgical instruments	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
surgical instruments	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
surgical instruments	Fisher Scientific	800-766-7000	www.fishersci.com
surgical instruments	VWR Scientific Products	800-932-5000	www.vwrsp.com
surgical instruments	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
surgical instruments	Fine Science Tools	800-521-2109	www.finescience.com
surgical instruments (micro)	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
surgical instruments (micro)	Fine Science Tools	800-521-2109	www.finescience.com
surgical instruments (micro)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
surgical instruments (micro)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
tattoo marking systems	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
tattoo marking systems	Ketchum Manufacturing Inc.	613-722-3457	www.ketchum.on.ca

Appendix A: Ear Punch Codes

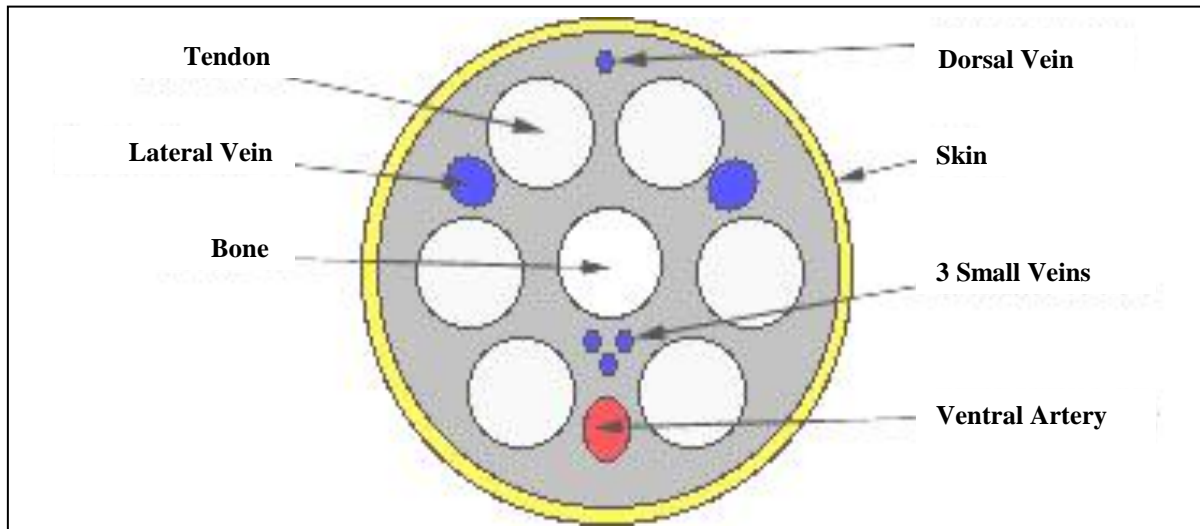
2.12 Pattern for ear punch numbering system



Drawn by William Ober (2001)

From page 86, Figure 31 in *Manipulating the Mouse Embryo, A Laboratory Manual*, 1986, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Appendix B: Rat Tail Anatomy


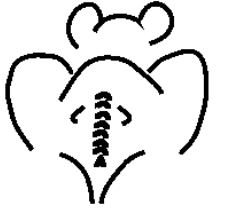

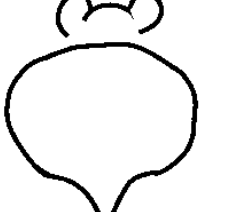



Cross-section of a rat's tail. Drawing adapted from Vanhoutte et al. 2002

<http://www.ratbehavior.org/RatTails.htm>

Appendix C:
Body-condition (BC) scoring

Can be applied to rats

	<p>BC 1</p> <p>Mouse is emaciated.</p> <ul style="list-style-type: none">◦ <i>Skeletal structure extremely prominent; little or no flesh cover.</i>◦ <i>Vertebrae distinctly segmented.</i>
	<p>BC 2</p> <p>Mouse is underconditioned.</p> <ul style="list-style-type: none">◦ <i>Segmentation of vertebral column evident.</i>◦ <i>Dorsal pelvic bones are readily palpable.</i>
	<p>BC 3</p> <p>Mouse is well-conditioned.</p> <ul style="list-style-type: none">◦ <i>Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.</i>
	<p>BC 4</p> <p>Mouse is overconditioned.</p> <ul style="list-style-type: none">◦ <i>Spine is a continuous column.</i>◦ <i>Vertebrae palpable only with firm pressure.</i>
	<p>BC 5</p> <p>Mouse is obese.</p> <ul style="list-style-type: none">◦ <i>Mouse is smooth and bulky.</i>◦ <i>Bone structure disappears under flesh and subcutaneous fat.</i>

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

From "Body Condition Scoring: A Rapid and Accurate method for Assessing Health Status in Mice" Laboratory Animal Science, June 1999.

Appendix D: Decapitation Notes

Some text borrowed from: <http://iacuc.ufl.edu/Policy%20for%20Decapitation%20of%20Rodents.pdf>.

Important:

DO NOT perform this procedure unless properly trained and authorized.

DO NOT perform this procedure unless approved as part of an IACUC protocol.

DO NOT depress the guillotine lever unless the rodent's head is **fully engaged in the guillotine**.

DO NOT depress the guillotine lever unless the rodent's head is **immobile**.

DO NOT depress the guillotine lever unless **your fingers** are **out the way**.

DO NOT depress the guillotine lever unless you are confident that the rodent's head will be removed in one clean stroke.

DO NOT allow any distractions in the room during this procedure.

Decapitation Procedure:

- 1) Procedure should be performed in a room that is isolated from all other rodents.
- 2) The guillotine will be placed upon a clean and stable benchtop or other stable surface, and the sharpness and smooth operation of the guillotine will be verified before introducing any rodent.
- 3) The rodent will be removed from its home cage, or experimental environment, and carried to the guillotine. The researcher will make every effort to adjust the transport of the rodent until it appears calm (note that the affective state of the animal may be determined by the experimental conditions). Although not required, use of de-capi-cones is suggested.
- 4) The researcher will hold the rodent securely, and place the rodent on the stage at the entrance to the guillotine, then gently and assertively move the head forward until the neck is directly above and below the upper and lower blades.
- 5) When the head is in position, pause momentarily and **verify the head is completely through the opening** of the guillotine, and that your hand and **fingers are clear of the blade path**.
- 6) Smoothly, quickly, and assertively depress the guillotine lever, decapitating the rodent.

Comments: Sometimes – depending on the method of restraint, rodents will put their arms/paws through the opening with the head – this does not matter. It is better to minimize stress to the animal-- just proceed with the decapitation, don't spend any time trying to get the paws out.

Another comment: Some people who have performed this procedure before recommend gently swinging or swaying the animal to disorient it prior to decapitation. I do not recommend this.

Care of the Guillotine:

- 1) After each decapitation, rinse and/or wipe down the guillotine and surrounding area to remove all blood and tissues.
- 2) At the end of each day of use, thoroughly wash the guillotine with detergent and water, and dry it. After drying, oil the moving parts with light machine oil (e.g. 3-in-1 oil), and run the blade up and down several times to spread the oil.
- 3) Sharpen or replace blades whenever they are dull. Contact either the vendor of the guillotine for specific instructions, or Scientific instruments here on campus will perform this.

Contact Scientific Instruments' Machine Shop at UW:

(206) 616-8905 or
mshop@u.washington.edu

Appendix E: UW Policy on Beyond-Use Dating (BUD) of Extemporaneously Compounded Drugs

Background:

In the field of pharmacology, **extemporaneous compounding** is the mixing of drugs to produce a medication for a specific purpose when no commercial forms are available.¹ Many injectable drugs used in research animals at the University of Washington are extemporaneously compounded. A common example is the mixture of ketamine and xylazine, or ketamine, acepromazine, and xylazine, used for rodent anesthesia. Other examples include drugs, such as buprenorphine, that are diluted for appropriate dosing in rodents.

According to the U.S. Food and Drug Administration (USFDA), compounding may only be performed using FDA-approved animal or human drugs and only when no approved animal or human drug is available, in the relevant dosage form and concentration, to treat the diagnosed condition.²

Extemporaneously-compounded drugs are assigned a "beyond-use date" (BUD), instead of an expiration date, which represents the last date at which there is confidence that, with normal use, the components have not changed stability, potency, or sterility. The BUD is never later than the expiration date of any of the component drugs.³

There is little information available on the stability and potency of compounded drugs. In addition, stability information gives no assurance regarding sterility unless sterility testing is performed.⁴

For extemporaneously compounded drugs, the limiting factor for safety and efficacy is more likely to be sterility than physical stability of the components,^{5,6} especially when compounded drugs are placed in a multi-use container and used by several people over time. The U.S. Pharmacopeia (Chapter 797) requires medication multi-dose vials for injections be given a beyond-use date that is 28 days after initial stopper penetration.⁷

Policy:

In order to ensure the safety and efficacy of extemporaneously compounded drugs, the following steps must be followed:

- 1) A drug dilution must be made with sterile diluent.
- 2) A drug must be mixed in a sterile container.
- 3) The dispensing container must be labeled with the name of the drug (s), manufacturer expiration date(s), date of dilution or compounding, and the beyond-use date (BUD).
- 4) **The beyond-use-date (BUD) is 4 weeks (28 days) from the date of compounding.**
- 5) The drug must be stored according to manufacturer instructions (e.g., refrigerate, protect from light, etc.).
- 6) The rubber stopper must be wiped with alcohol prior to each time the drug is removed.
- 7) A sterile needle must be used each time the drug is removed.
- 8) The compound must be fully resuspended before removal from container.
- 9) Drug must never be returned to the bottle after removal.
- 10) The bottle must be discarded at the BUD, or expiration date of the individual components, whichever comes first.

References:

- ¹Kairuz TE, Gargiulo D, Bunt C, Garg S (2007) Quality, safety, and efficacy in the 'off-label' use of medicines. *Curr Drug Saf* 2: 89-95.
- ²USFDA website: Compliance Policy Guidelines Sec 608.400, Compounding of Drugs for Use in Animals <http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074656.htm>
- ³USFDA website: Expiration dating of unit-dosed repackaged drugs: compliance policy guide, 2005. <http://www.fda.gov/CDER/GUIDANCE/6169dft.pdf>
- ⁴U.S. Pharmacopeia website (2010) <http://www.usp.org/audiences/pharmacist/797FAQs.html>
- ⁵Taylor BJ, Orr SA, Chapman JL, Fisher DE (2009) Beyond-use dating of extemporaneously compounded ketamine, acepromazine, and xylazine: safety, stability, and efficacy over time. *JAALAS* 48(6): 718-726.
- ⁶Trissel LA, ed. (2009) *Trissel's Stability of Compounded Formulations*, 4th ed. American Pharmacists Association, pp. 313-314.
- ⁷US Pharmacopeial Convention, Inc. General Chapter ,797. *Pharmaceutical Compounding-Sterile Preparations*. The United States Pharmacopeia, 32nd Revision and The National Formulary. 27th Ed, Rockville, MD: United States Pharmacopeial Convention; 2009. p. 318-54.