UNIVERSITY of WASHINGTON

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, other training might be required. Contact the Animal Use Training Session (AUTS) Program for training requirements.

1.1 Practicing Techniques:

You are not allowed to train/practice on your own animals unless 1) you have training approved in your protocol or 2) the animals are directly being used in an experiment. Example: You are NOT allowed to practice on animals because you are culling your colony unless it is in your protocol. Utilize the training program for your training needs. If you are trained in your lab, it must be documented. If it is a certifiable technique, the training must be performed by an individual designated by the AV.

1.2 Preparing for Class and Laboratory Work:

It is important to prepare yourself, work space, and animals before you start working. Laboratory work can be stressful and you are often on your feet. Wear comfortable closed toe shoes, lightweight comfortable clothing, eat a healthy meal, and stay hydrated. Have your work space set up to limit repetitive and uncomfortable movement. Ensure your animals are healthy before you start work and know how to contact Veterinary Services if you have any issues. Always give yourself extra time to finish your work; rushing can lead to mistakes and injured animals.

1.2.1 Preparing for your Hands-on Class:

1. Read the handouts included with the reminder email before coming to class.
2. Know what specific techniques (if any) you need to learn.
3. Wear lightweight comfortable clothing because you will be wearing a surgical gown.
4. Wear comfortable shoes because you will be standing for 1 or more hours.
5. There may be a potential for exposure to animal allergens, so come to class prepared if you have allergies (e.g., bring a mask).
6. Eat a meal and drink water before coming to class. Sometimes class can be stressful, particularly if you are new to working with lab animals, or are not comfortable with needles and get squeamish around blood. During times of stress, one can get light headed and faint. Eating a meal will help prevent fainting during a stressful situation.

1.3 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 appropriate methods of euthanasia.

1.4 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

1.5 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/

1.6 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)

1.7 Rat Anatomy:

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

1.8 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 m^2
- 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 H20
  - Urine pH 7.3-8.5
  - Urine specific gravity 1.04-1.07

1.9 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 the Rodent Health Monitoring Program (rhmp@uw.edu) for information on having tissues and cell lines tested.

1.10 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.

1.11 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).

1.12 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.

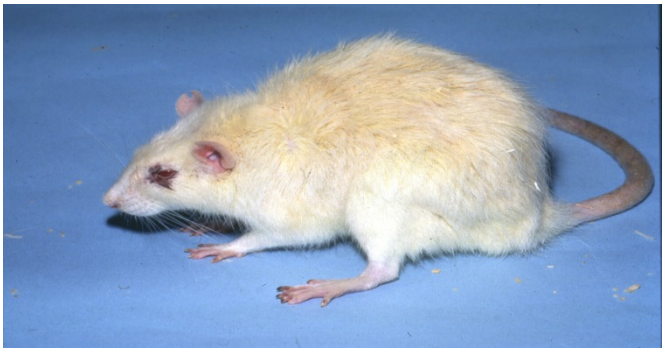
1.13 Purchasing Rats:

- 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.
  - Charles River Laboratories - http://www.criver.com
  - ENVIGO (formerly Harlan Laboratories) - http://www.envigo.com/
  - Taconic Biosciences – http://www.taconic.com
  - DCM Shared Rodent Service - https://catalyst.uw.edu/gopost/board/animals/8509/
- Bringing Rats into a UW Facility - All rats must be ordered through Animal Purchasing and cleared with RHMP. 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.
  - Specific Pathogen-Free (SPF) - Free of specific pathogens known to cause disease in some strains of rats.
    - All Comparative Medicine rodent facilities are SPF housing (K-wing, T-wing, 6th Floor, Foege, ARC, Brotman, SLU 3.1, HR&T, Roosevelt, Guthrie, and CHDD).
  - 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 lab-managed facilities that can support conventional animals.

2. Laboratory Outline:

2.1 Health Monitoring:

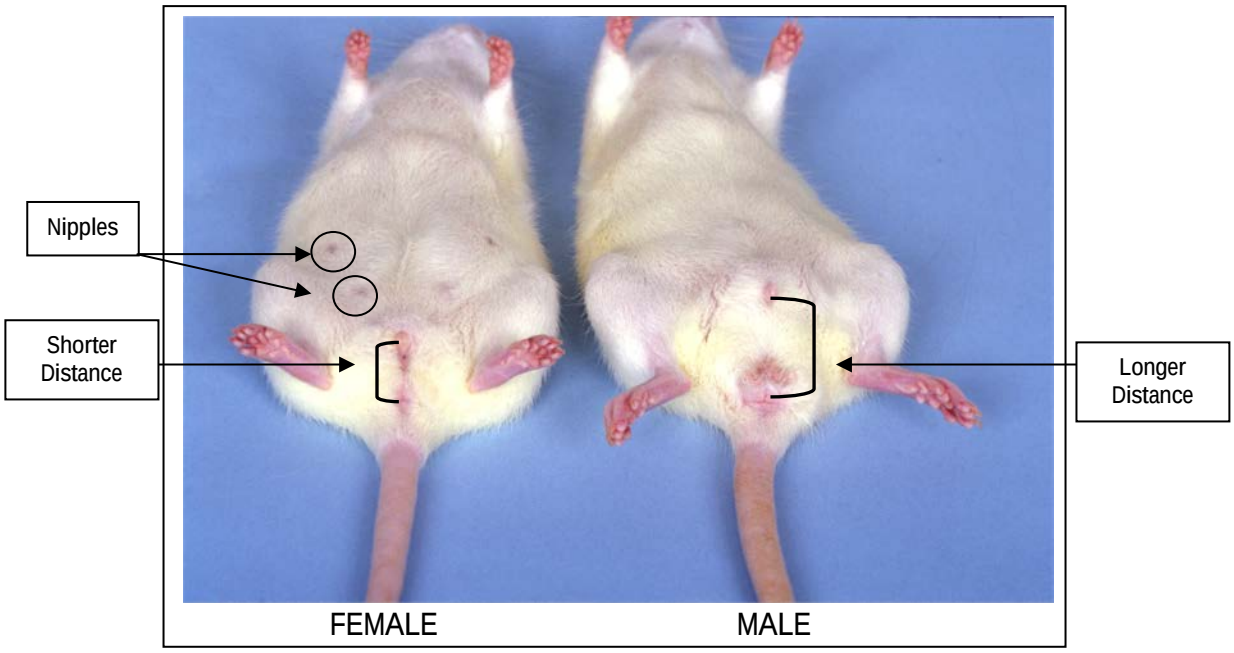
- It is important to be familiar with the characteristics of a healthy rat and normal rodent behavior in order to recognize an unhealthy animal. Normal rats should be active, have a smooth and shiny haircoat, bright clear eyes, and good body mass.
- Signs of disease or discomfort: huddles in corner, 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.



Sick rat: hunched, rough hair coat, porphyrin staining

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 Weaning:

Generally, rodents are weaned at 21 days of age. Weaning is when a litter is removed from the cage were the female is housed.

1. Place animals into fresh new cages. DO NOT mix males who are from different litters. Mixing males can cause fighting.
2. Check for enrichment (tube) in cage. If not present, place in cage
3. Place fresh food in one side of the clean wire top so it is flush with the surface. Do not over or under fill the food.
4. Check water source. If working in a facility that uses water bottle, check to see water bottle is full, not leaking and the sipper tube on the water bottle is down. If working in a facility with an automatic watering system, toggle water valve.
5. Put on a lid with a cage card holder.
6. Put on new cage card with all the required information.

2.4. Cage Cards:

Cage cards must be legible and include:

1. PI Name *(required by The Guide)*
2. Protocol Number *(required by The Guide)*
3. Budget Number *(needed for DCM to bill efficiently)*
4. Contact Name and Number *(required by The Guide; needed in order to reach someone regarding animal care issues with that cage)*
5. Birth Date or Date of Arrival (age/wt = when ordered) *(required by The Guide)*
6. Source (vendor; sire and dam if breed in house) *(required by The Guide)*
7. Strain/Stock *(required by The Guide)*
8. Sex *(required by DCM)*
9. Release # (DCM cage cards only) *(used for tracking purposes by DCM)*

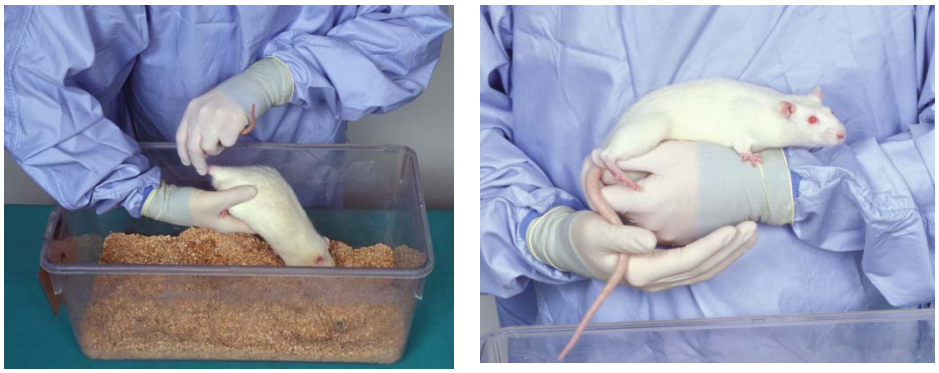
Note: cage cards can be printed at no cost by DCM staff (breeding or colony cards)

2.5 Restraint and Handling:

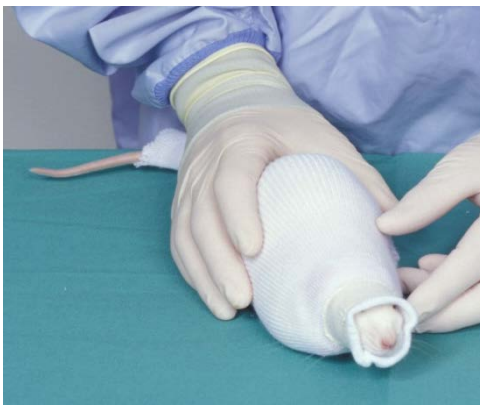
Rats are intelligent and respond well to gentle handling. When handling, include "petting" sessions so your rats associate you with pleasant activities. Rats can bite, but rarely do. Use of bite protection gloves is not usually needed. Note that rats can bite through thin leather gloves. Gloves heavy 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



Stockinet Restraint

- 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).

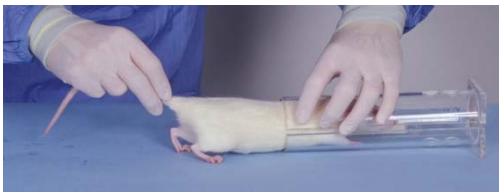
- DecapiCone® Restraint: Designed for use with a guillotine, but also provides good access to tail and limbs. Caution, rats can overheat in these bags (see Vendor List).

- 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).

Shoulder Restraint

Shoulder Restraint

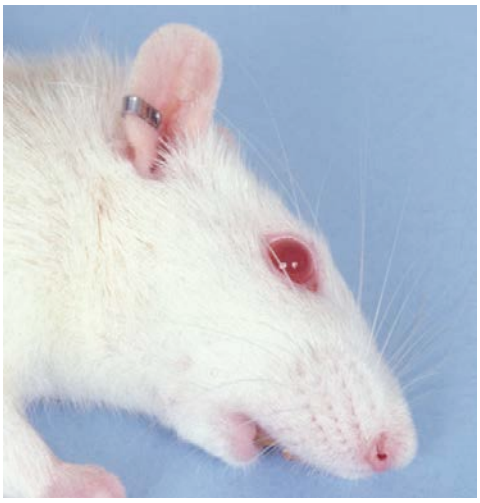
DecapiCone® Restraint



Broome Restraint

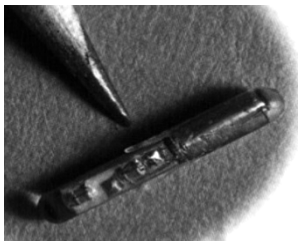
2.6 Methods of Identification:

- Temporary - Tail or haircoat marking with a non-toxic permanent marker, such as a Sharpie marker (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



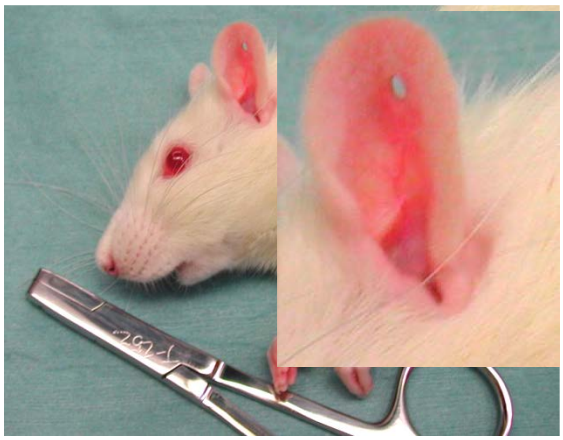
Ear Tag

Sharpie® Marker



Microchip

Tattooing Forceps



Ear Punch

2.7 Injection Techniques:

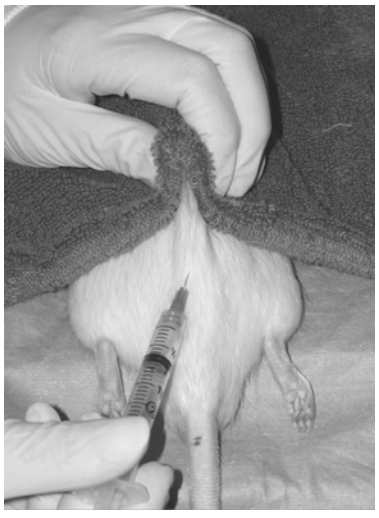
A 25 gauge (g) or smaller needle is recommended. (Larger numbers indicate smaller gauge needles.)

2.7.1 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.

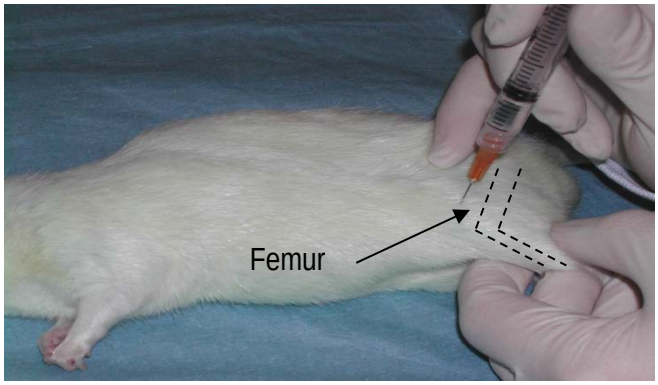
2.7.2 Intramuscular:

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



Subcutaneous Injection

the technique.



Intramuscular Injection

Intraperitoneal Injection

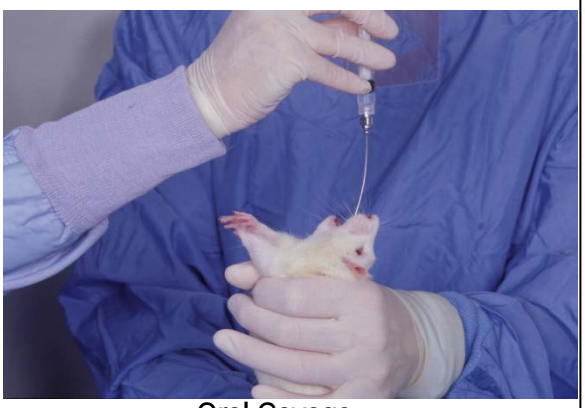
2.7.3 Intraperitoneal:

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.

2.7.4 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.

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



Oral Gavage

Intravenous Injection

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).

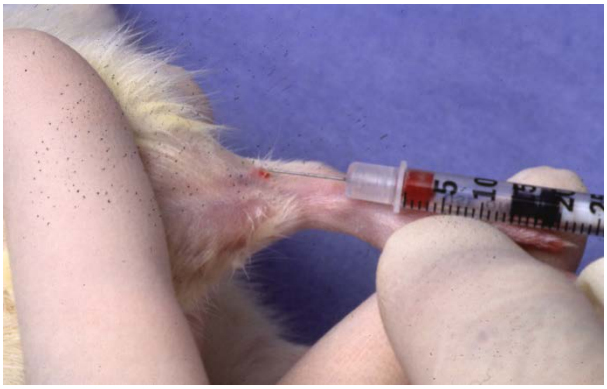
Restraint: Hold rat upright against your lateral midsection, immobilize its rear legs with your wrist and forearm. An assistant may restrain the animal. Extend the neck of the rat to allow passage of the needle into the esophagus.

2.8 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.

2.8.1 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

2.8.2 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

2.8.3 Ventral Tail Artery (see Appendix B):

- Start at the proximal portion of the tail.
- Your approach angle should be 45º-90º.
- 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.

Lateral Tail Vein Bleed

2.8.4 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.

Ventral Tail Bleed

2.8.5 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 with form.
- You can obtain slightly more blood by gently milking the tail.

2.8.6 Retro-Orbital Bleeds

Retro-orbital Bleed

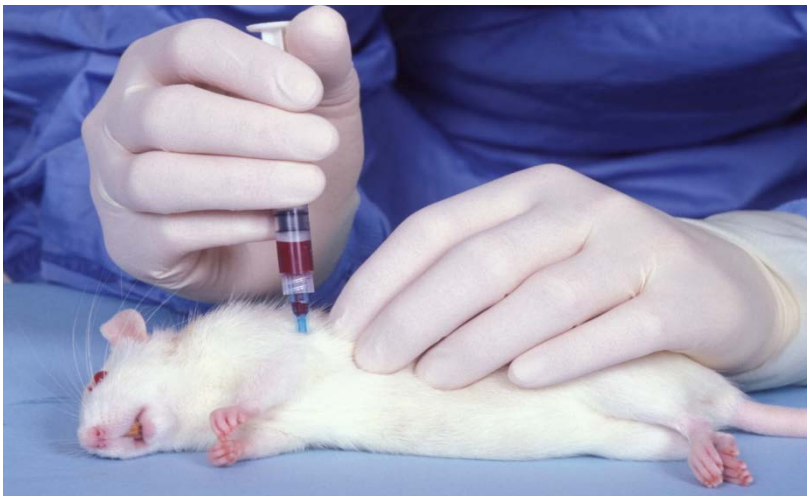
- Anesthesia is required.
- Certification is required.
- Provides venous blood.
- Pipette location begins in medial canthus of eye and glides to dorsal aspect of orbit.
- After sample is obtained, risk of suborbital hematoma may be minimized by using gauze sponge to apply gentle pressure over closed 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 AUTS to schedule this certification auts@uw.edu.

2.8.7 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

2.9 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

2.10 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.

2.10.1 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 1 month 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.

2.10.2 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.

2.11 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 (contact auts@uw.edu).

2.12 Surgical Requirements:

- 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) and ensure individuals are certified.
- Non-Survival Surgery
  - Animals are not allowed to recover from anesthesia.
- Survival Surgery
  - Animals are allowed to recover from anesthesia.
- Non-Aseptic Surgery
  - 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."
- Aseptic Surgery
  - Must be performed by an individual certified by a designated training. See policy here (https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies).
  - Aseptic 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 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.

Hot Bead Sterilizer

2.13 Euthanasia:

General Information: Animals communicate with sounds and smells humans cannot perceive. Do not expose animals to the death of others. Be sensitive to other animals and people in the vicinity. Ensure all equipment is in good working order. Species should be separated and never over crowded. Acceptable methods of euthanasia are injectable anesthetics, inhalation anesthetics, carbon dioxide, cervical dislocation*, and decapitation*. All methods must be approved on your IACUC protocol. Per the AVMA

Guidelines on Euthanasia (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf), decapitation requires scientific justification and approval by the IACUC.

*NOTE: Individuals performing decapitation of unanesthetized rats older than 14 days and/or cervical dislocation of unanesthetized or anesthetized rats weighing less than 200 grams at the UW must be certified by a designee of the Attending Veterinarian.

FETUSES - Rodent fetuses do not have to be removed from the uterus and separately euthanized after the mother has been euthanized.

- Anesthetic overdose
  - Inhalant - 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 agent) or place rat in a closed receptacle and introduce anesthetic from a vaporizer. Continue exposure to fumes for 3-5 minutes after respiration has ceased (at least 10 minutes total).
  - Injectable - Calculate injectable agent dosage at 4-5 times normal anesthetic dosage.
- Always use the timer to 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.
    - Clean up between cohorts of animals. This includes the hood, chamber, and work surface. Animals release pheromones when being euthanize that can induce stress in other animals.
    - Neonatal rodents will NOT die with CO2 alone (i.e., animals that are 10 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 CO2 tank.
      6. Pups should be anesthetized 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 10 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 CO2 tank.
  6. They should not be moving or breathing when removed from the cage.
  7. Remove dead animals from CO2 cage.
  8. Perform the secondary method. 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 "Euthanasia of Research and Teaching Animals": https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies
     - ✓ 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 when performed on all rats regardless of age. Learn this technique by initially practicing on anesthetized rats with an experienced trainer; contact personnel in the Animal Use Training Program for assistance.
- Decapitation – Must be performed using clean, sharp surgical scissors or guillotine of the appropriate size for the animal being euthanized. The equipment used to perform decapitation must be maintained in good working order and records demonstrating service of the equipment must be maintained. See Appendix D: Decapitation Notes for more information.
  - NEONATES (0-14 days old) - Decapitation of neonates must be performed using clean, sharp surgical scissors. Neonates can be anesthetized using CO_2 prior to decapitation; this reduces movement of the rat and makes decapitation easier. If you are unable to use CO_2, 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.

- ADULT RATS - Rat should be placed in a DecapiCone® prior to decapitation. Certification is required if the animal is to be decapitated while awake. Anesthetized decapitation does not require certification.

ONLINE RESOURCES

Animal Use Training Program	http://depts.washington.edu/auts/
AVMA Guidelines on Euthanasia	https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
DCM Animal Purchasing	http://depts.washington.edu/compmed/animal/index.html
DCM Shared Rodent Service-free	https://catalyst.uw.edu/gopost/board/animals/8509/
Ear Punch Numbering System	http://www.medicine.virginia.edu/research/cores/transgenic/all-mice-considered/ears-page
Environmental Health & Safety	http://www.ehs.washington.edu
Instrument Sharpening	http://auk.wanprc.org/services/
Office of Animal Welfare	http://oaw.washington.edu/
Rat Anatomy	http://www.biologycorner.com/worksheets/rat_intro.html

CONTACT INFORMATION

Department of Comparative Medicine

Veterinary Services
- *6th Floor, CHDD, Guthrie, Roosevelt, T-wing, and all Lab-Managed Facilities* 543-6257
  - *E-mail* ..... vs6floor@uw.edu
- *ARCF* 221-2250
  - *E-mail* ..... vsarcf@uw.edu
- *Brotman* 897-1508
  - *E-mail* ..... vsmercer@uw.edu
- *Foege* 221-4803
  - *E-mail* ..... vsfoege@uw.edu
- *Harborview* 897-5065
  - *E-mail* ..... vshrt@uw.edu
- *K-wing* 616-8716
  - *E-mail* ..... vskwing@uw.edu
- *SLU 3.1* 685-6605
  - *E-mail* ..... vsslu3@uw.edu

Central Animal Surgery 543-6150
Rodent Health Monitoring Program (RHMP) 221-2441
- RHMP E-mail ..... rhmp@uw.edu
- RHMP Web Page ..... https://depts.washington.edu/compmed/rhmp/index.html

Pathology Services 685-3040
Office of Animal Welfare (OAW) 685-7363
- OAW E-mail ..... oawrss@uw.edu
- OAW Web Page ..... http://oaw.washington.edu/

Animal Use Training Program 221-7709
- Training E-mail ..... auts@uw.edu
- Training Web Page ..... http://depts.washington.edu/auts/

Animal Purchasing 543-0640
- Purchasing E-mail ..... animals@uw.edu
- Purchasing Web Page ..... http://depts.washington.edu/compmed/animal/index.html

Drug Services

Ordering
- Website ..... http://depts.washington.edu/drugsvcs/home/
- Voicemail 598-6058
- Fax 598-3808
- Email ..... drugsvcs@uw.edu

Environmental Health and Safety

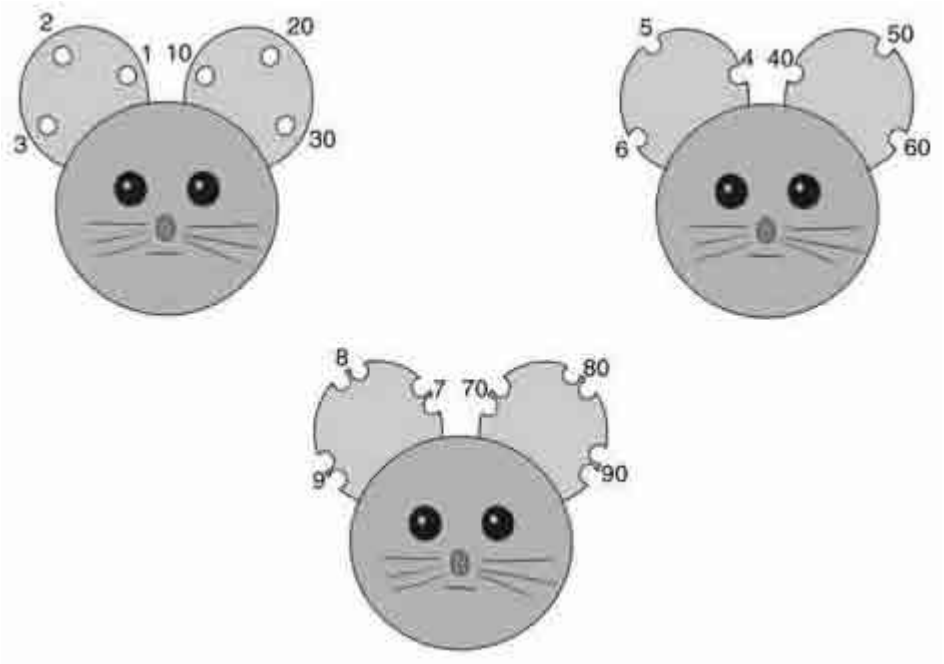
Occupational Health Nurse Consultant 221-3025
Occupational Health Clinic – Hall Health 685-1026
Environmental Health & Safety Web Page ..... http://www.ehs.washington.edu

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
guillotine & scissor sharpening	WaNPRC Machine Shop	206-543-1039	http://auk.wanprc.org/services/
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

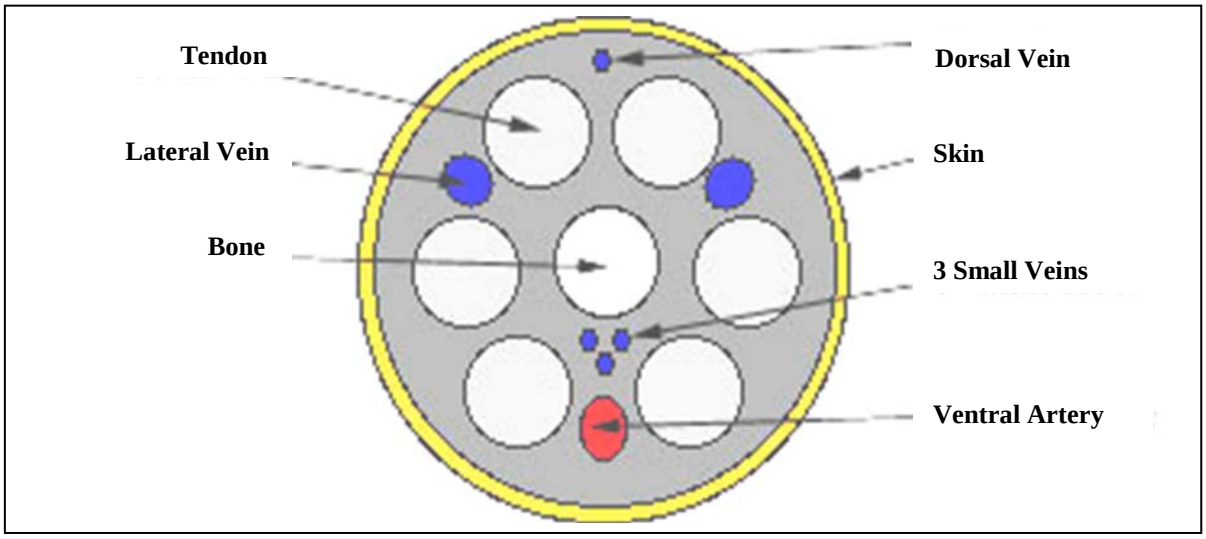
2.14 Pattern for ear punch numbering system



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

BC 1

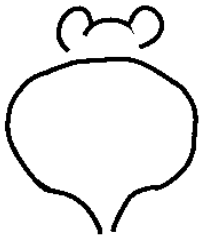
Mouse is emaciated.
- ***Skeletal structure extremely prominent; little or no flesh cover.***
- ***Vertebrae distinctly segmented.***

BC 2

Mouse is underconditioned.
- ***Segmentation of vertebral column evident.***
- ***Dorsal pelvic bones are readily palpable.***

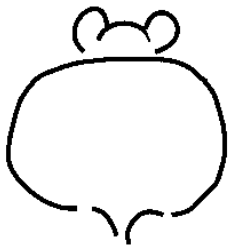
BC 3

Mouse is well-conditioned.
- ***Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.***



BC 4

Mouse is overconditioned.
- ***Spine is a continuous column.***
- ***Vertebrae palpable only with firm pressure.***



BC 5

Mouse is obese.
- ***Mouse is smooth and bulky.***
- ***Bone structure disappears under flesh and subcutaneous fat.***

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 finger**s 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 the WaNPRC Machine Shop on the main UW campus will perform this.

Also see UW IACUC Policy: Euthanasia of Research and Teaching Animals

Contact the WaNPRC Machine Shop at UW:
(206) 543-1039
grega@uw.edu
http://auk.wanprc.org/services/