Introduction

Welcome to the Working with Rats in Research at UAB (AU_RA) Course Material. The goal of this course material is introducing you to working with rats in research at UAB. However, some procedures may be different according to your approved protocol. If you have questions or need further instruction, contact the UAB ARP Veterinary Staff.

Objectives

At the conclusion, participants should be able to:

1. Care for and monitor rats used in research.
2. Comprehend the physiological and environmental issues when working with rats.
3. Identify who to contact for guidance if there are questions.

Care and Husbandry

Housing

Solid Floor Shoebox Cages

Solid floor shoebox cages contain a hardwood chip bedding material that absorbs liquid wastes. Research shows rats prefer having solid flooring with bedding as opposed to suspended wire flooring. The solid flooring with bedding provides rats with comfort, warmth, an opportunity for burrowing, and is beneficial for breeding since it protects the pups from hypothermia.

Minimum Space Recommendations

The Guide for the Care and Use of Laboratory Animals describes the minimum space recommendations for laboratory rats. For more information, see the IACUC Housing Density SOP for Rats.
Acclimation and Quarantine

**Acclimation**

Acclimation is the process of becoming adjusted to a new environment or situation and is necessary due to factors like stress from transportation. This stress could alter various blood parameters, immune cell function, and animal behavior. Upon arrival at UAB, rats must have an acclimation period of at least 48 hours before use in research studies. It may be desirable to extend the acclimation period beyond 48 hours. If possible, one week of acclimation is best.

**Quarantine**

The quarantine period is eight to ten weeks. The goal of quarantining and separating animals is preventing the transmission of diseases between new animals and animals already present in established colonies. ARP coordinates all animal acquisitions ensuring animals from new or unknown sources undergo the required quarantine period. Animals from approved and pathogen-free sources do not require quarantine.

A quarantine period is vital for avoiding loss of investigator time and valuable research data due to the introduction of disease, and the cost of controlling or eliminating infections escaping into established colonies.

Acclimation and quarantine periods can run at the same time even though they serve different purposes. For more information about acclimation and quarantining your rats, contact the UAB ARP Veterinarians.
The Sentinel System

Monitoring for the appearance or reoccurrence of a causative pathogen or parasite defines a sentinel. Health surveillance of animals at UAB monitors for the presence or recurrence of causative pathogens or parasites. The rodent health surveillance program uses a dirty bedding sentinel program. Colonies maintained by Principal Investigators (PIs) must participate in the Sentinel Program. For instructions and information, contact the UAB ARP Veterinarians.

This system protects all UAB rodent colonies by:

- Identifying potential outbreaks as quickly as possible
- Limiting the spreading of potential pathogens
- Mitigating research disruption
- Facilitating animals exported from UAB colonies to other institutions

The Sentinel Cage

Colony animals do not have to be tested directly to provide colony health information. A sentinel cage containing a specific pathogen-free and immune-competent rodent is used to represent colony animals. One sentinel cage represents up to 150 colony cages.

How This Works

- Mixing a portion of dirty bedding from each cage into the sentinel cage at cage change.
- Every six months survival samples are collected from sentinels and tested for viral pathogens. Sentinels are euthanized and replaced periodically to perform a gross exam and test for internal and external parasites, and viral pathogens.

If ARP identifies a problem within a colony, they will contact the PI to arrange suitable management solutions.
Temporary identification examples are marking the tail, back, or head with a permanent marker and usually lasts for two or three days on adults. However, it must be reapplied daily on rat pups.

Permanent

The most common permanent method is the ear punch. Other means of permanent identification include microchips, tattooing, or ear tagging. The use of anesthesia may facilitate some of these methods. Check with the UAB ARP Veterinarians for more information.

Enrichment

Enrichment promotes an animal’s natural behaviors by providing a stimulating and interactive environment. Environmental enrichment leads to improved health in a rat colony.

The Handbook of Laboratory Animal Management and Welfare identifies three points to consider when assessing enrichment:

1. Relevance to the species (positively impacting welfare and promoting normal species-specific behaviors).
2. It will not significantly impact science.
3. It will not interfere with animal housing management.
Examples

- Nestlets are a standard form of enrichment provided for rodents at UAB. Enviropaks are bags of shredded paper used for nest building and supplied in clean rodent cages.
- Disposable or sanitizable huts are used in the cage to provide a place to hide and feel secure, improve breeding success, reduce aggression between animals, and promote natural activities.
- Nylabones or Manzanita Wood Sticks take advantage of natural chewing behavior. These can be placed either on the cage floor or suspended from the wire bar to provide additional activity.
- Polycarbonate or PVC Elbows provide additional feelings of security and control over their environment.

Please do not add enrichment items without first checking with the UAB Animal Behaviorist. See the Enrichment and Social Housing of Animals SOP for more information.

Biological Features

Though rats share many anatomical and physiological features with humans, rats have many unique biological characteristics. Researchers should be aware of the practical features of the rat’s anatomy and biology.

Anatomy

Ocular System

Porphyric staining is where rats develop red staining around the eyes and nostrils due to accumulating porphyrins. Lacrimal porphyrin is a regular component of tears in rodents and usually removed through frequent grooming. When distressed, an animal will groom less often, and the porphyrin will visibly gather around the eyes.
Teeth

Rats have incisors that are open-rooted, meaning that these teeth grow continuously throughout their adult life. Misalignment of the upper and lower jaw or malocclusion causes tooth overgrowth. Staff must be alert to detect any signs of this condition and seek appropriate treatment.

Gastrointestinal

Inability to Vomit

Rats do not vomit; therefore, withholding food and water before surgery is not usually necessary.

Gall Bladder

Rats do not have a gallbladder. Bile passes directly from the liver into the bile duct and then into the duodenum.

Coprophagy

Plant material in rats is broken down by microbial action in the cecum. To utilize the microbial byproducts of digestion, the rat regularly eats its feces, a habit known as coprophagy. Stomach digestion and intestinal absorption of this fecal material yield essential nutrients to rats.

If a study requires fasting for scientific reasons, be aware rats will consume their feces and there may be fecal material in the GI tract.

Metabolism

High Rate of Metabolism

Since rats generally have a high rate of metabolism, drugs rapidly clear the body. This high rate of metabolism means rats should receive drug doses scaled to their metabolic rate. If you have questions, contact the UAB ARP Veterinary Staff.
High Surface Area

Rats have a large body surface area relative to their body volume and have many hairless body parts (tail, ears, and feet).

Sedation and anesthesia induce hypothermia due to the drug effects on the hypothalamus and sometimes causes a rapid drop in core body temperature. Because of the rat’s high surface area, there is a potential for profound hypothermia when using sedation and anesthesia.

Rats should have a source of warmth during procedures requiring anesthesia and afterward until they recover the ability to regulate their body temperature.

Albinism

Albinism is an inherited disorder, which has an impact on pigmentation (skin, fur, eye color, etc.) and other body systems. Neurologically albinos have abnormalities and functional impairments affecting hearing, equilibrium, and vision. The lack of pigment in the eyes of albinos results in retinal damage in brightly lit rooms. Studies comparing albino and pigmented animals show differences even in drug metabolism.

Delayed Bone Growth Plate Closure

Unlike most other species, these growth plates can remain open well after sexual maturity and stay open throughout the average life expectancy of a typical rat, which may directly impact orthopedic studies.

Behavior

Rats are primarily social creatures (tend to play and sleep in groups), generally do not fight, and are nocturnal. They actively investigate their surroundings due to natural curiosity. For more information, contact the UAB ARP Animal Behaviorist.
Handling

Proper Handling

It is essential you pick up rats by the base, not the tip or mid-tail. Picking up a rat by the base of its tail as a means of restraint is acceptable but only for short trips! The three common methods of handling rats are:

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<td>Forefinger and thumb crossed over the chest with one hand in front of the front legs and the other hand behind</td>
<td>Forefinger and index finger around the neck in a “V,” and the thumb and ring finger behind the front legs</td>
<td>One hand gathers up the skin around the scruff of the neck</td>
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Restraint

No matter how you manually restrain a rat, be careful not to restrict its breathing by squeezing its chest. There are commercial restraint devices available. Contact the [UAB ARP Veterinarian Staff](#) for more information.

Detecting Pain and Distress

Assessing pain and distress in rats is difficult at times because rats commonly conceal outward signs of moderate pain and suffering. Behavioral changes that reveal a rat's pain and suffering may be subtle and elude detection unless observations are thorough and made by a trained observer. Severe pain and suffering cause overt clinical signs in rats. Therefore, laboratory staff working with rats should be taught to check their activity level, behavior, appearance, body condition, and vital signs. For more information, see this comprehensive [checklist](#).
**Chronic State of Pain and Distress**

A chronic state of pain or distress can be difficult to detect until it becomes overtly apparent. The rat showed here, is presenting the following signs:

- Emaciation: evidenced by the wasted epaxial musculature along the spine
- Anemia: notice the pale color of the eyes and the skin of both the ears and face
- Generalized Alopecia: thinning or loss of hair (indicative of chronic disease)
- Piloerection or ruffled fur coat: a nonspecific sign of disease

**Procedures for Injections and Blood Collection**

**Volume Recommendations**

Exceeding 0.1 ml per 10 grams of body weight, or about 10% of total blood volume for collections produces a hypovolemic shock even when performing multiple collections over time. The regeneration time for blood cells is approximately two weeks. When removing large quantities of blood, it may be necessary to administer warmed physiological fluid to replace the volume of blood collected. For more information, see volume recommendations.

The recommended gauge size of a needle in blood collection and fluid administration is 23 to 27. Larger needles (meaning smaller gauged number) may be necessary for injecting large volumes or thick materials. When accessing the tail, lateral saphenous, and cheek veins anesthesia is not required, but sedation may be used to increase vein visibility by peripheral vein dilation and may help immobilize the animal. For hands-on training, contact the UAB ARP Veterinarian Staff.

**Methods Commonly Used for Blood Collection or Fluid Administration**

If you are not trained in blood or fluid collection techniques, contact the UAB ARP Veterinary Staff before attempting the procedure.
Tail Vein

Anesthesia is not required with proper restraint. Volumes allowed are approximately up to 1 ml. A tourniquet at the tail base is helpful to dilate the vessel. Dipping the tail in warm water also helps vessel dilation. Sedation can be used to enhance vein visualization by peripheral vasodilation (drug effect) and reduce the animal’s struggle due to distress.

Lateral Saphenous Vein

Anesthesia is required. Volumes collected are approximately up to 1 ml. The vein is punctured percutaneously after fur removal. Then blood is passively collected in capillary tubes as it pools on the skin. The collection is aided by applying petroleum jelly or stopcock grease to the area so that the blood creates a bubble instead of spreading out on the skin.

Jugular Vein

Anesthesia is required. You may remove volumes up to 10% of the blood volume from multiple collections over two weeks.

Cardiac Puncture

This method requires anesthesia and is a terminal procedure. There are two methods of collection: ventral midline and left lateral recumbency.

Retro-Orbital Puncture

Anesthesia is required and using a topical ophthalmic anesthetic is recommended. Volumes up to 1 ml are allowed. The optic nerve and other nearby structures are at risk of injury when using this method. Alternating the puncture site between eyes and corners reduces injury and discomfort for repeated collections.
Procedures for Injections

If you are not trained proper injection techniques, contact the UAB ARP Veterinary Staff before attempting the procedure.

**Intraperitoneal (IP)**

Inject the fluid into the lower quadrant of the abdomen. Both sides are acceptable (right or left). Avoid the midline to avoid the bladder. Enter approximately at the level of a relaxed knee to avoid the inguinal fat pad.

**Subcutaneous (DQ or SC)**

Tent the skin – typically over the back of the neck area. Insert the needle at the base of the tent. Pull back on the plunger to check for a flash of blood in the hub of the needle. If blood is present, reposition and try again.

**Oral Gavage**

Oral gavage requires a specialized gavage needle. The needle design helps to prevent instillation into the lungs. Contact the UAB ARP Veterinary Staff for specific training before attempting this technique.

**Intramuscular (IM) Injections**

The recommended injection location is in the cranial thigh muscles (quadriceps femoris). This muscle group is the largest available for injection. Also, it avoids complication of involvement of the sciatic nerve. Muscle mass is limited in rats; therefore, use this method only when necessary or required.

**Analgesics, Sedatives, and Anesthetics**

**Analgesics**

An analgesic is a medical term used for pain medicine or those drugs that help to control pain. The analgesic regimen should be chosen to match the

Under **no circumstances**, should pain medications not be provided following a painful procedure unless justified in your approved IACUC Protocol!
expected duration of pain or discomfort caused by the procedure. If you have any questions, contact the UAB ARP Veterinary Staff.

### Sedatives

Sedatives may blunt consciousness, but in normal doses do not do so sufficiently to ablate pain or other sensations. When combined with general anesthetics, they may be used to induce "balanced" anesthesia where muscle relaxation, unconsciousness, and analgesia are enhanced. Some sedatives also have analgesic effects. When combined with general anesthetics, balanced anesthesia is attained, and these sedatives improve analgesia through specific effects.

### Anesthetics

An anesthetic regimen should be chosen to match the duration of drug effects with the length of the procedure. Explosive agents, such as Ether, cannot be used.

- For long or short procedures, gaseous anesthesia using a non-explosive agent such as Isoflurane is often the most practical method to sustain uniformly adequate levels of anesthesia.

- You should not use short-acting agents or regimens for lengthy procedures. Repeat drug administrations may be necessary to prolong unconsciousness and do not provide a uniform level of anesthesia.

**Neonates**

Hypothermia may be used as an anesthetic for neonates (newborn) seven days of age or younger. Alternatively, inhalation anesthesia with an agent (such as Isoflurane) administered using a non-rebreathing system may be an acceptable alternative to hypothermia in neonatal rodents. For instruction in this technique, consult the UAB ARP Veterinary Staff.

### Surgery Techniques/Requirements

If you are planning to perform surgical procedures on rodents, you must complete Rodent Surgery. We also strongly recommend you complete Post-Procedural Care of Mice and Rats in Research, as well.
Euthanasia

UAB requires two methods of euthanasia listed on your approved IACUC Protocol. If you have not been trained to euthanize rodents, contact one of the UAB ARP Veterinary Staff before attempting the procedure.

**Primary Methods**

Following are examples of acceptable primary methods of euthanasia with IACUC approval:

1. Inhalants (e.g., CO$_2$ and Isoflurane)
2. Injectables (e.g., Fatal Plus, Ketamine/Xylazine)
3. Physical, (e.g., rapid decapitation, microwave euthanasia)

**Secondary Methods**

To ensure death, you must follow primary euthanasia (e.g., inhalants and injectables) with a secondary method such as:

*Cervical Dislocation*

Cervical dislocation is used in unconscious rodents and involves the physical separation of the skull from the vertebrae by applying force resulting in the severing the cervical spinal cord. This method is only acceptable in rats weighing less than 250 grams.

*Thoracotomy*

This method is used in unconscious rodents and involves cutting open the thoracic (chest) cavity which induces a pneumothorax prohibiting the expansion of the lungs and leads to irreversible oxygen deprivation and death.

*Exsanguination*

Exsanguination involves allowing an unconscious animal to “bleed out.” In essence, this acute loss of a large volume of blood results in death.
Decapitation

Decapitation is also used in unconscious rodents and involves physical removal of the head from the body. The equipment used to perform decapitation should be maintained in good working order and serviced regularly to ensure such.

Other Information

Restraint devices are available minimizing the chance of injury to personnel and improve the positioning of the animal for any procedure. Other techniques are available for rodent euthanasia and may be acceptable when scientifically justified by the user and approved by the IACUC. For more information on euthanasia techniques, consult with the UAB ARP Veterinary Staff, and see the IACUC SOP on Animal Euthanasia.

Carcass Disposal

Before placing euthanized rodents in a bag, you must make sure the rats are dead! Rats can stop breathing for a minute or more and can regain respiratory function and survive. Younger rats are resistant to Carbon Dioxide asphyxiation and take longer to succumb than adult rats.

Locations with an Animal Morgue

Non-Hazardous Carcasses

1. Place the carcasses in a black plastic bag
2. Place the black bags in the Stericycle box in the morgue

Hazardous Carcasses

1. Place the black bags in the Stericycle box in the morgue.
Locations without an Animal Morgue

Non-Hazardous Carcasses

1. Place the carcasses in a black plastic bag
2. Place the bag in the designated refrigerator

Hazardous Carcasses

1. Place the black bag inside a red bag marked with the hazard
2. Place it in the designated refrigerator

Carcasses Contaminated with Radioactive Hazards

Carcasses contaminated with radioactive hazards should be disposed of as indicated by the PI’s Radioactive Materials License.

Occupational Health Issues

Personal Protective Equipment (PPE) is required when working with rats. Always check the AUSI on the door (if posted) to ensure that you have on the right PPE. PPE is available at the entrance of each facility, and you must put on shoe covers to enter any facility.

- To enter an animal housing room, you must wear a clean, buttoned lab coat, a designated uniform or disposable gown. Respiratory equipment requires annual checks.
- You should wear appropriate gloves when handling rats. Never handle rats ungloved.
- When opening cages, wear a face mask, or conduct work in a changing station or Biosafety Cabinet.
- Remove your PPE in the proper place. Never wear possibly contaminated PPE outside the facility.

Allergens

People can develop an allergy to rat dander after having contact with them for some time. Persons who establish allergy symptoms should seek medical counseling and may have to discontinue working with this species.
Zoonotic Agents

The risk of transmission of zoonotic agents from laboratory rodents to research personnel is very low. Lymphocytic Choriomeningitis Virus (LCMV) and Campylobacter species are common agents that can be found in rodents and passed to humans. Avoid these and other agents by using proper Personal Protective Equipment (PPE) and consistent hand washing. Injuries should be cleaned with soap and water immediately and reported to your supervisor.

Conclusion

This section concludes the Working with Rats in Research at UAB (AU_RA) Course Material. You should take the assessment now. The passing score is 80% or higher. For a glossary, reference guides, and other information visit the IACUC website.

Other Required Training

- If you are responsible for breeding mice or rats at UAB, you must complete Rodent Breeding at UAB (AU_RBR).
- If you are responsible for monitoring rodents after any procedure, you must complete Post-Procedure Care of Mice and Rats in Research at UAB (AU_PP).
- If you are responsible for performing any procedures on a rodent, you must complete Rodent Surgery (AU_RS).
- If you are working with any animal at UAB, you must complete Using Animals for Teaching, Testing, and Research at UAB (AU_UA).