

Module 11. Care and Use of Rodents

Objectives:

- to understand the basic principles involved in the handling and care of mice and rats for use in teaching and research,
- to acquire an overview of the technical skills needed to use rats and mice as research subjects

Introduction

In preparation for research and or teaching using rodents, it is important to minimize the stresses of environment, handling, and experimental procedures in research involving rats and mice, and important to understand the behavioural characteristics of these animals. There are wide variations among various strains of rats and mice which can present major challenges in their housing and handling. Rodents are generally social animals although some can be aggressive depending on the breed and gender. Normal mouse behaviour usually involves climbing, digging, nesting and foraging, while rats exhibit similar behaviours but they are not apt to build nests. It is important to become familiar with the specific strain of mice or rat when planning a research program or to be used in teaching. Also, Module 5 should be consulted as several sections in this module are applicable to research using rodents.

Acquisition of Animals

At the University of Windsor, all mice and rats are ordered from designated (and reliable) suppliers or breeders at the request of the principal investigator with an approved AUPP. Upon arrival each container is inspected for damage and for the health status of the animals.

Depending on the type of animal, any animals exposed to the external environment during transport but otherwise look healthy, are placed in the quarantine room for at least one week.

Except for animals to be used in the barrier housing rooms. These should be euthanized.

Otherwise, any sick or unhealthy animals are euthanized as soon as possible so as not to stress

the animals in the shipment further and or contaminate other animals in the shipment or existing colonies. Often, the shipping containers are disinfected to avoid contamination of other rodents whose health status varies in the animal holding rooms.

After the age, sex and weight of the animals have been recorded and confirmed to be consistent with the animals ordered, the following action is taken;

- food and water are provided in the holding cages;
- any clinical abnormalities are recorded which will require treatment after diagnosis is reconfirmed and to ensure that animals adapt to new living conditions.
- The animals are closely observed for their consumption of food and water following the first 24 to 48 hr of receipt.
- Additionally, the animals should be observed for any clinical abnormalities as a result of transit or fighting due to altered cage populations.

Acclimation and Quarantine of Animals for Research

Acclimation of the animal following transport and exposure to a new housing environment provides a more stabilized animal for research use. However, additional stressors may occur after acclimation in animals that are naive to the experimental techniques such as handling, dosing, and restraint. Numerous studies demonstrate the beneficial effects of frequently handling research animals before initiation of study protocols as well as in early life. Animals habituated to a handler in early life show less handling stress in later life and react only to the particular experimental stimuli used in the study, whereas non-handled animals are much more likely to react to a new handler as well as to the experimental stress(es). Generally, mice and rats become tame and rarely bite when accustomed to being handled. It is generally agreed that exposure to minimal stresses such as handling before an unavoidable stressful event diminishes the negative effect of the secondary stress event and generates a potentially protective effect. This effect is preferable to a significant primary stress event in a naive animal never exposed to any type of stress.

The type of special diet, the ingredients, and the diet form (e.g., pelleted vs. powdered, crumbly vs. hard) should be evaluated and may result in additional requirements for the care of the animal. Diets high in fat often cause an animal's coat to become greasy and may delay

wound healing whereas diets high in salt increase an animals water consumption and require more frequent refilling of the water bottle.

The use of an unprepared animal in an experiment has a potentially significant effect on the data collected. Failure to acclimate and/or stabilize rodents may result in a need for increased animal numbers to determine statistically significant differences in experimental results. Such an outcome is inconsistent with the 3R's that animal researchers are expected to consider when planning their research.

Upon arrival mice and rats should be kept for a minimum of 3 days (one week if possible) in isolation in a quarantine room to monitor their health while allowing the animal to acclimate to its new environment. Subsequently, mice and rats should be kept in separate rooms with other like animals.

Housing

Social housing produces different effects on rodents as research models. There are degrees of sociality from simple aggregations to animals with highly intertwined and dependent social structures. Sociality also varies within a species due to many factors such as strain, age, and gender. It has been suggested that in some circumstances, individually housed rodents do not represent as robust a research model when compared with group-housed animals (two or more mice or rats per cage). The effects of stress on mice and rats as a result of long-term isolation include decreased food consumption and reduced weight gain. Abnormal behaviour such as aggression and excitement may occur when singly housed rodents are exposed to a cage cohort in a group housing situation. Additionally, abnormal behaviours include decreased male mating and decreased maternal care in long-term singly housed mice and rats. Of course, the behaviour pattern is influenced by the strain/stock of rodent used as well as the duration of isolation stress. The effects of stress due to transport have been shown to be diminished by group housing as a standard procedure. In the case of Sprague-Dawley rats, housing of four animals per cage showed a weaker and shorter stress response than SD rats housed singly or in pairs.

Although group housing appears to result in a more stable research animal, the effects of social hierarchies may result in decreased testicular weights, increased adrenal cortical function, and greater splenic weights in subordinate male rats. Any change in animal cage densities may result in stress and require stabilization of the animals during an additional acclimation period.

Handling rodents housed individually or after changes in animal cage densities may accelerate the acclimation process.

Mice should be housed in plastic cages where possible which is warmer than metal and more comfortable for the animals. Rats should be housed in metal cages with both mesh and solid floor spaces to avoid damage to their feet over the long term. Adult mice require a minimum of 15 square inches each and a cage height of 5 inches, while rats need a minimum of 40 square inches each and a minimum height of 7 inches. Bedding should be clean, non-toxic and absorbent (usually wood chips) and at least 2 inches in depth. Cages should be cleaned at least one per week and the bedding replaced. These tasks are usually done by the animal care technician, but research students are responsible for ensuring that their animals are properly cared for, including proper feeding and water containers filled – especially over weekends.

The optimal temperature for mice and rats is between 20-30°C with a relative humidity of 40-70%. Twelve hour light cycles are preferred, but this is dependent on the type of research undertaken. The newly renovated facilities in Biology and Chrysler Hall South meet the standards required by the CCAC for the housing of rodents. Rodent food (Purina lab chow) is typically dry pellets or blocks containing 20-25% protein, 4-5% fats, and 5-6% fibre.

Environmental Enrichment.

The CCAC now requires that vertebrate animals including rats and mice be provided with some kind of environmental enrichments to maximize species specific behaviours while minimizing stress-induced behaviours (see also Module 6). Environmental enrichment consists of a combination of complex inanimate and social stimulation. Some researchers have resisted environmental enrichments who wish to minimize the effects of ever-elusive non-experimental variables. However, the stresses associated with limiting an animal's ability to express basic species-specific behaviours and the subsequent potential effects of that stress on both animal welfare and research results must also be considered. For example, provision of nesting material gives an animal the ability to hide from potential threats (humans or cage mates), changes thermo-regulation, affects exposure to light, and provides for increased activity, thus leading to complex mechanistic and quantitative determinations of the effect of bedding material on the animal. However, the literature on the effect of environmental enrichment on data variability and animal numbers has been conflicting. Researchers should search the literature for “toys” that are

specific-specific for inclusion in the animals cage. Nevertheless, the researcher needs to be aware and control of non-experimental variables when designing a research project to minimize the number of stressors and the severity of the induced stress. Control animals need to be exposed to the same activities and environments as the experimental animals. In summary, well-implemented enrichment may reduce data variability.

Surgery on Rodents

No surgery should be undertaken by students without the proper instruction by an experienced researcher following an appropriate Standard Operating Procedure (SOP) for the type of surgery to be performed. Rodent surgery must be performed under aseptic conditions and with the appropriate anaesthetic (See Module 10) to avoid undue stress and subclinical infections. If an incision takes longer than a week to heal then the surgical technique needs to be modified and improved. Animals should regain their pre-surgical weight in a couple of days, otherwise poor surgical technique was used or poor post-operative care was administered.

Euthanasia

Carbon dioxide (CO₂) is commonly used for euthanasia of rats and mice in the laboratory because of its ease of use, relative safety, low costs and capacity to euthanize large numbers of animals at the same time. However, it should be kept in mind that the administration of CO₂ does cause stress in rats and mice and should only be used under controlled conditions and by the animal care technician. There is considerable research on finding a less stressful method to euthanize mice and rats, but the use of injectable anaesthetics is generally not practicable for a variety of reasons including the cost and regulations for handling and administering an overdose of a barbiturate such as pentobarbital.

Occupational Health and Safety

The greatest occupational risk to working with rodents is allergic reactions. Animal products such as dander, hair, fur, saliva and body waste contain powerful allergens that can cause both respiratory and skin conditions. Symptoms of an allergic reaction usually occur in the nasal passages, eyes, or skin. To avoid an allergic reaction wear nitrile gloves when working with mice and rats. Also, wash your hands before and after handling mice and rats. Laboratory

coats should also be worn at all times and, if necessary, a dust mask can be used to minimize aerosol transmission of zoonotic agents.

Potential zoonosis (disease of animals transmissible to humans) can occur when working with mice and rats, especially if the handler gets scratched or bitten by the rodent. However, the development of a zoonotic disease in humans usually requires a pre-existing state that compromises the immune system. If you are at risk, you should contact your physician should a bite or scratch occur when handling a mouse or rat. Some potential zoonotic diseases found in laboratory rodents include Leptospirosis, Yersinia, Rat Bite Fever, and Hantavirus infection. While these diseases are rare in research rats and mice, good hand washing and wearing of gloves are usually adequate protection from these diseases.

Researchers handling mice and rats must also avoid exposure to the inhalation of anesthetic gases as some can cause hepatic toxicity after repeated exposures.