Behavioral Biology of the Laboratory Mouse and Its Relevance to Welfare
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NOTE: This summary is based on a Brown Bag Seminar presented by Dr. Sherman during the fall of 2012.

Classified in the mammalian order Rodentia, the mouse (Mus musculus) has been associated with human agriculture practices since approximately 12,000 years BC. One subspecies in particular, the Western House Mouse (Mus musculus domesticus), has become specialized as the laboratory mouse. In terms of their life history, mice are well adapted to laboratory life. They are characterized by rapid maturity and high reproductive potential. Females can give birth to one litter per month. The lifespan of a free-living mouse is 100-190 days, while the lifespan of a laboratory mouse is up to 3+ years. In the wild, mice are high adaptable, with great behavioral adaptability to various habitats.

However, some characteristics are considered part of the behavioral biology of the species.

As stated in the most recent version of the Guide for the Care and Use of Laboratory Animals (8th edition, p. 121), “It is … essential that personnel caring for and using animals be trained in: species-specific and individual clinical, behavioral, physiologic, and biochemical indicators of well-being.” (p. 121) and the behavioral biology of the species they work with…to identify the development of adverse or abnormal behaviors.” (p. 53).

In spite of their short lifespan and ubiquity, mice kept in laboratories should be managed in order to ensure their well being. Not only is such an imperative consistent with recommendations in the Guide for the Care and Use of Laboratory Animals (2011), but such management will improve data quality by avoiding “stress” responses that can

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CO2 EQUIPMENT REQUIREMENTS
(Based on 2013 AVMA Euthanasia Guidelines)

The Duke IACUC has implemented changes to the Policy on Euthanasia in rodents using CO2, based on updates to the AVMA Guidelines (2013). The most important change involves prohibition of “pre-charging” the chamber. Cages, chambers must be filled gradually at a measured rate delivered through the use of a flow-meter. This requires three specific pieces of equipment:

1. A caging system that allows atmospheric air to begin the process of euthanasia.
2. A regulator that provides 50-60 PSI line pressure.
3. A CO2 flow meter that regulates to l/min

DLAR has provided several cages and lids which were unserviceable for animal housing (but perfectly good for euthanasia), which the OAWA modified to accept a new gas connection. While several options may be employed, the preferred approach is to use the animal’s home cage with the euthanasia lid if possible for euthanasia. But it that is not possible, then the use of a secondary with the euthanasia lid. The photo shows the modified euthanasia lid (with CO2 label properly affixed), which can be obtained by contacting Bill Wade (w.wade@duke.edu) to obtain a modified lid free of charge.

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Upcoming Deadlines

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Deadlines are 5 PM on the date listed!
lead to alterations from normal resting hematological, endocrine, and behavioral values. There is a human welfare perspective as well: studies suggest that caretakers and researchers express higher job satisfaction when the animals under their care are managed according to best welfare principles. From a safety perspective, "low stress" handling techniques can reduce the incidence of animal bites.

Studies of the behavioral biology of the laboratory mouse reveal information that we may use to optimize its welfare. These concepts should be incorporated into housing and hygiene practices for the maintenance of mice in the laboratory.

NESTS: When provided with appropriate substrates, mice build sleeping nests and maternal nests. In several choice studies, mice show strong preference for cages with material that may be used to construct a nest (Kawakami et al 2007). When offered various substrates that may be used to construct a nest, mice will show a preference (Blom et al 1996; http://oacu.od.nih.gov/wellbeing/RodentEE.pdf). Cloth and molded recycled paper are preferred over paper or wood shavings alone (Nam-Mi Gross et al 2011).

OLFACTION: Mice live in a rich olfactory world. In addition to olfactory mucosa, they have a specialized structure, the vomeronasal organ which directs aerosolized molecules into the olfactory epithelium. There is evidence that mice can use odor cues to identify a conspecific’s individual identification, its familiarity, its sex, and its reproductive and social status. Mice use scent glands to mark familiar areas or territories. The feces of recently frightened conspecifics and the feces of carnivores are aversive. In addition, olfactory marks are produced by the plantar glands on the feet of mice and they can navigate (in the dark) and return to a familiar area using these marks. The importance of familiar odor cues supports the behavioral changes mice exhibit with complete cage bedding changes.

HYGIENE. Although cage cleaning is necessary, it may also be stressful for the mice. Cage changes may disrupt behavioral homeostasis produced by familiar odors. There is evidence that a longer-than-conventional inter-cage change interval (2 week) may be behaviorally beneficial. One study was conducted with 15 cages of 5 ICR females/cage, with bedding unchanged for 17 days Cages contained aspen bedding and were individually ventilated. Although animal caretakers assessed the cages as “dirty,” after 17 days, there were no detectible effects on fecal corticosterone, bedding weight, mice mass, atmospheric analysis, abnormal behaviors per video analysis, or brochoalveolar lavage samples (Rosenbaum et al 2009).

FORAGING: In free-ranging situations, mice are omnivores. Depending on a number of variables, mice eat up to 20% of their body weight daily, spending much of their day foraging for seeds, nuts, fruits, roots, meat, living insects. For caged mice, methods that allow them to spend time foraging and manipulating foodstuffs will best simulate their natural behavioral repertoire and provide environmental and behavioral enrichment (Bayne et al 2002).

CAGE BEHAVIOR: Mice exhibit thigmotaxis (object orientation), expressed by orientation toward a wall or other vertical surface. Considered a heritable trait, thigmotaxis is a predator avoidance strategy which has been used as a measure of anxiety. It decreases with familiarity of the environment and the presence of conspecifics (Leppänen et al 2005, Simon et al 1994). Other signs of decreased well-being include stereotopies such as: barbering, excessive grooming, back flipping, bar mouthing. These signs may be improved or prevented with social and environmental enrichment (Olsson & Dahlborn, 2002).

HUMANE HANDLING: Rough handling can be a significant form of distress by mice. Picking mice up in cupped hands or plastic tunnel decreases “stress” and anxiety compared to tail handling as evidenced by decreased defecation & urination, decreased avoidance behaviors, and increased facilitation of maze entry (Hurst and West 2010).

ASSESSMENTS: How do we assess the welfare of mice in our care? Assessments of positive welfare include evidence of the expression of normal behaviors including nesting, resting, and positive social interactions (Guide for the Care and Use of Laboratory Animals, 8th edition, 2011). Similarly, the absence of abnormal behaviors such as thigmotaxis, stereotypies (barbering, cage circling) and aggressive social interactions are other indicators of welfare. In addition to behavioral measures, laboratory values and research outcomes may serve as welfare indicators.

In conclusion, our understanding of normal rodent behavior can suggest best methods for meeting the needs of mice as well-managed laboratory subjects.

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SELECTED REFERENCES

For
‘Behavioral Biology of the Laboratory Mouse and Its Relevance to Welfare’

REPORTING MISSING or ESCAPED ANIMALS

Even under the most controlled circumstances, adverse events may occur. When an animal escapes the holding cage, it is important that measures to capture the animal are engaged. It is not acceptable to consider it ‘returned to the wild’ or ‘free to roam the building.’ The goals of recapture are to: a) prevent an injury to an animal unaccustomed to the out-of-doors; b) to prevent a transgenic animal from passing their modified genes to other animals; and c) to prevent the spread of potential pathogens (if the animal is infectious).

According to Duke animal care program policies, all personnel who work with animals must be trained in handling, restraint, and capture of animals. The responsibility for ensuring appropriate training of the research staff lies with the PI (for PI managed spaces) and with DLAR (for DLAR managed spaces).

Select considerations for recapturing animals include:

- A rodent which has escaped should not be handled by hand. Use a hard container (e.g., a cup or empty cage) when capturing animals.
- Animals found in a trap or on the floor must be placed in a clean cage with food and water.
- A label using the word “compromised” must be affixed to the cage. This denotes that the animals may not be healthy and should be handled as if infected.
- A DLAR veterinarian must be notified immediately after the animal is captured.
- If the responsible PI can be determined, they will be notified immediately.
- If you suspect an animal is missing, check the room mortality log to see if an animal has died and the carcass removed for refrigerated storage.
- If you cannot determine that an animal is missing, or you know it is missing and cannot find the remains, then contact the DLAR supervisor.

It is especially important to notify DLAR management if the missing animal is a transgenic animal, KO/KI, or an animal with recombinant DNA. According to NIH policy, loss of these animals may require notification of the NIH Office of Laboratory Animal Welfare.

STANDARD OPERATING PROCEDURE REQUIREMENTS FOR ABSL2 CONTAINMENT AT DUKE

The Principal Investigator (PI) has the responsibility to inform the laboratory personnel of the appropriate research procedures. When using hazardous or regulated biological agents the PI must prepare a written Standard Operating Procedure (SOP) outlining the necessary precautions to safely conduct research. An SOP is a set of specific guidelines designed to address the methods that will be used and the safe handling of biological agents. The SOP must be available in the laboratory.

The SOP is a valuable tool and worth the preparation time. A well-written SOP can be used to satisfy several compliance requirements. SOP should be written for all procedures that pose an identified potential risk to the health and safety of the laboratory personnel, although a separate SOP does not need to be written for each individual experiment, procedures with the same hazards can be combined into one SOP.

The process of writing SOPs requires an individual to think through all steps of a procedure and perform a risk assessment before work has begun. The best approach to writing an SOP is to do it, write it and test it. Be brief and succinct; the shorter the better. A SOP template is available on OESO Biosafety Web Site:

http://www.safety.duke.edu/BioSafety/Animals.htm

OESO HAS SEVERAL GUIDELINES FOR SOP DEVELOPMENT OF HAZARDOUS AGENT USE IN ANIMAL PROTOCOLS

OESO Biosafety Division has a great web site which assists researchers with specific SOP development! For example:

- Guide for Developing an SOP for the use of Biohazards in Animals
- Guide for Developing SOP for the use of Hazardous Drugs
- SOP for the use of Toxic Chemicals in Animals
- Guidelines for the Safe Handling of Animals Exposed to LPS in Research
- Radiation Safety Animal Care and Use Protocol Wizard

You can reach this site and use this links be going to the OESO Biosafety site at:

http://www.safety.duke.edu/BioSafety/Animals.htm
Did you know that a child born today in the United States is expected to live into his or her late 70s, whereas the same child born at the turn of the 20th century would have been expected to live only into his or her 40s? Eating better and learning how to keep ourselves and our environment clean played a role, but medical research using laboratory animals was a vital factor driving this incredible progress.

What is medical research that uses animals?

Animals and people get many of the same illnesses. Certain types of animals can stand in for humans with particular diseases. The information we gain from these studies—about how we’re the same and how we’re different—benefits people and animals.

Medical research with animals is one type of medical research, but other types include experiments with cells and chemicals and simulations on computers. Animal research usually describes research involving vertebrates, such as cats, mice, frogs, pigs, and primates. Most animals used in research are specifically bred for use in medical research.

Another important type of research is clinical research, in which scientists conduct studies with humans. These studies almost always require the results of preliminary tests in animal research studies.

### Table 1. Medical Research with Animals Saves Lives

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Medical Benefit for People</th>
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<tbody>
<tr>
<td>Dog</td>
<td>Discovery of insulin</td>
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<tr>
<td>Monkey</td>
<td>Polio vaccine</td>
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<tr>
<td>Mouse</td>
<td>Rabies vaccine</td>
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<td>Pig</td>
<td>Skin grafts for burn victims</td>
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<td>Pig</td>
<td>Computer-assisted tomography (CAT) scans</td>
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<td>Rabbit</td>
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<td>Rat</td>
<td>Carcinogen screening</td>
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How do scientists decide to use animals in medical research?

All medical research is carefully planned, and this includes medical research with animals. Experts who review a scientist’s proposed experiment involving animals weigh several considerations before approving each study.

The most important thing is that the research must be relevant to human or animal health. Studies need to protect the animals’ welfare. That means that only the fewest number of the most appropriate species may be used. Under federal law, all animals must be treated humanely and undergo the least distress possible.
Who does medical research with animals?

Medical researchers who have Ph.D., D.V.M., or M.D. degrees oversee animal research studies. These scientists study animals because they are a lot like people when it comes to basic body functions like breathing, eating, hearing, and seeing. That’s because nature is extremely economical. Throughout vast evolutionary time—from bacteria to plants to people—the same biological processes are recycled over and over.

Veterinarians with specialized training in laboratory animal medicine are an integral part of a medical research team. As part of this research group, veterinarians assure the humane treatment of animals and provide medical and surgical support throughout research studies. Emergency veterinary care for research animals is available on a 24-hour basis.

How are animals protected?

Congress and the Public Health Service have set up laws, regulations, and policies to ensure humane treatment of all animals in research. The Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act give details about day-to-day animal care. (Links to these publications are provided at the end of the fact sheet.)

Scientists use this and other information to answer important questions about setting up the proper environment for research animals. How big should the cages be, and how warm or cool do the animals need to be to stay healthy? What kind of food is best, and how much noise do the animals like to have (some like it very quiet)? Do the animals like bright or dim light, and do they need other animals or toys to play with?
Why do medical research with animals?

Results from animal studies are crucial for closing knowledge gaps about health and disease in both humans and animals. Understanding cell and organ function—which is similar in all vertebrates—helps researchers design experiments to test new treatments in people.

Cell culture studies or computers are important but cannot at present take the place of research models that use animals. No single set of results from a particular model—whether animal, cell, or computer—can predict exactly what will happen, so researchers often ask the same questions in different kinds of studies. When different models yield similar results, the results are much more believable.

Computer Models in Research

Even though computer models are very valuable, they are limited by what is already known about a process or disease. Data for computer models often comes from animal studies. In turn, computer models reveal gaps for further study in living organisms. Thus, medical research with animals and computer modeling studies work together to increase our understanding of health and disease.

In Vitro Studies in Research

In vitro experiments are performed in test tubes and plastic dishes. These studies usually use tissues or cells obtained from animals or people. When scientists study living cells in laboratory containers, they cannot reproduce the whole, complex, interactive system that is present in an animal or a human. But researchers can learn a lot from in vitro studies. The results of these experiments help scientists design further experiments to conduct in an animal.

Where is medical research with animals conducted?

The National Institutes of Health funds most of the basic medical research in the United States and beyond. This research takes place at universities and medical schools in all 50 states. In turn, biotechnology and pharmaceutical companies, often in partnership with the NIH, expand on this foundation of knowledge to develop medical treatments.

Every academic institution funded by the NIH that conducts medical research with animals is required to have a committee called the Institutional Animal Care and Use Committee that oversees care of animals in research. These committees are responsible for making sure that all the researchers at the institution obey the animal welfare laws. Additionally, the government even has rules about who should serve on these committees.
When do research results in animal studies get applied to humans?

Sometimes quickly, sometimes slowly. Scientists don’t understand human biology enough to risk using new medical treatments or surgical procedures directly on people. Because research is a quest to understand the unknown, the rate of progress varies a lot. In research, one discovery builds upon another.

Nearly everyone considers finding lifesaving cures through biomedical research to be one of humanity’s highest purposes. Although research helps humans protect and provide for themselves, humans are also the only species capable of considering the needs of other species on the planet we share.

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<th>Discovery</th>
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<td>Research on viruses</td>
<td>Dog parvovirus vaccine</td>
</tr>
<tr>
<td>Research on reproduction</td>
<td>Breeding programs for endangered species (like pandas, white tigers)</td>
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<tr>
<td>HIV/AIDS research</td>
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<tr>
<td>Chronic disease treatments</td>
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Learn more at these Web sites:

- National Institutes of Health
  http://www.nih.gov
- Animals in Research
  http://science.education.nih.gov/animals
- Living Laboratories
- NIH Office of Laboratory Animal Welfare
  http://grants.nih.gov/grants/olaw/olaw.htm
- Public Health Service Policy on Humane Care and Use of Laboratory Animals
  http://grants.nih.gov/grants/olaw/references/phspol.htm
- Guide for the Care and Use of Laboratory Animals
  http://books.nap.edu/readingroom/books/labrats
- Animal Welfare Act
  http://www.access.gpo.gov/uscode/title7/chapter54_.html
- Office of Animal Care and Use Regulations and Standards
  http://oacu.od.nih.gov/regs/index.htm
- NIH Clinical Research
  http://grants.nih.gov/grants/funding/PHS398/instructions2/p2_human_subjects_definitions.htm
- ClinicalTrials.gov
  http://clinicaltrials.gov