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Office of Research Infrastructure Programs

Rigor and Reproducibility of Animal Studies: Extrinsic Factors Workshop

Session 2. Rodents

September 28, 2022
Virtual Meeting

Final Report

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Executive Summary

The Extrinsic Factors Workshop was held in three sessions to better understand extrinsic factors and their effects on biomedical research. Session 2 was focused on extrinsic factors in the use of rodent animals for biomedical research. Drs. Elizabeth Bryda, James Fox, and David Wiest served as the Session 2 co-chairs. Discussions in Session 2 addressed the effects of housing environment, equipment modernization, and new and emerging monitoring methods in addressing the need for rigor and reproducibility in rodent research. The speakers identified various extrinsic factors for consideration in research, including personnel, caging type, density, thermoregulation, food and water, bedding, enrichment, cage-change frequency, species-specific measures of behavior, the microbiome, housing density, lighting (e.g., quantity, spectral quality, duration), vibration, and air. The participants also discussed the need to balance energy-saving measures (e.g., retrofitting of light-emitting diode lighting) with scientific needs within facilities. In discussion, several participants noted that extrinsic factors in animal research never will be standardized fully across institutions, because some external variables always will be present. Additionally, it was proposed that variation within animal studies might better represent the biological systems of humans. The need for increased federal support on this topic, as well as for collaborations across both facilities and communities, was emphasized throughout the discussion.

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Elizabeth Bryda, Ph.D., University of Missouri
James Fox, D.V.M., M.S., DACLAM, Massachusetts Institute of Technology
David Wiest, Ph.D., Fox Chase Cancer Center

Presenters
Brian Berridge, D.V.M., Ph.D., DACVP, National Institute of Environmental Health Sciences
George Brainard, Ph.D., Thomas Jefferson University
Jeffrey Everitt, D.V.M., Duke University
Mitchell Galanek, Radiation Protection Officer, Massachusetts Institute of Technology
F. Claire Hankenson, D.V.M., M.S., DACLAM, University of Pennsylvania
Ken Henderson, Ph.D., Charles River Laboratories
Vivek Kumar, Ph.D., The Jackson Laboratory (JAX)
Neil Lipman, V.M.D., Memorial Sloan Kettering Cancer Center
Steve Niemi, D.V.M., Boston University
Randall Reynolds, D.V.M., M.S., Duke University
Karen Svenson, Ph.D., JAX

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Workshop Report

Opening Remarks

James Fox, D.V.M., M.S., DACLAM, Workshop Chairperson, Massachusetts Institute of Technology
Xiang-Ning Li, M.D., Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Oleg Mirochnitchenko, Ph.D., ORIP, DPCPSI, OD, NIH

Dr. Xiang-Ning Li welcomed the attendees to Session 2 of the workshop. Dr. Li reminded the participants of NIH's dedication to rigor and reproducibility, which was emphasized by Dr. Robert W. Eisinger, Acting Director, DPCPSI, during Session 1. In 2021, the Advisory Committee to the NIH Director (ACD) Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research recommended that the NIH encourage and support work to better understand, monitor, record, and report important extrinsic factors related to animal care that might affect research results. ORIP is modifying its infrastructure programs to address reproducibility in animal studies. The Extrinsic Factors Workshop seeks to better understand extrinsic factors and their effects on biomedical research.

Dr. Li also reminded the participants that ORIP has long devoted efforts to enhancing rigor and reproducibility, as emphasized by Dr. Franziska Grieder, Director, ORIP, during Session 1. ORIP has supported this effort through scientific research workshops (e.g., Zebrafish and Other Fish Models: Extrinsic Environmental Factors for Rigorous Experiments and Reproducible Results; Validation of Animal Models and Tools for Biomedical Research) and publications of future funding opportunity announcements (e.g., NOT-OD-22-039). This workshop is one of several steps toward fulfilling ORIP's Strategic Plan by addressing the important endeavor of enhancing animal study rigor and reproducibility in NIH-supported research.

Dr. Oleg Mirochnitchenko also welcomed the attendees. He provided examples of extrinsic factors related to animal research, which include temperature, humidity, noise, and lighting. Housing conditions—such as size and material of enclosure, number of animals per enclosure, bedding material and thickness, and cleanliness and cleaning schedules—also must be considered. Dr. Mirochnitchenko emphasized that the effects of extrinsic factors can be highly complex and often include multiple interactions. This issue has been understudied and under-documented. The goal of the workshop is to discuss the current status, needs, and strategies related to management, monitoring, and reporting of extrinsic factors to enhance the reproducibility and rigor of animal research. The focus is on the most widely and commonly used animal models, relevant extrinsic physical factors, and modern technologies. Dr. Mirochnitchenko expressed appreciation to the organizing committee members, speakers, and participants for their engagement.

Dr. James Fox, Workshop Chairperson and Session 2 Co-Chair, previewed Session 3, which will focus on large animals (i.e., nonhuman primates and swine). He emphasized that the topic of extrinsic factors is highly relevant to biomedical research, both for investigators and vivarium staff members. Dr. Fox also introduced Drs. Elizabeth Bryda and David Wiest, Session 2 Co-Chairs.

Keynote Presentation: Impact of Extrinsic Factors on Rigor and Reproducibility in Rodent Research

F. Claire Hankenson, D.V.M., M.S., University of Pennsylvania

Dr. F. Claire Hankenson presented on the ways extrinsic factors (as defined by the NIH Working Group) can affect rigor and reproducibility in rodent research. She emphasized the importance of explicit experimental planning to better control for these variables but noted that doing so has proven challenging. Many investigators have demonstrated improvement of reproducibility by enhancing external validity of

results. Dr. Hankenson also noted that the ACD Working Group was composed of various members of the research community, including research scientists, journal editors, statisticians and two veterinarians with expertise in a wide variety of animal models, as well as members of the internal NIH community.

Dr. Hankenson clarified the distinctions between reproducibility (i.e., getting consistent or duplicated results when starting from the same materials), replicability (i.e., getting consistent or duplicated results when using the same procedures or asking the same scientific question, but collecting new data), and generalizability (i.e., applying the results of a study in other contexts, situations, and populations). The Working Group was asked to consider various questions related to reproducibility, including what analyses can be performed to identify gaps and how the conditions in which animals are housed and bred affect experimental outcomes.

The Working Group identified the following extrinsic factors that can affect reproducibility: caging type, density, thermoregulation, food sources, bedding, water type, enrichment options, handling, cage-change frequency, species-specific measures of behavior, the microbiome, and refinements in care and well-being. Most of these factors are being tracked within animal facilities by animal care staff, but these data rarely are requested by scientific groups. Coordination between the veterinary and scientific communities therefore is needed. A review of relevant publications by members of the NIH Working Group also was performed and encompassed discussion on various species (rodents as well as larger animals and nonhuman primates), sex as a biological variable, neuroscience models, and statistical applications.

In its final report, the Working Group identified five themes in obtaining reproducible results for animal research: (1) improve study design and analytic rigor; (2) address bias, incomplete reporting, and questionable research practices; (3) improve relevance and use of animal models; (4) improve methodologic and results reporting; and (5) measure and evaluate effectiveness and costs. The Working Group expressed a shared foundational agreement, supported by the NIH Director, that animal studies contribute to significant findings and breakthroughs in both basic and translational research. Motivating problems that affect reproducibility were identified. First, transparent reporting of research methods is essential, yet frequent failures and shortfalls are present. Additionally, failure to record and report these factors degrades the reproducibility of findings. Furthermore, the completeness and granularity of reporting on animal husbandry factors is a quality issue and a topic for future research.

Dr. Hankenson highlighted several recommendations contained within the report's fourth theme. First, the NIH should expect that supporting data reported on animal research submitted in support of grant applications will include measures of quality and/or uncertainty for reported estimates and an interpretation of effect sizes within the context of the field. Additionally, the NIH should expect all vertebrate and cephalopod animal research to include the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 Essential 10 Checklist at the publication stage. Furthermore, the NIH should work to understand, monitor, record, and report important extrinsic factors related to animal care that can impact research results. In the report, the Working Group emphasized the value of open-source methods for sharing findings and data. Methods reproducibility is dependent on transparency, and inferential reproducibility relates to the concept of generalizability.

Dr. Hankenson emphasized that more discussion on extrinsic factors is needed. Recent studies have demonstrated the complexity of this issue. Factors for consideration include age, feeding, replications, behavioral assessments, housing conditions, the 3Rs (i.e., replacement, reduction, and refinement) and 3Vs (i.e., construct validity, internal validity, and external validity) of animal research, and therioepistemology. She asserted that the limits of reproducibility are not violations of the 3Rs. Sound and reproducible science ultimately affects one or more of the 3Rs and might affect investigators' abilities to conduct appropriate cost–benefit analyses if work must be repeated with additional animals. Good study design and good data analyses, however, also are essential from an ethical standpoint.

Dr. Hankenson also remarked that limits of reproducibility permit scientific study. Investigators still are learning how extrinsic factors affect research results, and this topic has emerged as its own discipline in recent years. Animal housing, handling, and husbandry will never be standardized fully across institutions, because other external factors (e.g., personnel, building and facility age, HVAC, weather/seasonal changes) always will be present. Additionally, human conditions of disease are not standardized as they are studied; it is unrealistic to expect that animal conditions be identical.
Dr. Hankenson encouraged the participants to consider the concept of reproducibility of scientific ideas and conclusions, rather than reproducibility of research. An acceptable level of research variability—one that permits trust in experimental outcomes—must be determined. She emphasized that this topic requires engagement of both veterinary specialists and researchers.

Discussion

- Ms. Karli Gilbert highlighted a recent paper indicating that the sex of experimenters has significant and consistent effects on mouse behavior across different laboratories. She wondered about efforts to include this variable in reports. Dr. Hankenson responded that this variable can be reported but will never be controllable. Researchers will never be able to account for every extrinsic factor. In response to a follow-up comment from Dr. Fox, Dr. Hankenson recalled that the study accounted for the sex of the experimenters but not the animal handlers.

- Dr. Reid Landes asked about the value of a laboratory's purposefully increasing the variability of extrinsic factors in experiments so that the controlled factors can be more robust. Dr. Hankenson spoke on the value of repeating experiments and considering how the variability of extrinsic factors affects the application of outcomes.

- Dr. Brianna Gaskill remarked on the importance of NIH-funded work to examine experimental variables, rather than simply reporting them. Dr. Hankenson agreed and noted that investigators bring unique perspectives on these factors into the discussion.

- Dr. David Ashbrook raised the need for support of researchers to use multiple genetic strains, thereby increasing genetic diversity in their studies. Dr. Peter Nathanielsz reiterated the importance of considering these differences in human and animal studies, particularly in regard to pregnancy studies. Dr. Richard Nakamura suggested consulting experts on the ethology of the experimental animals, in the context of the species in the wild.

- Dr. William Gause highlighted recent studies suggesting laboratory housing conditions might not reflect the environment in which mice evolved to live. For certain experiments, researchers might consider housing mice under more natural conditions.

- A participant noted that incorporation of more variables increases the cost of performing research, and NIH grant budgets are limited. This limitation is likely to affect experimental design and reproducibility.

Presentations: Housing Environment That Impacts Rigor and Reproducibility in Studies Using Rodents

Effects of Increased Housing Density in Research Mice

Karen Svenson, Ph.D., The Jackson Laboratory (JAX)

Dr. Karen Svenson discussed the physiological effects of housing density in mice. She began by sharing a brief history of the *Guide for the Care and Use of Laboratory Animals*. The eighth edition of the *Guide*, published in 2011, included a recommendation to limit cages with breeding females to 51 square inches.

This guidance suggests culling litters to accommodate the recommendation in practice and appears to eliminate the possibility of using a trio breeding format, which is a common practice in research facilities. Dr. Svenson also noted that the seventh edition of the *Guide*, published in 1996, encouraged animal studies based on sound science to further define institute-specific guidelines.

Dr. Svenson presented a schematic of duplex cage setups at JAX, explaining that the layout ensures compliance with current *Guide* recommendations for housing density. A team of JAX scientists designed a study to characterize the changes in well-being that occur with increased housing density in mice. They also investigated ways to measure well-being in studies to assess such effects. They increased cage density using two approaches: (1) increasing group size and maintaining a single cage size or (2) maintaining group size and using smaller, variable caged. Mouse "clinics" were used to perform broad-based live animal phenotyping via an internally adopted strategy for assessing multisystem physiology.

Physiological effects of housing density on C57B/6J mice were assessed over a 9-month period. Mice were housed in groups of either five or nine animals per cage. The researchers concluded that in B6 mice, housing at twofold density had no measurable adverse effects; in fact, heart rate and adrenal weight were reduced in the higher-density group for both sexes. Cage air temperature and quality were measured in the study, and the frequency of cage changing (i.e., 1 week vs. 2 weeks) was assessed. The higher-density cages were about 3˚C warmer than the lower-density cages and were closer to the animals' thermoneutral zone. Additionally, the animals consumed less food. Humidity and carbon dioxide did not differ with density. Follow-up density studies at JAX did not detect measurable adverse effects at any density. The research also examined litter culling, which did not lead to improved health.

Studies performed by other groups have contributed to a growing body of evidence indicating that most mouse strains can be housed at higher densities than is recommended currently by the *Guide* and maintain good health. Dr. Svenson noted that several relevant literature reviews have been performed. She identified remaining gaps in this area, which include effects of lower densities (e.g., comparing one, two, three, or four animals per cage), additional studies in static cages, and use of outbred strains. Dr. Svenson concluded by emphasizing that housing density is an important extrinsic factor, and various components (e.g., number of cages, type of cage ventilation, range of densities, single-animal housing, management of cage attrition, use of enrichment) should be reported in research.

Minimizing the Impact of Habitat Lighting on Experimental Reproducibility for Rodent Studies
George Brainard, Ph.D., Thomas Jefferson University

Dr. George Brainard presented on the influence of lighting in rodent research, with a focus on wavelength. He first outlined physical parameters of photic input: quantity (e.g., light irradiance, illuminance), spectral quality (i.e., wavelength), timing, and duration. Systemic effects of wavelength include changes to circadian behavior, testicular weights, accessory sex organ weights, spleen and thymus weights, lymphocyte counts, pineal melatonin, pituitary prolactin, pituitary hormones, plasma triiodothyronine and thyroxine, and plasma testosterone.

A profound difference is present in the wavelengths that influence the visual system, compared to those that influence rodent behavior and physiology. Melanopsin, a protein found in 1–3% of ganglion cells in the retina, provides the cells direct photosensitivity, allowing them to influence a wide range of physiological processes. Dr. Brainard highlighted a study comparing fluorescence and solid-state lighting in animal facilities. Dr. Brainard noted that light-emitting diode (LED) lighting offers several benefits over fluorescent lighting. The two lighting types cover a similar spectrum but display differences in peak patterns, including at the wavelength of melanopsin sensitivity. In the study, the only variable was spectral characteristics of the two light sources.

Changes in lighting affected both melanopsin content and melatonin rhythm. C3H mice maintained in LED lighting showed reduced food and water consumption and grew at rates representative of a more youthful phenotype. Neurohormonal changes also were observed. These factors are associated with the promotion of animal health and well-being and therefore might influence scientific outcomes.
Dr. Brainard emphasized that numerous scientific opportunities exist in this area, and this topic must be considered by investigators as lighting systems are retrofitted in the future.

Vibration as an Extrinsic Variable for Research Outcomes
Randall Reynolds, D.V.M., M.S., Duke University

Dr. Randall Reynolds discussed the effects of vibration on experimental outcomes. He explained that vibration can serve as a general stressor for animals. Effects can include changes to reproductive parameters (e.g., nursing in mice); increased heart rate and mean arterial pressure in mice; increased stress hormones in mice, rats, and swine; startle response and fear-related behaviors in mice, swine, and poultry; and changes in brain neuroendocrine levels and vascular reactive oxygen species in rats.

Dr. Reynolds introduced the concepts of vibration in relation to waveform, directionality, and resonance frequency. He highlighted data reflecting the principle of resonance frequency in mice's startle response to vibration. The results provided insight into the range of vibration frequency in mice. Dr. Reynolds noted that secondary harmonic frequency ranges also must be considered. Other important principles of vibration include sound-induced vibration, periodicity, and habituation. Sounds produced in the environment can cause another object to vibrate if the frequency of sound matches the object's resonance frequency. Periodic vibration might be more problematic than constant vibration, and responses to repeated episodes may decrease over time.

Options for vibration control include cork, rubber, springs, and synthetic materials (e.g., polyvinyl chloride sheets, polyurethane foam). Methods for passive control include reducing the magnitude of vibration at the object's resonance frequency (i.e., damping) and changing the vibration frequency to which an object is exposed away from its resonance frequency (i.e., isolation). Dr. Reynolds outlined approaches to control vibration during construction-related activities and on a routine basis. He listed four elements (i.e., administrative, procedural, equipment, and engineering) of a construction-based sound- and vibration-control plan for construction and considerations for minimizing vibration that is inherent within a laboratory animal environment (e.g.,, equipment, housing location, husbandry procedures, transportation).

Administrative actions include developing a plan of action with the construction company and coordinating with research investigators. Procedural actions include premanufacturing ducting, pipes, and other materials in as large dimensions as possible off the job site and performing activities that produce more sound and vibration during non-business hours. Equipment-related actions include removing cinder block walls with power tools, rather than a sledgehammer, and removing vinyl tiles with power machines instead of scrapers and chisel bits. Engineering-related actions include using barriers and screens to block the direct path of sound and using rubber mats on the floor during demolition.

Equipment-related actions related to minimizing vibration that is inherent to facilities include employing low-vibration-producing equipment and ensuring continued maintenance of the equipment and physical plant. Actions related to housing location include housing larger species, which generate more noise, away from more sensitive species and housing breeding rodents away from the cage-wash area, autoclaves, and elevators. Husbandry-related actions include educating employees and addressing high-impact activities in the facility that can cause vibration. Transportation-related actions can include using towels on large carts or carrying by hand. Dr. Reynolds noted that vibration during transportation was found to be significant, even when using these minimizing approaches.

Dr. Reynolds spoke on the need for standardization of research and reporting of vibration. Frequencies should be tested near the animal's resonance frequency, and the magnitude of vibration used should be limited to what is within reason for the environment when studying the effects of vibration. Additionally, the effects of sound should be controlled when studying vibration. He also highlighted the *Reporting Guidelines for Whole-Body Vibration Studies in Humans, Animals, and Cell Cultures*, which lists 24 factors for consideration on this topic. Gaps in vibration research include more precise minimal magnitudes and frequencies that cause physiological and behavioral changes; magnitudes, frequencies, and periodicity for habituation; differential effects of vibration in *x, y,* and *z* directions; design criteria that prevent resonant and harmonic frequencies from affecting animals; additional studies on the magnitude and frequency of vibrations produced during construction and their associated effects on animals; and transportation methods to mitigate vibration.

Dr. Reynolds concluded by emphasizing that vibration is an important extrinsic variable in animal studies. Sensitivity to vibration differs among species and is dependent on the frequency of vibration. The resonance and harmonic frequencies both must be considered. A comprehensive vibration- and sound-mitigation plan is essential for construction activities. He also encouraged the participants to consult with knowledgeable engineers during facility planning and demolition/construction phases. The *NIH Design Requirements Manual* is an important resource, as are previous studies.

Environmentally Associated Lesions in Rodent Toxicology Studies
Jeffrey Everitt, D.V.M., Duke University

Dr. Jeffrey Everitt presented on lesions associated with environmental factors in rodent toxicology studies. He began by asserting that toxicology studies often serve as an exemplar for rigor and reproducibility in rodent studies. Studies often are repeated in the same facilities and with identical test systems. Additionally, the studies often employ relatively large groups of rodents with robust data-capture systems in place. Quality systems are employed for safety studies in the regulatory environment, and methods and endpoints are well established. Furthermore, standard nomenclature for lesions also has been established. Comparative pathologists are experts in working with animal models, and they spend much of their time distinguishing between treatment effects and extrinsic effects. The rigor and reproducibility of pathology data in academic studies, however, often are lacking.

Numerous extrinsic factors can lead to lesions in rodents. Major factors include air, housing, and diet. Rodents are obligate nasal breathers, and the nasal cavity is known to be affected by the environment. Additionally, the olfactory mucosa shows high metabolic activity. Effects of olfaction can extend to numerous endpoints, including neurobehavior. Dr. Everitt presented data suggesting the effects of cage changing on nasal lesion development. Volatile pollutants from soiled bedding can affect the development of lesions in the rat nasal cavity; these effects could not be attributed solely to the high presence of ammonia. He emphasized that further investigation in this area is needed.

Dr. Everitt briefly highlighted other examples of extrinsic factors that affect lesions. Obstructive genitourinary lesions in mice have been shown to be modulated by housing. These effects are also genotype dependent. Wire caging influences the development of dermal tumors in transgenic mouse models for carcinogenicity. Diet also represents a complex issue in this area that encompasses numerous factors, including chemical contaminants, nutrient content, form of diet, feeding methods, storage conditions, natural versus chemical ingredients, and open versus closed formula. He underscored the importance of using data from multiple laboratories to understand variables in experiments.

Historical pathology databases can contribute to understanding the robustness and reproducibility of rodent studies. Best practices have been established in this area in the toxicologic pathology community for sampling histopathology and rodent organs. Dr. Everitt emphasized that such standardized approaches should be established within the animal modeling community. Additionally, historical databases must be

treated as living documents with standard nomenclature and multi-facility data. He also noted that many investigators have written papers with less-than-optimal generation of pathology data in academic research.

Dr. Everitt encouraged ORIP to consider the question of quality and rigor of histopathology, and pathology in general, in NIH-funded studies and to foster best practices that can be better standardized across institutions. He listed challenges in this area, which include lack of best practices approaches and peer review in academia, cost, and limited infrastructure and access. He emphasized that the NIH could build pathology infrastructure similar to that within the toxicologic pathology community. This would include a robust community of animal model pathologists with common interest in rodent model best practices, necropsy, and pathology protocols; utilization of databases that incorporate digital pathology tools; and using digital imaging tools to move from qualitative to quantitative assessment of animal models pathology.

Discussion

- Dr. Amy Keller remarked that her laboratory has noted significant vascular physiological differences of rats housed at thermoneutrality, compared with those housed at human room temperature. Dr. Svenson noted research indicating that mice can mount an immune response to tumor invasion more readily at thermoneutrality. Dr. Svenson also emphasized that dedicated funding is needed for robust studies of extrinsic factors. She added that these efforts can provide insight when interpreting study results.

- In response to a question from Dr. Emily Franklin, Dr. Svenson confirmed that the density studies were performed in individually vented caging.

- Dr. Brainard clarified that cage light density was kept equivalent for each of the racks, with no housing on the top row. He emphasized the importance of considering rack design and location in studies.

- Ms. Kerith Coulson asked about ultrasound comparisons between lighting systems. She commented that fluorescent ballasts are thought to create more ultrasound and therefore might contribute to another extrinsic factor in addition to light wavelength. Dr. Brainard agreed to examine this question further. He added that flicker of light also should be considered, particularly in regard to variation among commercial products.

- Dr. Gaskill wondered how to account for ultraviolet wavelengths that can be seen by mice. Dr. Brainard explained that a rodent-based toolbox is used for calculating alpha-optic values. He agreed that this could be factor in experimental results but noted that the specified fluorescent lights emit little ultraviolet light.

- Dr. Vivek Kumar noted that most rodents live in amber boxes that are fitted with a filter, and the boxes tend to wear over time. He asked whether the filtration of light is being considered. Dr. Brainard responded that several experiments on this question have been performed.

- Dr. Landes noted that if all animals in a cage are part of the same experimental group, the cage inadvertently becomes the "experimental unit" and thus reduces the power of a study if any cage-to-cage variability is present.

- Dr. Miguel Contreras shared a publication demonstrating immune and inflammatory genetic responses to fluorescent light in vertebrate organs.

- Dr. Enrico Radaelli shared a publication on the reproducibility of histopathological findings in experimental pathology of the mouse.

Presentations: Equipment Modernization That Enhances Rigor and Reproducibility in Studies Using Rodents

New Methods for Performing Irradiation in Rodents
Mitchell Galanek, Radiation Protection Officer, Massachusetts Institute of Technology

Mr. Mitchell Galanek discussed the differences between cesium- and X-ray-based systems. He explained that isotope-based irradiators have been the workhorse of animal and cell irradiations for the past 50 years. Low-dose irradiators typically are based in cesium 137, which has a half-life of 30 years. Irradiators can function for decades with minimal maintenance. Advantages of the cesium-based irradiator include the mono-energetic gamma ray, reproducible dose rates, historical data on animal models, ease of use, low maintenance requirements, and safety. Disadvantages of the cesium-based irradiator include the non-collimated field, difficulty of shielding unwanted exposures to experimental animals, facility and researcher security requirements, and cost of final disposal.

In recent years, the U.S. government has encouraged laboratories to consider X-ray-based irradiators. Advantages of the X-ray-based irradiator include the monodirectional beam, collimated beam, lack of facility and researcher security requirements, safety, and capability for X-ray and bioluminescence imaging. Disadvantages of the X-ray-based irradiator include the lack of a monoenergetic beam; preventive maintenance requirements and costs; mechanical reliability; heat generation; and lifetime of X-ray tubes, which are expensive to replace.

Mr. Galanek shared several users' perspectives on the cesium- and X-ray-based systems. The users expressed that the cesium irradiator requires less training and minimal power consumption; the system works well for whole- and partial-body irradiation in rodents, as well as in vitro studies. Good dose homogeneity and dose rate were noted. The system mimics clinical radiation therapy and can allow reparable DNA damage. The cesium irradiator was perceived, however, to be less safe, and targeted irradiation is difficult to perform in animals. Additionally, expensive source exchanges may be required for older irradiators. units. Decommissioning and security requirements were a concern to users, as well as shielding requirements and continuing source decay.

The users also expressed that the X-ray irradiator is safe, with a tunable dose rate, and can be used easily to perform targeted irradiation in animals. The treatment area and platform height can be controlled, and cameras allow direct visualization. Additionally, energies are clinically relevant. The X-ray irradiator, however, requires more training, and the radiation energy is lower than clinical relevance. One user noted differences between moderate- and low-energy X-ray systems, remarking that a graded filter offers a reasonable option of whole- and partial-body irradiations. It was also noted, however, that extra filtration lowers the dose rate. Extra dosing works well for irradiating cells, but not for whole- or partial-body irradiations.

The U.S. Department of Energy Office of Radiological Security is sponsoring efforts to move toward X-ray-based irradiators. The Cesium Irradiator Replacement Program (CIRP) provides financial incentives to replace Cs-based irradiators with X-ray based systems. Mr. Galanek shared a case example of the removal of a cesium-based system at the University of Washington; the cesium source could not be removed from the shield plug, and the methods employed to remove the source led to widespread radioactive contamination in the immediate work area. Ten individuals were found to have skin contamination and were decontaminated by the first responders. All individuals were monitored for both external and internal radiation exposure. The highest internal dose was 70 millirems and the highest

external exposure was 55 millirems. He stated that the resulting decontamination project resulting from the source handling mistake was highly costly. Since then, the removal process has been changed so that the cesium source is not removed onsite, the irradiator is packaged and shipped as the entire unit.

Mr. Galanek concluded by posing the question of whether facilities should continue to use cesium-based irradiation systems. He answered that the Cs-based systems should be kept if the research warrants the use of these tools. A combination of cesium- and X-ray-based systems likely is the best solution.

Enhancing Animal Study Translation: Physiological Monitoring as a Key Contribution
Brian Berridge, D.V.M., Ph.D., DACVP, National Institute of Environmental Health Sciences

Dr. Brian Berridge discussed physiological monitoring in the context of understanding extrinsic factors as a key contribution to improving the translational relevance of animal studies. He began by remarking that animal studies are an important translational modeling platform used to support the full spectrum of exploratory to confirmatory biomedical research interests, where rodents are the predominant species used. These uses can include targeting and validation, hit and lead discovery and optimization, candidate selection, preclinical safety studies, and clinical assessment. He emphasized that the translational process presents multiple challenges, and success rates vary across therapeutic areas. Clinical experience can provide insight into translational weaknesses.

Reproducibility is an ongoing challenge in research. Three primary challenges in this area are reporting standards (e.g., insufficient experimental details to replicate study conditions), study design and conduct (e.g., bias, insufficient statistical power, technical consistency), and biology (e.g., natural validity, comparative relevance to humans). Dr. Berridge emphasized that more work is needed in the context of biological challenges (i.e., external validity). Based on these factors, the ACD Working Group recommended enhancing training in animal study design, improving access to statisticians, enhancing peer review of study plans, increasing expectations for describing animal study plans in grant applications, applying ARRIVE guidelines for reporting, improving rationalization for animal model selection, registering animal study plans, increasing funding for large-animal models, improving understanding and reporting of external factors, and assessing costs of these increased expectations.

Dr. Berridge remarked that animals can model important anatomic, functional, and mechanistic features of the human condition, but numerous differences between animal models and humans are present. These differences should be considered in model selection and study design. Environmental effects also must be considered. He noted that general health checks are standard in clinical care but typically are not monitored in the context of animal research. These technologies have been developed but often are not applied. He also highlighted the importance of monitoring behavior as a translational physiologic endpoint; new technologies are expanding capabilities in this area. Dr. Berridge briefly highlighted opportunities for monitoring physiologic and behavior endpoints in research.

In summary, Dr. Berridge encouraged investigators to think of their animals as the patients that they are intended to represent. He emphasized that animals will never be a perfect surrogate for patients, but translational relevance can be improved through more human context. Organ system function is a clinically important context for morphologic and molecular endpoints and measures. Additionally, technology solutions provide an opportunity to improve the human relevance of animal studies, as well as to optimize care and welfare.

Smart Cages Require Smart Management
Steve Niemi, D.V.M., Boston University

Dr. Steve Niemi spoke on the need for smart management of smart cages. He defined a smart cage as equipped to monitor various intra-cage parameters digitally and continuously, and inform personnel about

the status of those parameters remotely. Therefore, smart cages can provide researchers continuous information on the status of housed mice and represent a new generation of large-scale housing. Dr. Niemi began by explaining that mice must be observed at least once daily in accordance with regulatory requirements and good quality care. This practice can be challenging for institutions maintaining thousands of rodent cages daily. He presented data from an anonymous program indicating that most rodent health concerns were reported on weekdays, i.e., when the technical team was fully staffed, versus fewer health concerns reported on weekends and holidays when skeleton crews were used which indicated a need for more effective routine monitoring for better animal welfare especially during times when fewer personnel are on site. He hypothesized that similar effects might have occurred during the beginning of the COVID-19 pandemic when staff access to facilities was highly restricted.

Many options for housing are now available to researchers, including new platforms that can provide after-hours alerts of changes in intra-cage conditions, such as flooding, excess ammonia levels, unwanted temperature excursions, and animal activity. These capabilities address the need to inform staff and make these options more accessible to investigators. Rather than replace an institution's entire rodent caging inventory, Dr. Niemi proposed the use of smart caging on a limited scale for monitoring post-operative recovery and pain management, severe endpoints, difficult breeders, and hostile cage mates. Other opportunities include pilot studies to assess the effects of various factors (e.g., bedding, enrichment, room environment, housing density) on behavior and activity, staff and investigator training, and troubleshooting (e.g., suspect environmental controls, environmental disturbances).

Dr. Niemi also envisioned other "smart" cage accessories, such as food hoppers and water bottles that would monitor and broadcast if and how fast their contents were emptying. These enhancements could help researchers determine or confirm consumption of critical experimental components, such as medicated food or drinking water, and adequate agitations of chemical suspensions. He emphasized the importance of fostering collaborations between investigators and lab animal program managers to explore other benefits.

Highly Scalable and Reproducible Preclinical Rodent Behavioral Assays Using Machine Vision
Vivek Kumar, Ph.D., JAX

Dr. Kumar presented on the development of preclinical rodent behavioral assays using machine vision. He emphasized the critical need for new psychiatric treatments and better preclinical animal models, particularly rodent models. He explained that many current behavioral tests have low reproducibility and throughput. His work is focused on improving reproducibility by developing approaches that use novel instruments and equipment. Dr. Kumar is striving to achieve ethologically relevant monitoring of high-resolution outputs from neural circuits of multiple animals over long periods of time. He is working to manipulate the environment, nervous system, genetics, and pharmacology.

The field of computational ethology has expanded in recent years. Major advancements in statistical learning now are being applied to real-world problems. Dr. Kumar emphasized the need to democratize these new technologies. One opportunity in this area is automated annotation of animal behavior. Dr. Kumar briefly presented a representative recording using this method and explained how recordings can be used to detect behaviors in mice (e.g., grooming, gait, posture). He presented a readout of data annotation, explaining that multiple extrinsic factors (e.g., time, tester, light, noise, season, room of origin) can be considered.

Dr. Kumar proposed that highly reproducible and scalable motor assays could substitute for complex cognitive traits for screening purposes. He also spoke on the context of index-based phenotypes for generalizability in various contexts. For example, multiple behaviors contribute to developing these indices for biological age. He also presented an example of data monitoring to characterize social interaction; these data were found to be replicable.

The integrated mouse phenotyping platform involves steps of data acquisition, behavior annotation, classifier training, behavior characterization, and data integration. Dr. Kumar underscored the value of a standardized, high-quality data pipeline and a standardized analytic pipeline, with minimal human intervention. These systems would allow data comparisons across laboratories, location, and time. To achieve this outcome, sharing of data, hardware, software, and annotations will be needed. More infrastructure developments in this area are needed.

Discussion

- Dr. Ashbrook asked about factors related to genetic variation. Researchers tend to use single-genome rodents to model humans, which are genetically variable. Dr. Ashbrook noted that "translation" even between mouse strains sometimes is unsuccessful. Dr. Berridge responded that researchers are exploring ways to incorporate genetic variation into mouse studies. He suggested increasing the depth of evaluation in individual animals.

- Dr. Craig Franklin remarked that "pet shop" mice experience a higher antigen experience through exposure to a richer microbiome; this antigen experience correlates with immune system development that better replicates the adult human immune system. Moreover, a simpler microbiome might be more susceptible to change, which could impact reproducibility.

- A participant wondered whether any facilities are breeding "dirty" mice that still are classified for research. Dr. Wiest noted that investigators at the University of Minnesota are performing research using pet shop mice.

- Dr. James Burkett remarked that the ability to know immediately about adverse conditions does not necessarily imply that an immediate response is required for animal welfare. A reasonable response time could be defined. Dr. Niemi noted that the appropriate action is dependent on current circumstances, as well as the culture of the institution. He added that these practices now must be defined, rather than assumed.

- Dr. Wiest remarked that his facility is organized into different areas of health status. He noted that the smart caging technologies provide new opportunities but necessitate staffing considerations.

- Dr. Kumar commented that many of the smart sensors have been in place for decades but have not been implemented at scale in vivaria. He emphasized the need for scalable and affordable technologies. He proposed that a single sensor could be used for multiple modalities of monitoring. Dr. Niemi agreed, noting that specialized, premium equipment could be used for specialized situations. Scalable systems could be used in more general contexts.

- Dr. Wiest wondered about the use of machine learning to detect subtle behaviors, such as grooming versus scratching. Dr. Kumar replied that the algorithms are highly sensitive; for example, the breathing rate can be determined to distinguish sleep states. He added that frame rate and resolution must be considered.

- Dr. Wiest wondered whether the video systems could be implemented in cages. Dr. Kumar responded that this design would be feasible, but certain behaviors—such as strides—might be challenging to determine in cages. Other behaviors—such as sleep—might be easier to determine. He added that the algorithms are flexible and require only high-quality video and training data.

- In response to a question from Dr. Leah Villegas, Dr. Contreras noted that the ORIP small business programs could be applied for development of smart caging systems.

- Dr. Joseph Newsome remarked that the transition between isotope- and X-ray-based systems must be validated to ensure consistency in physiological effects.

Presentations: New and Emerging Monitoring Methods That Enhance Rigor and Reproducibility in Studies Using Rodents

Influence of Housing and Pathogen-Control Measures on Host Physiology and Reproducibility
Neil Lipman, V.M.D., Memorial Sloan Kettering Cancer Center and Weill Cornell Medicine

Dr. Neil Lipman spoke on the influence of housing and pathogen-control measures in the context of host physiology and reproducibility. He first presented a history of changes in rodent caging systems, which have evolved from wood to stainless steel and glass and, as currently utilized, thermoplastics. Several seminal events have shaped this industry. In the late 1950s, the first isolator cage and biological transfer hood was developed. In the 1980s, the design standard for most current isolator cages was developed.

Around this time, researchers began detecting new issues associated with these cages which affected mice health. The isolators led to changes in the microenvironment, particularly regarding ammonia levels. Bedding also plays a significant role in the accumulation of ammonia. As a result, researchers began exploring the concept of ventilated cages. This design helped to reduce ammonia accumulation in cages, it provided an additional level of protection for the cage occupants through intracage pressurization, and increased cage housing density. Dr. Lipman explained that this development occurred in parallel with the emergence of new approaches for genetic engineering in mice, which greatly increased the demand for their use in biomedical research.

Today, most facilitates use individually ventilated cages. Dr. Lipman noted that specific designs vary greatly across systems. Installation differences also should be considered. Dr. Lipman remarked that researchers often fail to adequately report the characteristics of the housing systems used in their studies, even when the studies are evaluating the systems. Often, researchers do not fully understand these systems. Dr. Lipman also noted that the ARRIVE guidelines provide inadequate details in this area. Information that should be reported could include airflow mechanics, rack ventilation, air-change rate, cage design, and intra-cage airflow dynamics. Dr. Lipman noted that reporting needs vary based on the type of study.

Dr. Lipman also discussed the use and processing of thermoplastics in animal research. These materials are used extensively in the production of rodent caging and water bottles. The plastics have been shown to degrade over time releasing bisphenols and other components. Bisphenols function as endocrine disrupters mimicking estrogen. This concern relates to the fundamental question of whether rodent caging should be routinely sterilized, because this process exacerbates the breakdown of caging materials. He presented data suggesting that cage-washing at industry standards might be sufficient to eradicate most murine pathogens. He concluded by underscoring the importance of understanding how practices and operations can introduce additional variables in animal studies.

Circumventing Challenges with Rodent Microbe Detection in Research Vivaria by Incorporating PCR-Based Environmental Screening Methods
Ken Henderson, Ph.D., Charles River Laboratories

Dr. Ken Henderson presented on the use of PCR-based methods for rodent microbe detection in animal facilities. He began by highlighting the work of Dr. Lisbeth Kraft, who described details of the microenvironment of research animals in 1957. Dr. Henderson briefly outlined examples of ways in which diseases can affect experimental outcomes in rodent research. Researchers must decide which pathogens in their rodent populations must be characterized and reported.

Methodology for PCR was first established in the 1980s, but this approach originally was viewed only as a good confirmation tool or as a secondary tool to traditional diagnostic methods. In 1998, researchers began using PCR to identify contact and air transmission risks for rat parvoviruses. They also detected externally released viruses from exhaust ducts. In 2004, the first proof-of-concept report of exhaust air duct collection on an individually ventilated cage rack was published.

At this time, many researchers were hesitant to change their current practices of using soiled bedding sentinels. Several years later, however, laboratory veterinarians became concerned about the use of these sentinels for quarantine. A fecal quarantine study was performed, and high-throughput PCR was incorporated. A larger-scale study was performed using pet shop mice, and researchers found that most infectious agents not detected in soiled bedding sentinels were detected via direct sampling for PCR.

Dr. Henderson explained that issues have occurred with the transition from open-top to microisolator caging. The effects on soiled bedding samples with regard to routine infectious agents were not considered. He highlighted additional advancements in biosecurity, which include cage-changing stations, use of surface decontaminants, decontamination of husbandry materials, and cell line and research biologics testing before use in animals.

Around 2009, researchers became aware of the prevalence of fur mites in research animals. Dr. Henderson was involved in efforts to better characterize these effects. In this process, the researchers developed the concept of routine environmental sampling for pathogen screening by PCR on individually ventilated cage racks. In recent years, several publications have supported the use of environmental PCR testing methods. Dr. Henderson began testing cage filters, which appeared to be more effective than the sentinels but still required a mouse for agitation. Additionally, in certain cage designs, the filters were difficult to remove.

A recent approach has involved manual agitation of soiled bedding with contact media. This approach does not require a mouse, and the data support good sensitivity for a small group of agents. Additionally, this method can be used with any cage type. Dr. Henderson collaborated with other groups to determine challenges within this system. They reported that cage shaking was cumbersome, and data for commonly excluded agents were limited. Standardized methods for agitation and evaluation of different contact media are needed. Furthermore, submissions have not been standardized.

Based on these challenges, the group standardized and optimized the agitation approach. They identified an optimal contact media treatment schedule and evaluated more than 20 contact media to select high-binding candidates. The cage was replaced with a collection box, which was used to agitate the collection media with the soiled bedding. This approach eliminates the need for soiled bedding sentinels.

Dr. Henderson concluded that environmental and exhaust dust sampling methods for PCR detection of rodent infectious agents are being used today by many institutions. These methods detect infectious agents typically found by traditional soiled bedding sentinel use, as well as a larger group of agents that are not. A better knowledge of which agents are present is important in understanding their potential effect on research outcomes. He emphasized the value of pursuing equivocal or superior methods that eliminate animal use in research.

Discussion

- No discussion occurred.

Group Discussion and Summary

- Dr. Wiest commented on the finding that LED lighting was associated with less feeding and drinking. He noted the link between caloric restriction and aging. He asked whether LED exposures lead to sustained reductions in feeding and whether this variable would be of interest to the aging research community. Dr. Brainard responded that the study was performed over 12 weeks, and he agreed on the importance of this effect. Continued investigation in this area is needed.

- Dr. Kumar noted that light pollution in facilities (e.g., from equipment) could be an area of concern. Dr. Brainard agreed, noting that darkness is relative and difficult to achieve in facilities. This is a particular issue for rodents, because they are nocturnal. In response to a follow-up comment from Dr. Fox, Dr. Brainard added that the effects of light cycles should be considered in this context. Light duration can prompt seasonal responses, triggering numerous physiological effects. The current edition of the *Guide* does not provide guidance in this area.

- Dr. Lipman asked whether LED lights can be tuned to address the observed effects in ganglia. Dr. Brainard commented that the International Space Station has been retrofitted with a tunable LED light source with a pre-sleep mode for astronauts. This tuning eliminates stimulation of the system. Similar strategies are being applied in other human studies. Dr. Brainard noted that tunable LED systems likely are not needed in all animal facilities at this point, but the engineering capacity has been established.

- Dr. Fox asked about the effects of environmental monitoring and energy-saving measures in the context of extrinsic factors. Dr. Lipman explained that air ventilation rates at his institution are controlled by various factors. The newly implemented system is designed to adjust air exchange as needed. Temperature and humidity are important factors for consideration. The new system is more cost-effective than previous systems, with a high return on investment. He noted that ventilation rates can be adjusted based on the presence of animals. Dr. Lipman added that the need for ventilation is driven primarily by the heat generated by the animals and equipment. He added, however, that such automated systems could introduce a new variable. Dr. Brainard emphasized that changes in facilities should be driven primarily by scientific needs.

- Dr. Lipman noted that the microbiome has emerged as an important topic in recent years. He added that as transgenic mice have been shipped across the globe, researchers do not truly know what new agents have been introduced to their facilities.

- Dr. Burkett asked about strategies to determine whether animal racks are being exposed to problematic vibrations. Dr. Reynolds noted that animals often exhibit stress responses, such as food grinding, reproductive issues, and cannibalization. Additionally, researchers can measure vibration directly within facilities, but problematic levels can be challenging to define.

- A participant asked about the duration of response to one-time significant vibration incidents. Dr. Reynolds replied that the response is dependent on numerous factors, and may be different for animals *in utero*. Direct testing would be needed to understand the effects fully.

- Dr. Gordon Lithgow shared information on the National Institute on Aging's Interventions Testing Program, which is designed to identify agents that extend life span and health span in mice.

- Dr. Lipman underscored the need for an NIH funding mechanism to study extrinsic factors in animal research. Dr. Li agreed on the importance of this issue. He noted that ORIP's recent concept clearance could provide some support in this area. Additionally, the outcomes of this workshop will be helpful in setting criteria for evaluation of grant applications that are addressing needs in this area. If NIH programs identify gaps in their funding portfolios, new funding opportunities can be developed.

- Dr. Everitt suggested that the NIH encourage inter-institutional studies to foster a better understanding of extrinsic factors. He reiterated the need for standardized methods. Dr. Fox noted that in private industry, many experiments are being performed by contracted laboratories. Dr. Everitt agreed that this practice can create challenges but noted that confirmatory studies, which are common practice within the pharmaceutical industry, have contributed to a stronger system of peer review.

- Dr. Marta Chesi wondered how the information discussed during the workshop could be incorporated into the *Guide*. Dr. Everitt remarked that numerous extrinsic factors are present, and researchers cannot account for every variable in research. He spoke on the need for tailoring controls to the type of research being performed. Dr. Wiest agreed, noting that researchers can take one of two approaches in addressing extrinsic factors: controlling for every variable or conducting studies on animals that are more representative of biological organisms in the real world (e.g., pet shop mice).

- Dr. Berridge emphasized that the issue of extrinsic factors will require large-scale collaborative efforts among the NIH, professional societies, and private industry entities. The NIH could help foster partnerships in this area. Dr. Lipman also suggested that ORIP convene a panel of experts to develop recommendations related to the use of LED lighting in facilities.

Session Wrap-Up and Adjournment

Drs. Fox and Mirochnitchenko thanked the speakers, organizers, and participants for their engagement during the meeting. Dr. Mirochnitchenko encouraged the participants to consider how the principles discussed during the meeting apply to other types of model organisms. Dr. Fox also encouraged the participants to register for Session 3. Dr. Mirochnitchenko adjourned the meeting.

Appendix A: Meeting Agenda

Session 2. Rodents
Virtual Meeting
September 28, 2022

12:00–12:10 p.m.	**Opening Remarks** *James Fox, D.V.M., M.S., DACLAM, Workshop Chairperson, Massachusetts Institute of Technology* *Xiang-Ning Li, M.D., Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)* *Oleg Mirochnitchenko, Ph.D., ORIP, DPCPSI, OD, NIH*
12:10–12:30 p.m.	**Keynote Presentation: Impact of Extrinsic Factors on Rigor and Reproducibility in Rodent Research** *F. Claire Hankenson, D.V.M., M.S., University of Pennsylvania*
12:30–1:50 p.m.	**Presentations: Housing Environment That Impacts Rigor and Reproducibility in Studies Using Rodents**
	Effects of Increased Housing Density in Research Mice *Karen Svenson, Ph.D., The Jackson Laboratory (JAX)*
	Minimizing the Impact of Habitat Lighting on Experimental Reproducibility for Rodent Studies *George Brainard, Ph.D., Thomas Jefferson University*
	Vibration as an Extrinsic Variable for Research Outcomes *Randall Reynolds, D.V.M., M.S., Duke University*
	Environmentally Associated Lesions in Rodent Toxicology Studies *Jeffrey Everitt, D.V.M., Duke University*
1:50–2:00 p.m.	**Break**
2:00–3:20 p.m.	**Presentations: Equipment Modernization That Enhances Rigor and Reproducibility in Studies Using Rodents**
	New Methods for Performing Irradiation in Rodents *Mitch Galanek, CHP, Massachusetts Institute of Technology*
	Enhancing Animal Study Translation: Physiological Monitoring as a Key Contribution *Brian Berridge, D.V.M., Ph.D., DACVP, National Institute of Environmental Health Sciences*
	Smart Cages Require Smart Management *Steve Niemi, D.V.M., Boston University*

	Highly Scalable and Reproducible Preclinical Rodent Behavioral Assays Using Machine Vision *Vivek Kumar, Ph.D., JAX*
3:20–3:30 p.m.	**Break**
3:30–4:10 p.m.	**Presentations: New and Emerging Monitoring Methods That Enhance Rigor and Reproducibility in Studies Using Rodents**
	Influence of Housing and Pathogen-Control Measures on Host Physiology and Reproducibility *Neil Lipman, V.M.D., Memorial Sloan Kettering Cancer Center*
	Circumventing Challenges with Rodent Microbe Detection in Research Vivaria by Incorporating PCR-Based Environmental Screening Methods *Ken Henderson, Ph.D., Charles River Laboratories*
4:10–4:40 p.m.	**Group Discussion and Summary**
4:40–4:50 p.m.	**Session Wrap-Up**
4:50 p.m.	**Adjournment**

Appendix B: Participants List

Session 2. Rodents
Virtual Meeting
September 28, 2022

Kristin Abraham, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH)
Stephanie Achilles, The University of Alabama at Birmingham
Yoko Ambrosini, Washington State University
James Amos-Landgraf, University of Missouri
Laura Anderson, The Jackson Laboratory (JAX)
Amanda Lee Armijo, Massachusetts Institute of Technology
Matthew Arnegard, Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), NIH
David Ashbrook, The University of Tennessee Health Science Center
Charles-Antoine Assenmacher, University of Pennsylvania
Julia Bachman, National Institute of Neurological Disorders and Stroke (NINDS), NIH
Alanna Backx, Massachusetts Institute of Technology
Amanda Barabas, Case Western Reserve University
Ashley Barnes, ORIP, DPCPSI, OD, NIH
Taylor Bennett, National Association for Biomedical Research
Zorana Berberovic, The Centre for Phenogenomics
Michelle Bernard, Oregon Health & Science University
Brian Berridge, National Institute of Environmental Health Sciences (NIEHS), NIH
Scott Birks, Boise State University
Himadri Biswas, The University of Toledo
Auke Boersma, University of Veterinary Medicine Vienna
Courtney Bouchet, Colorado State University
Aleaya Bowie, Vanderbilt University Medical Center
Felicia Boynton, Mayo Clinic
George Brainard, Thomas Jefferson University
Samantha Brims, University of Southern California
Mallory Brown, Johns Hopkins University School of Medicine
Patricia Brown, Office of Laboratory Animal Welfare (OLAW), Office of Extramural Research (OER), OD, NIH
Elizabeth Bryda, University of Missouri
James Burkett, The University of Toledo
Angelica Cabrera, Bristol Myers Squibb
Edgar Angelats Canals, August Pi i Sunyer Biomedical Research Institute
Jessie Carder, U.S. Department of Agriculture
Selen Catania, National Heart, Lung, and Blood Institute, NIH
David Cavazos, The University of Texas Health Science Center at San Antonio
Shreaya Chakroborty, National Institute on Aging (NIA), NIH
Susan Chandran, ORIP, DPCPSI, OD, NIH
Chris Chao, National Institute of General Medical Sciences (NIGMS), NIH
Sarah Chapman, University of Notre Dame
Naomi Charalambakis, Federation of American Societies for Experimental Biology
Julia Charles, Brigham and Women's Hospital
Lee Chaves, University of Kansas Medical Center

Amanda Chen, National Institute of Allergy and Infectious Diseases (NIAID), NIH
Marta Chesi, Mayo Clinic
Sangyong Choi, University of Connecticut
Debanik Choudhury, University at Buffalo
Eleanore Chuang, NIAID, NIH
Megan Clark, OLAW, OER, OD, NIH
Joyce Cohen, Emory University
Lesley Colby, University of Washington
Giancarlo Colombo, National Research Council of Italy, Istituto Di Neuroscienze
Karina Concha, Florida Atlantic University
Ronald Conlon, Case Western Reserve University
Miguel Contreras, ORIP, DPCPSI, OD, NIH
Kerith Coulson, University of Cape Town
Devon Crawford, NINDS, NIH
Joette Crews, Emory University
Lani Cupo, McGill University
Chi-Ping Day, National Cancer Institute (NCI), NIH
John Dennis, U.S. Food and Drug Administration (FDA)
CJ Doane, University of Arizona
Abigail D'Souza, Louisiana Tech Research Institute
Adrienne Duran, MD Anderson Cancer Center
Samantha Earlywine, Nationwide Children's Hospital
Catalina Echeverri, Rockefeller University
Samantha Edell, Cytokinetics
Mark Eichelberg, American Physiological Society
Michael Eichner, Office of Research Services, Office of Management, OD, NIH
Michael Ellis, JAX
Peter Ernst, University of California, San Diego, and University of California, Davis
Laverne Estanol, University of California, Santa Cruz
Rachel Sarabia Estrada, Mayo Clinic
Jeetendra Eswaraka, Rutgers University
Marissa Eudaley, University of Southern California
Jeffrey Everitt, Duke University
Angelika Fath-Goodin, ParaTechs Corporation
Chuhan Feng, McGill University
Steve Festin, Charles River Laboratories
Cameron Fili, FDA
Kelsey Finnie, The University of Tennessee, Knoxville
Ann Flenniken, The Centre for Phenogenomics
Craig Fletcher, The University of North Carolina at Chapel Hill
Loren Fong, University of California, Los Angeles
James Fox, Massachusetts Institute of Technology
Craig Franklin, University of Missouri
Emily Franklin, Massachusetts Institute of Technology
Julien Freeman, Massachusetts Institute of Technology
Ashley Gaffey, Georgetown University
Mitch Galanek, Massachusetts Institute of Technology
Judit Peix Gallofré, August Pi i Sunyer Biomedical Research Institute
Chelsea Garrison, Boise State University
Brianna Gaskill, Novartis
William Gause, Rutgers New Jersey Medical School

Karli Gilbert, Georgetown University
Sarah Gillis-Smith, Massachusetts Institute of Technology
Sylvia Gografe, Florida Atlantic University
Rafael Moreno Gómez-Toledano, Universidad de Alcalá
Neera Gopee, OLAW, OD, OER, NIH
Ernesto Gulin, Universidad de Buenos Aires
Bryan Hackfort, University of Nebraska Medical Center
Travis Hagedorn, Kansas University Medical Center
David Hamilton, The University of Tennessee Health Science Center
F. Claire Hankenson, University of Pennsylvania
Susan Harper, Inwood Animal Center
Melissa Harrington, Delaware State University
John Hasenau, Lab Animal Consultants
Alissa Hatfield, American Physiological Society
Hami Hemati, University of Kentucky
Ken Henderson, Charles River Laboratories
Beate Henschel, Indiana University
Renee Hernandez, GlaxoSmithKline
Jennifer Hess, Nationwide Children's Hospital
Deb Hickman, Purdue University
Nancy Hitt, NINDS, NIH
Tuan Hoang, Fluid Synchrony, LLC
Craig Hodges, Case Western Reserve University
Heather Holliday, Clemson University
Mary Holtz, Medical College of Wisconsin
Maureen Humphrey-Shelton, U.S. Army Medical Research and Development Command
Courtney Hunter, Vanderbilt University Medical Center
Sandra Jablonski, Georgetown University
Glenn Jackson, Cornell University
Caitlin James, Roswell Park Comprehensive Cancer Center
Naveena Janakiram, NCI, NIH
Remi Jawando, Seagen
Walter Jeske, Loyola University Chicago
Weidong Jiang, Georgetown University
Crystal Johnson, Georgetown University
Katherine Johnson, Boise State University
Philip Jordan, Uniformed Services University of the Health Sciences
Vijay Kanth, Qatar University
Christopher Keator, Western Michigan University
Roseann Kehoe, Rutgers University
Amy Keller, Rocky Mountain Regional VA Medical Center
Lois Kelsey, The Centre for Phenogenomics
David Kennedy, The University of Toledo
Yong-Hwan Kim, Delaware State University
Angela King-Herbert, NIEHS, NIH
Julia Kissling, National Aeronautics and Space Administration
Madison Klanke, Turner Scientific
Kim Klukas, The Hormel Institute, University of Minnesota
Lauren Gerard Koch, The University of Toledo
Sailaja Koduri, NIGMS, NIH
Shannon Kramer, Texas A&M School of Dentistry

Vivek Kumar, JAX
Donna Kupniewski, Monell Chemical Senses Center
David Kurtz, NIEHS, NIH
Kelsey Lambert, Wake Forest University School of Medicine
Lorissa Lamoureux, University of Illinois Chicago
Reid Landes, University of Arkansas for Medical Sciences
Chelsea Landon, Duke University
Louise Lanoue, University of California, Davis
Elizabeth Lavin, Cornell University
Karen Lencioni, California Institute of Technology
Jori Leszczynski, University of Colorado Denver and University of Colorado Anschutz Medical Campus
Louis Leung, Martineau & Associates, Inc.
Denyse Levesque, Emory National Primate Research Center
Xiang-Ning Li, ORIP, DPCPSI, OD, NIH
Xiaohong Li, The University of Toledo
Neil Lipman, Memorial Sloan Kettering Cancer Center
Gordon Lithgow, Buck Institute for Research on Aging
Chang Liu, University of Kentucky
Eric Liu, The Hospital for Sick Children
Carla Lobina, Cittadella Universitaria di Monserrato
Christina Loftin, Texas A&M University–Corpus Christi
Kerith Luchins, The University of Chicago
Andrea Luker, NIAID, NIH
Courtney Lunger, Massachusetts Institute of Technology
Yibo Luo, The University of Toledo
Cat Lutz, JAX
Gabrielle M. Robbins, University of Minnesota
Paola Maccioni, National Research Council of Italy, Istituto Di Neuroscienze
Diogo Magnani, University of Massachusetts
Sophia Mahoney, University of Colorado Boulder
Elena Makareeva, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), NIH
Leah Makaron, University of Pennsylvania
Laura Mamounas, NINDS, NIH
John Manker, Turner Scientific
Kati Marshall, Oregon National Primate Research Center
Shinobu Matsuura, Boston University School of Medicine
Allison Maurice, Mirimus
Lois McKennett, Leidos
Colin McKerlie, The Hospital for Sick Children
Derek McLean, Office of AIDS Research, DPCPSI, OD, NIH
Pihu Mehrotra, University at Buffalo
Ana Melero, University of Valencia
Hongsheng Men, University of Missouri
Vinal Menon, University of Minnesota
Istvan Merchenthaler, University of Maryland
Anne Merley, Brown University
Reginald Miller, Icahn School of Medicine at Mount Sinai
Nahir Miranda-Rathbone, U.S. Army Institute of Surgical Research
Oleg Mirochnitchenko, ORIP, DPCPSI, OD, NIH
Jennifer Mitchell, MD Anderson Cancer Center

Robert Molestina, American Type Culture Collection
Beverly Montgomery, Boise State University
Elizabeth Moore, Cornell University
Manuel Moro, NIA, NIH
Christopher Morrison, Pennington Biomedical Research Center
Stefan Muljo, NIAID, NIH
Judy Murray, Charles River Laboratories
Nagalakshmi Nadiminty, The University of Toledo
Richard Nakamura, National Institute of Mental Health, NIH (retired)
Peter Nathanielsz, University of Wyoming
Meera Navaratnam, InterVivo Solutions
Hend Nawara, Georgetown University
Allison Neely, University of Kansas Medical Center
Joseph Newsome, University of Pittsburgh Medical Center
Steve Niemi, Boston University
Ipe Ninan, The University of Toledo
Richard Noel, Georgia Institute of Technology
John Norton, Duke University
Tai Oatess, Vanderbilt University Medical Center
Albert Gris Oliver, August Pi i Sunyer Biomedical Research Institute
Alan Olzinski, GlaxoSmithKline
Carly O'Malley, Charles River Laboratories
Glen Otto, The University of Texas at Austin
Monica Ouellette, JAX
Scott Perkins, Tufts University
Kim Perron, JAX
Katy Phillip, The University of Arizona
Mahesh Pillai, The University of Toledo
Roser Pinyol, August Pi i Sunyer Biomedical Research Institute
Kathleen Pritchett-Corning, Harvard University
Michael Pryor, Vanderbilt University Medical Center
Jeanette Purcell, University of Illinois Chicago
Enrico Radaelli, University of Pennsylvania
Carol Raymond, U.S. Army Institute of Surgical Research
Gregory Reinhard, University of Pennsylvania
Francisco Rendon-Gonzalez, Regeneron Pharmaceuticals
Jessica Revolorio, Georgiamune, LLC
Randall Reynolds, Duke University
Lisa Root, The University of Toledo
Shelby Rorrer, The University of Alabama at Birmingham
Chris Royer, California National Primate Research Center
Jan Rozman, Institute of Molecular Genetics
Kenneth Salleng, Florida Atlantic University
Melissa Sanchez, U.S. Army
Nalini Santanam, Marshall University
Sarah Schlink, University of Missouri
Caroline Schomer, The University of Texas at Austin
Mohammed Selloum, Institut Clinique de la Souris
Terri Shaffer, The Abigail Wexner Research Institute at Nationwide Children's Hospital
Linshan Shang, University of Minnesota
Meaghen Sharik, Mayo Clinic

Anuj Sharma, Office of Research Integrity, U.S. Department of Health and Human Services
William Shawlot, The University of Texas at Austin
Bhupinder Singh, Rutgers University
Purva Singh, Hospital for Special Surgery
Anna Skorupski, University of Pittsburgh
Gillian Sleep, The Hospital for Sick Children
Diane Smith, Boise State University
Heather Smith, Office of Animal Care and Use, Office of Intramural Research, OD, NIH
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