FOSTERING RIGOROUS RESEARCH:

FOSTERING RIGOROUS RESEARCH:

Lessons Learned from Nonhuman Primate Models and Charting the Path Forward

February 18-19, 2020
NIH Campus, Bethesda MD

National Institutes of Health

Table of Contents

Executive Summary

The National Institutes of Health (NIH) has announced several policies and initiatives related to enhancing the rigor, transparency, and reproducibility in the research it funds. Toward that goal, NIH recently hosted a workshop on fostering rigor and reproducibility in nonhuman primate (NHP) research. The workshop convened experts and stakeholders from a wide array of backgrounds (e.g., biomedical researchers, bioethicists, veterinarians) to discuss lessons learned from research using NHP models and leveraging these lessons to inform future research. Sessions were focused on ensuring rigorous study design, the influence of external factors on study outcomes, data sharing, research challenges due to new and emerging technologies, and future directions. Several overarching themes emerged over the course of the workshop that can be used to inform the conduct of NHP research.[1]

Ethical considerations for NHP studies should extend beyond legal and regulatory requirements

The standards for animal care and use in the US, including husbandry and welfare, are set by Federal laws and regulations. Various frameworks can supplement these codified requirements to ensure that ethical considerations are accounted for in the best interest of rigorous study design and interpretation of outcomes. For example, the 3Rs (Replacement, Reduction, and Refinement) provide an important framework for minimizing animal use, improving animal welfare by addressing harms to animals, and supporting high-quality science that can be a benefit to society. In addition, a recently published framework in Principles of Animal Research Ethics provides a more robust basis for ethical considerations in NHP research, even beyond the 3Rs framework, by incorporating additional principles in social benefit and animal welfare. These principles provide a framework for investigators to go beyond the legal and regulatory requirements for conducting animal research and focus on research that is grounded in ethics. Researchers should continually be mindful of the balance between societal benefit and harm to the animal, so that harm never outweighs benefit.

Frameworks can also be applied when developing new technologies. Emerging technologies, such as genetically-modified NHPs and mitochondrial replacement therapy have raised ethical concerns that warrant new discussions within existing ethical frameworks.

It is essential to have a robust understanding of both the NHP model and the human condition under study

Rigorous study design can be achieved by having a reproducible model that closely mimics the human condition and posing discrete questions and hypotheses that are answerable with the chosen model. Therefore, a thorough understanding of both the primate model and the human disease state (including clinical presentation and treatment regimen) are needed to maximize the potential translatability of findings from the NHP model. Ideally, the primate model will have similar characteristics to affected human populations (e.g., age, sex) as well as closely mimicking the human condition. Mimicking the human condition is not demonstrated by simply describing shared symptoms between the primate model and the patient, but also sharing causative underlying biology.

[1] The discussions reflected in this summary document represent the views and presentations of the workshop participants and do not necessarily represent those of the NIH, the Department of Health and Human Services, or the US Government.

Consider the influence of environmental, behavioral, and husbandry factors on experimental results

A number of environmental, behavioral, and husbandry factors (e.g., social housing, space allocation, interactions with research personnel, PRT) affect the welfare, behavior, and physiological and mental health of NHPs. These effects can significantly influence experimental results. Therefore, these external factors should be carefully considered along with the goals of the research to increase the data validity of the studies. It will be critical for researchers to regularly review existing data or conduct new research on factors such as refinement, social housing, and positive reinforcement and to consider how each of those factors may affect experimental results.

There is a need to share data and results as soon as possible; however, open data sharing is not without challenges

Data sharing enhances rigor and reproducibility by enabling other scientists to validate the original findings, facilitate cross-study comparability, and leverage existing knowledge into new discoveries. For animal studies, transparent reporting entails making information about study design, methods, results, and related data openly available. This sharing includes both positive and negative data with the latter needing to be just as robustly validated in order to maximize societal benefit. Additionally, widely sharing failures (e.g., rigorously designed studies that produced negative results, rigorous animal studies that do not translate to clinical research) is beneficial for the community. Learning from failure can provide context and information that leads to future successes. Ideally, data should be collected longitudinally, and this can be enhanced by tracking both the animal (e.g., with unique IDs) and, in the case of infectious disease, the viral challenges. However, timely data sharing poses a number of problems for researchers. The effort required to share data can be considerable and initial interpretations of results may gain traction even though they may not hold up over time as new data is obtained. Issues related to credit for academic advancement and compromising of potential intellectual property also preclude some groups from sharing.

Rigorous and reproducible science requires a virtuous cycle of learning – a learning research system where basic science informs clinical research or practice and vice versa

Ideally, research conducted in NHPs will seamlessly translate into clinical research to improve the health of the nation. However, that does not always occur in practice. One approach for increasing the translatability of NHP research is establishing a continuous feedback cycle between pre-clinical research, including NHP studies, and clinical research, with both informing the other. For example, research conducted in NHPs may identify a molecular mechanism or drug target to investigate in a human population. Key components of clinical research that may inform the design of an NHP study include uniform inclusion and exclusion criteria, sharing of standard operating procedures, agreement on clinically-relevant endpoints, reducing design-imposed confounders, reduction of variability to achieve higher power with smaller numbers, and embracing novel study designs such as n-of-1 clinical trials. Together, this cycle of learning will increase the validity and value of both clinical and pre-clinical research.

Conclusion

In conclusion, rigor and reproducibility are influenced by numerous factors. Good experimental design and execution is of the highest priority to funders, researchers, and society. Decreasing the number of animals used in studies, taking advantage of advanced statistical techniques, and ensuring studies are neither overpowered nor underpowered to address the experimental question will all result in more

reproducible results while maintaining high ethical standards. Broad frameworks that promote rigorous and ethical study designs can help mitigate failure and maximize data quality and validity. Considering these factors, controlling for welfare and environmental variables, and transparent reporting can lead to increased reproducibility and data validity of NHP research. Infrastructure and capacity are also needed to support these goals especially around training, standards development, and ensuring adequate housing space and transport to support future studies.

FOSTERING RIGOROUS RESEARCH: Lessons Learned from Nonhuman Primate Models and Charting the Path Forward

Background/Context

NIH's mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability.[2] As part of that mission, NIH has an obligation to ensure that the science it funds is held to the highest standards of accountability, and the welfare of both human participants and animals used in NIH-funded research is a priority.

Rigorously conducted and reproducible science is necessary for advancing the biomedical research enterprise. Appropriately-powered and well-controlled study designs using rigorous methodologies, analyses, and interpretations provide a foundation for ensuring reliable observations and testing of hypotheses. Open communication about the conduct and outcome of scientific research is a necessary component of rigorous science as it enables other researchers to reproduce results and test scientific claims.

NIH is committed to promoting research reproducibility, including in NHP research, through enhancing scientific rigor and transparency, while maintaining the highest standards for animal care and use in the research it supports. Specific examples include the "Enhancing Reproducibility through Rigor and Transparency Policy" which is meant to exemplify and promote the highest level of scientific integrity, public accountability, and social responsibility in the conduct of science;[3] the "Clinical Trials Stewardship Reforms" effort that is directed toward improving the quality, relevance, feasibility, efficiency, accountability, and transparency of NIH-funded clinical trials;[4] and the "Enhancing Reproducibility and Rigor in Animal Research" effort which aims to identify gaps and opportunities to improve the rigor, reproducibility, translational validity, and transparency of studies involving animal models.[5] In 2016, NIH also hosted a Congressionally-mandated workshop titled "Ensuring the Continued Responsible Oversight of Research with Non-Human Primates", which set the stage for a focused discussion on enhancing rigor by integrating ethical and animal welfare considerations into NHP research.[6]

While NIH's efforts span the entire spectrum of biomedical research, NHP models may have some unique considerations and lessons to share with regard to rigorous science. On February 18-19, 2020, NIH convened a workshop of experts and stakeholders to foster dialogue on the challenges and opportunities for improving rigor, reproducibility, and translatability in NHP research at the intersection of experimental design, animal welfare, and ethical considerations.

The workshop was videocast live and archived for future reference on the NIH Videocast website.[7,8]

---

[2] https://www.nih.gov/about-nih/what-we-do/mission-goals
[3] https://grants.nih.gov/policy/reproducibility/index.htm
[4] Hudson, K. (2016). Toward a New Era of Trust and Transparency in Clinical Trials. JAMA, 316(13), 1353-1354.
[5] https://acd.od.nih.gov/working-groups/eprar.html
[6] https://osp.od.nih.gov/wp-content/uploads/NHP_NIH_Workshop_Report_01_18_2017.pdf
[7] Day 1 Videocast: https://videocast.nih.gov/summary.asp?live=35537&bhcp=1
[8] Day 2 Videocast: https://videocast.nih.gov/summary.asp?live=35541&bhcp=1

Session Summaries

Keynote Address: Experimental Rigor is Multi-Faceted

The keynote presentation focused on the need to maximize rigor in NHP research, taking into account ethical concerns and practical challenges.

Rigor should inform all aspects of scientific research from hypothesis development to model selection to study design. Practical, scientific, and ethical considerations can all be impactful for the rigor of a study. Significant practical hurdles include the shortage of some NHP species, a dearth of well-trained NHP researchers, and a small number of transgenic NHP lines that can be developed and made available to researchers. Scientific challenges include accounting for the heterogeneity of NHPs (for example they are outbred) in study designs, power calculations for hypothesis testing, interpretations of observations, and the robustness of conclusions. Ethical considerations include the contemplation of alternative approaches, the significance of the scientific questions, model selection for translational studies, and tradeoffs between welfare and study methods.

For many research topics, NHPs will remain critical to scientific progress for the foreseeable future as they cannot be replaced by noninvasive human research, rodent models, or computational models. When selecting a model, a number of criteria should be applied – the relevance of the translational model to the research question being addressed (including the comparative genetic architecture of the traits under study and evolutionary conservations vs differences); an understanding of what questions can and cannot be addressed; and whether the study can be adequately powered to answer the question at hand. When evaluating statistical options, it is critical to use the appropriate statistical models and not confuse statistical for biological significance. Studies must also be powered appropriately, avoiding the use of more animals than needed but also avoiding underpowered designs that will most likely fail to test the research hypothesis. It was noted that, underpowered studies can lead to the increased use of subjects due to the need for repeated studies that are better designed.

Rigor and reproducibility could be further supported by the open sharing of data among research groups. This may require the development of data-sharing infrastructure and long-term support, including cloud-based data storage and computing facilities that support collaborations and meta-analyses. Consortia that offer standardized methods and shared designs could also be beneficial in answering specific research questions.

Summary of Session I: Ensuring Rigorous Study Design

During this session, presenters and participants discussed lessons learned and generalizable approaches across scientific disciplines and experimental aims regarding rigorously designed and conducted research studies using NHP models.

Infectious Disease Models

Use of NHPs has enabled fundamental advances in our understanding, treatment, and prevention of human infectious diseases. They have allowed researchers to ask fundamental questions about pathogenesis that cannot be addressed using rodents or human tissues and have been essential to studying diseases such as Ebola, HIV, tuberculosis, and avian influenza. Rigorous study design can be achieved by having a reproducible model that closely mimics the human condition, posing discrete questions and hypotheses that are answerable with the chosen model, and employing an experimental design based on solid preliminary data.

Rigorous Study Design Strengthens the Translational Bridges to Clinical Success in Transplantation

NHP models have meaningfully informed drug and regimen development for clinical transplantation for over 40 years based on their similar physiology and underlying biology to humans. The complex immune system of NHPs comparably mimics what is seen clinically in patients making them a good model for conducting preclinical safety and efficacy studies in a similar fashion to how a normal treatment regimen might be provided to a human patient (e.g., timing, dosing). The question being asked must be carefully considered, however, to ensure that use of the NHP model is appropriate.

Lessons learned from clinical trials show that rigor can be obtained by having uniform inclusion/exclusion criteria, sharing of standard operating procedures, agreement on clinically relevant endpoints, reducing design-imposed confounders, and the reduction of variability to achieve higher power with smaller numbers. Rigor can also be improved by employing a two-way strategy where research not only informs clinical practice (forward translation), but clinical practice also informs research (reverse translation). When a therapy fails and a patient rejects a transplant, the understanding from that failure should be instructive for new *in vitro* experiments that can be carried through various models (e.g., mouse, NHPs) until ultimately reaching practice again – essentially a continuous circle of learning and improvement. Non-adherence, inappropriate subjects, treatment factors, patient factors, and illness factors are all areas where clinical studies struggle and thus are targets for reverse translation.

Increased data accuracy and reduced variability can also be obtained through the way we handle or care for animals. Behavioral management strategies provide NHPs with an opportunity to build relationships with caregivers, thereby promoting adherence and fostering quality interactions. It also allows NHPs to learn study procedures using Positive Reinforcement Training (PRT) in low-stress conditions. This allows for the collection of physiological data with less or no restraint or removal of the NHP from the group. PRT requires an average of 3-5 hours of training, produces better data due to improved adherence to the study protocol, and increases the accuracy of outcome measures.

Understanding the Disease Being Studied

NHPs have been a valuable resource in testing therapeutic options for Parkinson's disease (PD), but it is not just essential to have the right model; you have to also apply that model correctly. A preclinical model of PD was established by treating NHPs with a neurotoxin (MPTP) that imparts symptoms similar to clinical cases of PD. The NHPs in the study were then treated with GDNF, a naturally occurring protein that has been shown to promote dopaminergic neuron survival, and the study showed initial success in restoring and regenerating neurons. While these studies showed improvement in NHPs, attempts to replicate these effects in human clinical trials did not translate. In evaluating why this translational failure occurred, it was realized that the underlying disease was not the same as the chemically-induced state of the NHPs. Studies in humans showed that at four years post-diagnosis there are no remaining neuronal fibers in the target region in the brain where GDNF was being delivered, making regeneration impossible.

Lessons learned suggest that translational researchers need to have a better understanding of the clinical course of the human disease and appreciate the limitations of the models being used even more than their similarities to the human disease. This can be essential to successfully modelling conditions in the experimental setting, which can help to contribute to improved success in translational research.

What Failure Can Teach Us in NHP Research

NHP studies have led to the development of some important pharmacological treatments, such as tenofovir (PMPA). Patented in 1998, NHP studies showed the feasibility of preventing Simian Immunodeficiency Virus (SIV) using this compound and today, tenofovir is used by millions of humans as an HIV treatment. Despite this critical success, NHP studies failed both before and after the landmark work showing tenofovir's utility. Maximizing the value of these failed studies to improve rigor in future studies can occur through increased transparency and urgency. With respect to transparency, a more robust disclosure of study design, methods, results, and data needs to occur. In order to track animals through multiple studies, some National Primate Research Centers (NPRCs) have developed a unique identifier for each NHP, which allows researchers to study animals throughout their lifespan regardless of where they are or what study they are on. Additional disclosure is also needed on identifying the viruses used to challenge NHPs. The University of Wisconsin has sequenced more than 40 stocks in its viral registry and offers to sequence viruses for free with the goal of enabling cross-study data comparisons.

With respect to urgency, there is a need to share data and results with other researchers prior to publication, especially for emerging diseases. Free preprint servers, such as bioRxiv, are now available which allow researchers to post non-peer-reviewed findings immediately.[9] Open source platforms such as LabKey can also help researchers to analyze and share complex biomedical data.[10] However, open data sharing is not without challenges. The effort required to share data can be considerable and initial interpretations of results may gain traction even though they may not hold up over time as new data is obtained. Issues related to credit for academic advancement and compromising of potential intellectual property also preclude some groups from sharing.

Group Discussion

- *Strategies to reduce NHP numbers*. Some basic research studies, such as understanding neural processing in the central visual system, can require a small number of NHPs (n=1 or n=2) by using rigorous design that includes good statistical analysis. A key differentiator with these studies is the capture of data from a significant number of neurons and repeating those results in just the one or two NHPs. Power analysis, selection of appropriate outcome measures, adaptive trial design, developing a limited number of research questions, and overall good study design can, in some instances, lead to the use of a smaller number of NHPs without inappropriately underpowering studies. Effect size can also greatly influence the number of NHPs necessary for a study.
- *Good experimental design*. Good experimental design using advanced statistical techniques can help increase study power. This is critical as researchers may at times seek to increase power by increasing the number of animals, rather than focusing on good design. Free tools are available to help researchers design robust experiments more likely to yield reliable and reproducible results.[11] A deep understanding of the disease that is being modeled and staging is critical to properly designing studies and having open access to data from many investigators can increase the knowledge base for all.

[9] https://www.biorxiv.org/
[10] https://www.labkey.com/
[11] https://eda.nc3rs.org.uk/

- *Individual vs. cluster studies*. In some studies, each animal can be considered an individual data point. However, in other studies, such as neurophysiology studies, one or more NHPs can provide data clusters. It is important to keep in mind that the analysis of cluster data does not follow the same power rules as does analysis of individual animal studies. The number of subjects used for cluster studies may therefore differ.
- *Essential use of NHPs*. NHPs have been found to be essential to answer some types of research questions. For example, with highly pathogenic flu, mouse models do not reflect the human disease. To better understand HIV, SIV can be studied to better understand early events after infection. This is difficult to do in humans as a person can acquire HIV but not know it or show symptoms for some time. In addition, NHPs are essential for understanding motor movement and cognition, as the mouse motor cortex is significantly different than that of humans.

Summary of Session II: Considering External Factors and Interpretation of Findings

In this session presenters and participants examined approaches for addressing the intersection of species selection, animal behavior, biological outcomes, and other external factors in study design. The session also explored how these factors may affect interpretation of study findings when using NHP models.

The Impact of Environmental Variables on the Welfare and Behavior of Laboratory NHPs

Environmental variables not only influence the welfare and behavior of NHPs but also have the potential to affect the quality of the research outcomes and study goals. The scientific literature shows the beneficial effects of environmental and social enrichment on animals and scientific studies. Incorporating environmental variables such as social housing, space allocation, and PRT in the study design can enhance the animal's welfare while ensuring rigor and reproducibility of scientific studies.

Managing animal welfare and behavior should be harmonized with the goals of the research. Incorporating social housing enables NHPs to perform species-appropriate behavior and reduces abnormal behavior, buffers against environmental and social stressors, and reduces stress/cortisol levels. When NHPs are sheltered in long-term single housing, studies have shown an increased level of abnormal behavior – such as self-biting and pacing – and can lower experimental external validity. Space allocation (cage height and volume) should address the housing needs of the animal to reduce tension-related behaviors such as abnormal behavior and aggression. Finally, utilizing PRT can improve the quality of research and reduce stress by conditioning the animals for desired behaviors through positive reinforcement. PRT is used to increase efficiency, improve the ability to conduct study procedures, and reduce environmental variables including stress associated with clinical and husbandry procedures.

Social Environment Influences Neuroendocrine Regulation and Homeostasis in NHPs

Understanding the social needs of NHPs in studies is important for providing an appropriate social environment and for enhancing the quality of research data. Socially grouping primates is often beneficial, having both physical and psychological benefits. For example, a social companion can provide a buffer to a stress response by reducing HPA activity and cortisol response. In fact, positive response from a preferred partner or family member has been shown to decrease stress levels. Additionally, utilizing PRT and transporting animals with a social companion can reduce stress. Scientists should consider social needs in their study design to maximize the collection of quality data.

Fostering Rigorous Research with NHPs: A Funder's Perspective

The guiding principles that underpin the welfare of animals in scientific research are the 3Rs (replacement, refinement and reduction). International regulations and guidelines for the conduct of safety studies in animals require implementation of the 3Rs. The National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) is an independent, UK-based scientific organization dedicated to replacing, refining, and reducing the use of animals in research and testing.[12] It funds research and reviews all NHP proposals for 28 funding organizations internationally to ensure that design is robust, the number of animals to be used is justified, and high animal welfare standards are promoted. It also advises on opportunities to implement the 3Rs. NC3Rs has developed the ARRIVE guidelines, which are used to maximize the output of animal research and avoid unnecessary animal use.[13] These guidelines have been adopted by more than 1000 journals, funders (including NIH), universities, organizations, and societies worldwide.

Common contributors to failed translational research are flawed study designs, incomplete reporting, and lack of reproducibility of preclinical studies. The ARRIVE guidelines prioritize the use of ten essential items to help mitigate such failures: study design, sample size, inclusion/exclusion criteria, randomization, blinding, outcome measures, statistical measures, experimental animals, experimental procedures, and results. Environmental enrichment such as social housing, PRT, and pain relief are crucial for NHP welfare and maximum experimental validity. Good behavioral management can affect an experimental endpoint and improve scientific outcomes by reducing inter-animal variation and enhancing model validity. Standardization of methods should reduce variability and more detailed reporting of the characteristics of NHPs used in experiments can improve reproducibility and lead to new areas of investigation. Transparent reporting of animal studies is necessary to realize the anticipated benefit of research studies and reduce animal use. Research that is not reported in enough detail or with unreliable findings cause more harm than good, skewing the harm/benefit analysis and making research involving animal pain or distress unethical.

Funders can play a major role in improving standards and reproducibility, ultimately maximizing the value of their investment in the NHP research. Introducing an infrastructure to promote transparency and complete reporting would speed the development of methods and tools that support reproducibility by strengthening the reliability and rigor of results. Utilizing the 3Rs concept is both a robust framework for minimizing animal use, improving animal welfare (addressing the harms to animals), and a means of supporting high quality science and translation (addressing the benefits).

Group Discussion

- *Behavioral science and social housing.* The potential for abnormal behavior of single-housed animals can have an impact on research outcomes. Social housing is, therefore, an incentive for those pursuing both reproducible research and animal welfare. It is important to keep in mind that additional funding may be required to convert some existing US facilities to better support social housing.
- *US housing standards*. More studies are needed to augment the evidence regarding the size of animal enclosures in the US Guidelines, which were developed in the 1980s and have not been revised since.

[12] https://nc3rs.org.uk/

[13] https://www.nc3rs.org.uk/arrive-guidelines

- *Randomization*. Randomization is an important component of rigorous experimental design. However, animals may be excluded from studies by veterinarians who believe they are fractious or needle shy, which impacts true randomization. A rolling enrollment period could help address this through repeated assessments to determine if behaviors have ameliorated or changed enough to meet the study's qualifications. Another strategy is to use behavioral management to make sure all animals in the study have the same level of coping skills.
- *Species differences*. Researchers should keep species differences in mind when it comes to social housing and other challenges. Some species may need a particular protocol to introduce an animal to a group or pair it with another animal. Having professionals that understand species differences as part of the team can be beneficial.
- *Reporting results*. While there is broad support for the ARRIVE guidelines, sufficient follow-up may be lacking to ensure that such guidelines are followed in the reporting and publishing of study results. Compliance with guidelines such as ARRIVE in published manuscripts can help increase reproducibility.
- *Role of funders and institutions*. Requirements from funders can help improve rigor and reproducibility. For example, data sharing or social housing could be a requirement for funding[14]. However, such requirements may incur additional costs, and funders may need to compensate for these activities. Institutional bodies such as IACUCs could help to assess rigor, as this is part of their charter.

Summary of Session III: Reporting Results and Sharing Data

Participants in this session discussed opportunities for maximizing data and metadata sharing from NHP research studies, including the research culture and infrastructure needed for ensuring data quality and replication of study design.

Sharing methods and data in systems neuroscience

The first presentation of the session focused on the value of using consortia for data sharing in systems neuroscience research. The International Brain Laboratory (IBL) was formed in 2017 and consists of 22 laboratories from around the world. While the IBL uses species other than NHPs, the lessons learned from IBL can be co-opted by other consortia, including those using NHPs. The IBL software has many features that are ideal for data sharing in general – shared data, shared resources, and shared code. Members of the IBL have an agreement to share data with other members within 12 months and have published white papers on data standardization and sharing.

Robust data sharing requires adequate infrastructure. One example of a large data sharing paradigm is EBRAINS, a platform providing tools and services which can be used to address challenges in brain research and brain-inspired technology development. The goal of EBRAINS is to provide tools and services to assist in collecting, analyzing, and integrating brain data. EBRAINS is open to all investigators around the world and can be used to integrate any neuroscience data. However, such a platform is costly; it required a significant financial investment from the European Union.

Data Sharing in NHP Genetics and Genomics

The genetics field has a strong culture of sharing genomic data, stemming from data sharing policies of the Human Genome Project. Researchers already deposit raw genomic data prior to or upon publication

[14] www.nc3rs.org.uk/primatesguidelines

of their data. Currently, two databases – developed by the NPRCs and the University of California, Santa Cruz – exist for the sharing of genomic and annotated phenotypic data from NHPs. However, the types of data that can be deposited are limited, and there are a number of opportunities to increase the types of data that are shared as well as increase the number of NHP species that can be represented in the databases.

Reporting Results & Sharing Data: Experiences from Zika virus studies

Despite the fact that many individuals had never heard of the Zika virus before 2016, there are publications that are more than 70 years old describing the discovery of the Zika virus in rhesus macaques. The surge of Zika infections in 2016 led many investigators to rapidly study the virus, necessitating wide-spread collaboration and data sharing. Several NPRCs formed a working group to establish, and learn from, NHP models of Zika infection. Working group members met frequently in person and by phone to share data and findings as well as to aggregate data from different studies. In order to aggregate the data, the working group needed to establish several standards and data elements. When possible, the working group used quantitative data or reduced data elements to binary options (e.g., yes/no) to simplify analyses. The rapid collaboration resulted in successful publications and can serve as a model for future urgent health needs.

Group Discussion

- *Benefits and challenges of consortia.* The consortia and collaborations discussed by the panelists all have different roles and characteristics. IBL is a closed consortium with a limited number of members. EBRAINS is open to anyone, but the value of data is variable and is dependent on what is deposited in the database. Many genomics collaborations are smaller than consortia; however, data sharing in genomics is rapid and plentiful. Still, one concern is that there is little filtering of the data. Finally, the Zika working group, composed of NPRC members, was rapidly assembled to address a pressing topic. Each type of consortia or collaboration has value and has its own role to fill.
- *Role of trainees in consortia.* Attendees expressed concerns about the careers of trainees and how they may be affected by participating in large consortia. Experiences with the IBL suggest that trainees are very engaged in the work. Additionally, participants predict that the future of science will be large collaborations rather than smaller labs conducting independent research. Thus, participants felt that collaborations will ultimately benefit the careers of young scientists.
- *Data from individual animals.* Questions were raised regarding data from a single animal that may be an anomaly in a study. Panelists suggested sharing the data from that animal with as much metadata as possible to allow other researchers to draw their own conclusions, as appropriate.
- *Publishing negative results.* The attendees were generally supportive of publishing negative results; however, several attendees expressed caution. Negative data are only as valuable as the experimental design used to generate them. Therefore, experiments generating negative data must be conducted and analyzed as rigorously as other data.
- *Limitations to data sharing.* In the past, data sharing was limited by storage for the data. However, data storage has become significantly more affordable, and data sharing is now limited by computing power. Increasing computing power will require substantial resources that are out of the realm of possibility for a single lab. In addition to physical limitations of data

sharing, there is concern about the intellectual investment needed to increase data sharing. Those sharing data will need to be compensated for the time to do so.

Day 1 Wrap Up Discussion: Lessons Learned and Generalizable Strategies

The goal of the first day of the workshop was to help figure out how to continue to maximize the scientific value of NHP research by optimizing experimental design in terms of rigor, sharing of methods, tools, and data. Several key themes that emerged from the day included:

- *Appropriate numbers of animals.* Various workshop participants indicated that larger number of animals may be needed to conduct experiments, and this need is very apparent when doing experiments that require transgenic animals. While increasing the number of animals is counter to the goals of the 3Rs, researchers should view the 3Rs as a framework to support ethical and high-quality science using the appropriate number of animals to answer a particular scientific question. Using either too many or too few animals would be unethical.
- *Using the appropriate model for the disease.* Understanding the animal model and understanding the human disease are fundamentally different, and it's critical to have a thorough understanding of both to conduct rigorous science. This knowledge should be used to choose the appropriate model, which is often the least complex model that can answer the research question. Sometimes the ideal model will be NHPs, but many times it will not.
- *Transgenic models*. Genetic modification of NHPs is already being carried out in other countries. While some efforts have taken place in the US, a national initiative is lacking. Without an organized and funded program, it may take years before there will be enough transgenic NHPs for US researchers to conduct studies. For NPRCs to launch a major initiative in genetically modified animals, it would require something akin to a program providing supplements specifically for that purpose.
- *Timing and limitations on data sharing*. Data sharing is vital for reproducibility and scientific progress. However, barriers exist to data sharing, including releasing data prior to publication. Two approaches to support data sharing could involve timing and limitations. For example, researchers may want to stipulate *when* the data are shared. In some fields, such as infectious diseases, data could be shared earlier while in others it might be appropriate to share later, in some circumstances, even after publication.
- *Standardization*. Sharing data across centers may require adoption of a variety standards to ensure interoperability and comparability of datasets. However, there are challenges to what can be standardized, as a big factor influencing an animal's experience are the personnel it interacts with. This is a component that is not easily controlled or standardized, but it can be accounted for if it is recorded.

Summary of Session IV: Research Opportunities and Challenges for the 21st Century

Technical advances or transformative breakthroughs have the potential to pose new scientific or ethical challenges related to the design or conduct of rigorous research studies. Participants in this session discussed ethical considerations that have arisen in the past related to novel technologies as well as considerations for the future.

Technological Advances that May Pose New Opportunities and Challenges to Rigorous Research

Experimental genetics, personalized medicine, and technology-driven increases in data generation, reuse, and integration all provide new opportunities and challenges in NHP research. The use of

marmosets provides an opportunity to accelerate experimental genetics in NHPs; however, the infrastructure for significant marmoset use may not be available. One provocative option to address this issue is to genetically accelerate the life cycle of the rhesus macaque to make them more amenable for experimental genetics. However, the use of genetically-engineered NHPs is not without challenges. One challenge is the issue of space constraints and other infrastructure associated with rapidly expanding an NHP colony. Additionally, researchers should not create or expand these models without establishing clear research objectives.

Targeting genes in NHP somatic cells provides the opportunity to generate hypotheses for personalized medicine and treatment of genetic disorders.[15] In order to achieve this objective, researchers would need to be more comfortable with small sample sizes and may need to consider the framework established by n-of-1 clinical trials.[16]

Finally, advances in data generation, reuse, and integration provide opportunities to extend the influence of data. Dense longitudinal measurements allow for comparison over long periods of time and can be used by multiple research groups. Given the similarity of the genomes, integration of human and NHP genetic data can improve the validity of human genetic analyses. Furthermore, integration of NHP and human histology data in combination with artificial intelligence provides an opportunity to increase the validity of these approaches.[17,18] The ability to develop technology and approaches to increase generation, reuse, and integration of data will require an investment in necessary infrastructure.

Challenges and Opportunities of New Developments

Genome editing and brain imaging both pose new opportunities for the advancement of science. However, both are hindered by available resources and infrastructure. The ability to generate genetically-engineered marmosets is impeded by inefficiencies in *in vitro* fertilization, mosaicism, and the number of animals needed to establish founders and breeding colonies. These difficulties currently limit experiments to studying monogenic diseases which reduces the utility of the models since many human diseases are polygenic.

Significant effort has been devoted to establishing methods for imaging the brains of mice. These analyses have been very extensive and resource intensive. While it will be important to extend these studies to NHPs, the needed resources may be limiting. As such, the community should think critically about designing experiments, establishing cores and centers, or developing comprehensive data sharing plans.

Principles of Social Benefit and Animal Welfare

When designing and conducting experiments using animals, researchers should weigh the societal benefit against the cost to the animal's welfare. A recently published book on ethics of animal research

[15] Schork, N. (2015). Personalized medicine: Time for one-person trials. Nature, 520 (7549), 609-611.
[16] Lillie, EO. (2011). The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? Per Med, 8(2): 161-173.
[17] Fu, Y. (2020). Pan-cancer computational histopathology reveals mutations, tumor composition and prognosis. bioRxiv preprint. DOI: 10.1101/813543.
[18] Sundaram, L. (2018). Predicting the clinical impact of human mutation with deep neural network. Nat Genet, 50 (8), 1161–1170.

in applied science, Principles of Animal Research Ethics,[19] brings forward three principles in social benefit (principle of no alternative medicine, principle of expected net benefit, and principle of sufficient value to justify harm) and three principles in animal welfare (principle of no unnecessary harm, principle of basic needs, and principle of upper limits to harm). Comprehensively considering the six principles will take researchers beyond the 3Rs when designing and conducting experiments using NHPs. The principles provide a framework for investigators to go beyond the legal and regulatory requirements for conducting animal research and focus on research that is grounded in ethics.

Group Discussion

- *Beneficial technological advances*. Various technological advances and discoveries in NHPs have impacted humans directly. One of them is the mitochondrial replacement therapy which was originally pioneered at the Oregon NPRC. This has allowed individuals to have children who are free from debilitating mitochondrial diseases. This discovery has been adopted for clinical use in the United Kingdom and the first children have been conceived using this technique.
- *Gene therapy*. Transgenic NHP studies could help to better understand some conditions, such as autism. Additional NHP studies involving gene therapy could help to improve monogenic disorders. Many monogenic disorders include high levels of disability which might be alleviated through gene therapy.
- *Ethical frameworks*. Ethical frameworks, such as the recently published Principles of Animal Ethics, are important because they allow researchers and the general public to have pivotal, in-depth discussions on harm, social benefit, and overall ethics.
- *Ethics and new technologies*. New technologies and the development of new NHP models may create new opportunities for ethical discussions. The development of new technologies, such as facial recognition cameras that can evaluate NHP behavior, may also allow researchers to test and evaluate animals in less restrictive conditions where they may not need to remove them from their environment to manually evaluate their behavior.
- *Influence of private funding*. Private donors can influence science and discovery by funding discrete projects. However, funding is sometimes tied to studying a specific condition, such as a particular rare disease. The converse of this benefit is that the research may not be focused towards the greater good. More discussion may be needed among researchers and the public to prioritize NHP research to meet public goals.

Summary of Session V: Strategies for the Future

Participants discussed generalizable strategies for continuing to maximize the value of NHP research through strategies to: 1) Facilitate translation through study design, 2) Evaluate ethical considerations by incorporating evidence-based approaches, 3) Identify cross-discipline competencies to ensure robust research training paths, and 4) Conduct animal well-being and welfare research.

Facilitating Rigorous Science by Standardizing Behavioral Management

Research using NHPs often requires complex data collection and behavioral management. This research now includes molecular, electrophysiological, behavioral and imaging technologies to identify the underlying mechanisms of disease. As NHP research continues to become more complex, behavioral

[19] Beauchamp and DeGrazia. (2020). Principles of Animal Research Ethics. Oxford University Press. ISBN: 9780190939120.

management may not only help to ensure that animals are physically and psychologically healthy but can also continue to enhance rigor and reproducibility efforts through standardization of tools and measures. The NPRCs are working through a Behavioral Management Consortium to monitor and improve animal welfare and NHP research rigor by developing consensus on enrichment and other behavioral management strategies. Three areas of standardization that they are focused on in research include terminology, tools, and housing.

Terminology has been used inconsistently, hampering the broader integration of results from different studies. Language must be universal, with researchers consistently referring to the same terminology in the same way. It would be helpful to have consensus on definitions for terms such as "pair housing," for instance. In some facilities this is defined as animals who are continuously paired, while in others it is applied to animals that are separated overnight. The differentiation in terminology can disrupt coordinated research on individual-level processes and group-level outcomes and decrease the success rate of collecting meaningful and comparable data.

Standardization of tools is necessary to comparably assess animal behavior and welfare. For example, some centers have developed scoring systems to assess alopecia and self-injurious behavior. By using the same tools, two different centers can assess alopecia the same way, thereby increasing the reproducibility of research. New technology offers exciting new opportunities to continue standardization of behavioral observations and imaging.

Housing status can differ within and between fields. For example, data on social housing in infectious disease studies from six NPRCs showed that 34 percent of NHPs were single-housed for part of the study while 20 percent were single-housed for the entire study. Variations such as these may influence experimental outcomes, and more research is needed to examine this effect. Individual variation in terms of the individual needs of the animals as well as other factors such as the animal's temperament and stress sensitivity also influence behavioral needs and study outcomes. For these reasons, housing standardization is ideal. Standardization does not have to be a one-size-fits-all approach; however, housing decisions must account for the individual needs of the animal.

Statistical Approaches to Study Reproducibility

Only a fraction of animal research studies can be reproduced. In some cases, even the original authors could not reproduce the same studies in their own lab, using their own reagents. Studies that failed replication shared the following six characteristics, of which most involve experimental design or statistical analyses:

- Experiments were not blinded;
- Basic experiments were not repeated;
- Not all results were presented (e.g., omitting negative results);
- There were no positive or negative controls;
- The reagents were not validated;
- The statistical tests were not appropriate.

To address variability, researchers should include blinding, factorial, and/or randomized block designs as minimum standards. Invariant methods such as Student's t-tests are less powerful and often inflate sample size. Employing advanced "human" statistical methods to control for uncontrollable variation in

the analysis rather than in the experiment avoids the dilemma of standardization. However, this is the lowest hanging fruit in terms of positively changing the issues of reproducibility and rigor by changing our perspective away from thinking of research animals simply as tools. Instituting the infrastructure and framework to educate study groups, proactively identifying and cross-training biostatisticians, program officers, and the IACUC on these matters could help enforce such standards. Providing more funding for research on animal welfare, reproducibility, and translation may have strong impacts on standardization and culture changes in the future. These steps may increase the reproducibility rates of animal research, work to effectively increase translation into clinical settings, and increase the inherent value of animal research.

Convergence and its Influence on Translation, Rigor, and Reproducibility

Complex scientific questions often require that scientists from a variety of scientific disciplines work collaboratively, or "converge", on a problem in order to solve it. A similar approach could be taken by converging many types of datasets on the same cohorts of NHPs. The California NPRC has used this approach to study diseases related to aging. Most aging studies are cross-sectional, meaning that data from young animals and older animals are compared to each other. The California NPRC has built a "pipeline" of young and aged (greater than 19 years) NHPs in its colony to study cognitive function and Alzheimer's disease. The researchers have full datasets and lifelong veterinary records for each of the animals, allowing them to address many research questions over the lifespan of the animals. The longitudinal monitoring of health indices allows for convergence of many datasets, providing a holistic picture of the health of the animal. Such efforts, however, would require significant and long-term funding.

Group Discussion

- *Funding support*. Funding support is needed to develop infrastructure for paired housing and complex social housing. Studies converging many types of datasets on the same cohorts of NHPs will also require a different type of funding mechanism. Increasing the number and size of NHP colonies can be done at some primate centers but will also require additional funding.
- *Capacity building.* Capacity building is needed in various areas including training individuals in behavioral training, PRT, standardized evaluation, experimental design, biostatistics, developing large multi-disciplinary studies, and other areas.
- *Genomic characterization*. One of the reasons for the lack of reproducibility in a study could be genetic differences among animals. Characterizing the genomes of the NHPs used in research could help to better understand how genetic variation affects reproducibility. A national program for genomic characterization would be needed to do this at a large scale.
- *Phenotypic characterization*. Phenotypic characterization could help researchers to identify which NHPs should be used for a particular study. For example, in autism studies at UC Davis, animal behavior was well-characterized in the first year of life. This helped researchers identify NHPs believed to be socially abnormal, carry out behavioral observations to confirm this, and then move forward with the study.

Day 2 Wrap-Up Discussion: Charting the Path Forward

- *Openness to failure and negative results.* Negative results are key to scientific progress. "Failure" from otherwise rigorously designed research can be used to generate both scientific and ethical value. For example, publishing and openly sharing negative results provide information and

context to improve the experiments in the next round of inquiry. There needs to be more acceptability towards publishing negative data in the scientific community.

- *Knowing your animals.* Rigorous and reproducible studies require a good understanding of the influence of both internal and external factors on the animals. Different species of NHPs exhibit a wide range of genetic and genomic variations. Additionally, environmental variables and natural histories can all significantly affect animals' physiology and behavior. It is important to understand and characterize the influence of these variables on specific experimental measurements, data interpretation, and welfare considerations.

- *Improving NHP research infrastructure.* The demand for and value of NHPs in biomedical research will likely increase in the future given the growing availability of advanced research tools brought up by recent science and technology progress.[20] It is important to recognize the differing strengths of NPRCs and individual university labs in NHP research. While NPRCs can leverage resources for studies involving a larger number of subjects, small groups at universities also conduct important work and have relatively easy and ready access to a multidisciplinary team of professionals. Many US institutions and primate facilities are facing the challenge of complex housing needs and declining primate transport capacity. There is clearly a need for more support for infrastructure development and capacity building to guarantee researchers' access to NHP models.

- *Enhancing data sharing.* Data sharing increases transparency and rigor. It is helpful for the community to learn from successful examples of data sharing, such as some of the large-scale storage and data sharing established by large initiatives (e.g., EBRAINS). Effective data sharing necessitates development of robust infrastructure and a culture change. There is a need for a federated, interoperable database system to maximize the value of data sharing. Both financial and strategic guidance are needed to develop the infrastructure for data sharing. Many entities in the private sector (e.g., Amazon, Google) possess the subject matter expertise and experiences in data sharing and can help build the infrastructure as federal contractors. A culture change can be stimulated by incentives to data sharing, which can be given to the entire research group so that sharing of data is not perceived as an additional burden. NIH should consider providing resources, such as staffing, that can help support data curation and sharing among scientists. Some have also called to include a data scientist in every research team to help structure the data for sharing from the very beginning. A centralized hub of experts could support scientists who want to share data but may not have the expertise to do so. Such effort is already underway in the field of genetics with the Sequence Read Archive[21] —NIH's primary repository of high-throughput sequencing data.

- *Science of animal welfare.* Good animal welfare equals good science. While many activities have been undertaken with regard to rigorous science, there are rarely conversations nor funding for fostering rigorous welfare. There is clearly a need to invest in animal welfare research, which provides the evidence base for good welfare and its impact on science. New ways of thinking and continuing discussions can help identify areas for optimization without the need for new regulations to be put in place.

- *Open communication to the public.* As recipients of public funds, it is important for scientists to engage and communicate their work to the general public. Such engagement could start within

[20] National Institutes of Health (2018). *Nonhuman primate evaluation and analysis part 2: report of the expert panel forum on challenges in assessing nonhuman primate needs and resources for biomedical research.*

[21] https://www.ncbi.nlm.nih.gov/sra

the communities at the research universities. It was suggested that scientific data and findings could be shared with the public through open and trustworthy resources. There could also be a website showcasing scientists' dedication to science and animal welfare. Such efforts are already underway. For example, the NPRC publishes news from various centers on its website.[22] Some universities also have public-facing websites demonstrating their commitment to animal welfare.[23] Communication to Congress is also important to help understanding and to inform public policies. It was noted that scientists need to improve their communication skills when speaking to the public.

[22] https://nprc.org/news/

[23] http://med.stanford.edu/animalresearch.html

Appendices

Appendix 1: Common Acronyms

ARRIVE	Animal Research: Reporting of In Vivo Experiments
GDNF	Glial Cell Line Derived Neurotrophic Factor
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic–pituitary–adrenal
IACUC	Institutional Animal Care and Use Committee
IBL	International Brain Laboratory
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NC3Rs	National Centre for the Replacement, Refinement, & Reduction of Animals in Research
NHP	Nonhuman primate
NIH	National Institutes of Health
NPRC	National Primate Research Center
PD	Parkinson's Disease
PMPA	Tenofovir *or* 9-[9(R)-2-(phosphonomethoxy)propyl]adenine
PRT	Positive Reinforcement Training
SIV	Simian Immunodeficiency Virus
SRA	Sequence Read Archive

Appendix 2: Workshop Participant Biographies

SIMON BARRATT-BOYES, BVSC, PHD, DACVIM

Dr. Barratt-Boyes is a professor in the Department of Infectious Diseases and Microbiology in the Graduate School of Public Health with a secondary appointment in the Department of Immunology in the School of Medicine at the University of Pittsburgh. He graduated with a Bachelor of Veterinary Science from Massey University in New Zealand in 1984 and did residency training at the University of California, Davis. Dr. Barratt-Boyes earned a PhD in comparative pathology from UC Davis in 1993 prior to postdoctoral training in immunology at the University of Pittsburgh. He joined the faculty of the University of Pittsburgh in 1998. His research interests are in viral immunology and pathogenesis, with an emphasis on infectious diseases of importance to global human health.

ELIZA BLISS-MOREAU, PHD

Dr. Bliss-Moreau is an associate professor in the Department of Psychology and a Core Scientist at the California National Primate Research Center at the University of California, Davis. She completed her undergraduate (SB in biology and psychology) and graduate training (PhD in psychology) at Boston College, and postdoctoral training in NHP neuroscience, primatology, and systems science at UC Davis. Her lab's multi-method, multi-level, multi-disciplinary, multi-species research program is focused on understanding the biological mechanisms that generate healthy and unhealthy emotions and social behavior, with the goal of developing new effective treatments and interventions for emotion-related psychopathology and understanding how and why emotions evolved. Her research program adopts a lifespan approach, studying NHPs from infancy through old age – what the Bliss-Moreau Lab refers to as womb-to-tomb affective science.

KRISTINE COLEMAN, PHD

Dr. Coleman is the head of the Behavioral Services Unit and an associate professor in the Divisions of Comparative Medicine and Neuroscience at the Oregon National Primate Research Center (ONPRC). She has over 25 years of experience in animal behavior, with an emphasis on individual differences in temperament and stress sensitivity. She received her PhD in behavioral ecology from Binghamton University before moving to Oregon as a postdoctoral fellow. For the past 19 years, she has overseen the ONPRC behavioral management program, where she has studied ways to improve the psychological well-being of laboratory macaques. She is particularly interested in how individual differences in temperament can affect behavioral management practices and inform management decisions. In addition, Dr. Coleman is Vice-Chair of the ONPRC IACUC, co-chair of the American Society of Primatologists' Primate Care committee, and an ad hoc specialist with AAALAC International.

DON CONRAD, PHD

Dr. Conrad is an expert in genetics/genomics and the development of bioinformatics pipelines/platforms for the efficient assessment and management of genetic data. He played a leading role in mapping and characterizing the functional impact of human chromosomal structural variants. He also developed novel statistical methods for identifying de novo point mutations from next-generation sequencing data and used this to estimate germline mutation rates from parent-offspring trios as part of the 1000 Genomes Project. A major emphasis of his current research includes defining the origins of mutation and the distribution of mutation frequencies that impact gametogenesis, fertilization, and pregnancy. He is founder of the Genetics of Male Infertility Initiative (GEMINI), which is an international network of andrology investigators. Dr. Conrad was trained at the University of Chicago and the Wellcome Trust

Sanger Institute prior to joining the faculty at Washington University in St. Louis, where he led a successful research group in the Department of Human Genetics. He joined the ONPRC in 2018 to lead the newly established Division of Genetics.

ROBERT DESIMONE, PHD
Dr. Desimone is the director of the McGovern Institute and the Doris and Don Berkey Professor of Neuroscience at Massachusetts Institute of Technology. Prior to joining the McGovern Institute in 2004, he was director of the Intramural Research Program at the National Institutes of Mental Health, the largest mental health research center in the world. The Desimone lab is interested in how the brain deals with the challenge of information overload and investigates the brain mechanisms that allow us to focus our attention on a specific task while filtering out distractions. Dr. Desimone is currently developing primate genetic models for brain disorders. He is a member of the National Academy of Sciences and the American Academy of Arts and Sciences, and he was recently the Secretary of the Society for Neuroscience.

JOSEPH GARNER, DPHIL
Dr. Garner received his doctoral degree from the Department of Zoology at the University of Oxford, Great Britain, where he studied the developmental neuroethology of stereotypes in captive animals. His postdoctoral research in animal behavior and well-being was undertaken at UC Davis. He served as an assistant and an associate professor of animal behavior and well-being in the Department of Animal Sciences at Purdue University, where he also held a courtesy appointment in the Department of Speech, Language, and Hearing Sciences. Dr. Garner joined the Department of Comparative Medicine at Stanford as an associate professor in 2011. There, he runs Stanford's Technique Refinement and Innovation Lab, which provides a wide range of support services to assist researchers on campus maximize the efficiency of their work and the well-being of the animals involved. Recognition of Dr. Garner's work includes awards from the National Center for the 3Rs (UK), the American Association for Laboratory Animal Science, the Swiss Laboratory Animal Science Association, and the Universities Federation for Animal Welfare.

MELANIE GRAHAM, MPH, PHD
Dr. Graham is an associate professor in the Departments of Surgery and Veterinary Population Medicine as well as the director of the Preclinical Research Center (PCRC) at the University of Minnesota. She earned her MPH in epidemiology from the University of Minnesota and her PhD from Utrecht University. Her research is centered on the development of cell-based therapies for the treatment of diabetes, specifically extrahepatic delivery of islets. Dr. Graham is also widely recognized for her expertise in the characterization and refinement of animal models of chronic disease to enhance translation to the clinic. This work proved pivotal to the first demonstration of successful long-term diabetes reversal after adult pig islet xenotransplant in NHPs. Dr. Graham is serving on the North American 3Rs Consortium steering committee, the NIAID NHP Transplantation Tolerance Cooperative, and NIAID Immunobiology of Xenotransplantation Cooperative Research Program. Her research is supported by the State of Minnesota, JDRF, and NIH.

STEVEN E. HYMAN, MD
Dr. Hyman is the director of the Stanley Center for Psychiatric Research at Broad Institute of MIT and Harvard, a core member of the Broad Institute, and Harvard University Distinguished Service Professor of Stem Cell and Regenerative Biology. Dr. Hyman served as provost of Harvard University, the university's chief academic officer. As provost, he had a special focus on establishment of collaborative

initiatives in the sciences and engineering spanning multiple disciplines and institutions. From 1996 to 2001, he served as director of the U.S. National Institute of Mental Health (NIMH), where he emphasized investment in neuroscience and emerging genetic technologies and initiated a series of large practical clinical trials that were forerunners of comparative efficacy studies. Dr. Hyman is past president the Society for Neuroscience and the American College of Neuropsychopharmacology; founding president of the International Neuroethics Society; and previously served as editor of the *Annual Review of Neuroscience*. He is a member of the US National Academy of Medicine where he has served on the council. Dr. Hyman received his BA summa cum laude from Yale College, an MA from the University of Cambridge, which he attended as a Mellon fellow studying history and philosophy of science, and an MD cum laude from Harvard Medical School.

LYRIC JORGENSON, PHD

Dr. Jorgenson is the deputy director for the Office of Science Policy at NIH. In this position, she provides senior leadership in the development and oversight of cross-cutting biomedical research policies and programs considered to be of high-priority to NIH and the US Government. Most recently, she was also the deputy executive director of the White House Cancer Moonshot Task Force in the Office of the Vice President in the Obama administration, where she directed and coordinated cancer-related activities across the Federal government and worked to leverage investments across sectors to dramatically accelerate progress in cancer prevention, diagnosis, and treatment. Prior to joining the Office of Science Policy, she was a senior science policy advisor and analyst under the NIH Deputy Director for Science, Outreach, and Policy and assisted in the creation of new, high impact science and policy initiatives such as the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative and the National Center for Advancing Translational Sciences (NCATS). She was also an AAAS Science and Technology Fellow and has received numerous awards in recognition of her accomplishments and service. Dr. Jorgenson earned a doctorate degree from the Graduate Program for Neuroscience at the University of Minnesota-Twin Cities where she conducted research in neurodevelopment with a focus on learning and memory systems. She earned a bachelor's degree in psychology from Denison University.

JEFFREY KORDOWER, PHD

Dr. Kordower is an international authority in the area of movement disorders with special expertise in pathophysiology and experimental therapeutic strategies in Parkinson's disease, Alzheimer's disease and Huntington's disease, especially using NHP models. He has published landmark papers in the area of cell replacement strategies, including the first demonstration that fetal dopaminergic grafts can survive, innervate and form synapses in patients with Parkinson's disease that was published in the *New England Journal of Medicine*. His recent demonstration that long-term grafts in such patients can form Lewy bodies was recently published in *Nature Medicine*. Dr. Kordower has published over 400 peer-reviewed papers and chapters, has lectured all over the world, has been on over 20 editorial boards, and is on the scientific advisory boards (SAB) of many biotech companies and scientific organizations. He is a past councilor and past president of the American Society for Neural Transplantation, past chair for the Committee for the Use of Animals for the Society for Neuroscience, and is a founding SAB member for the Michael J. Fox Foundation as well as a past member of their Executive SAB.

MARGARET LANDI, VMD, DIPACLAM, MBIOETHICS

Dr. Landi is chief veterinarian for GlaxoSmithKline and heads their Office of Animal Welfare, Ethics and Strategy. She is a diplomate in the American College of Laboratory Animal Medicine and its past president. Dr. Landi is the chair of the Council of the Institute of Laboratory Animal Research, part of the National Academy of Science, Engineering and Medicine. She is a veterinarian with a master's degree in

comparative medicine. She also recently received a master's in bioethics from the University of Pennsylvania. Dr. Landi currently serves on the Board of Trustees for the Scientists Center for Animal Welfare and the Board of Directors for Chimp Haven, the only federally funded sanctuary for chimpanzees. She has received several awards, including the University of Pennsylvania's Veterinary School Alumni Award, and the Harry Roswell Award from the Scientists Center for Animal Welfare. Dr. Landi served on the Institute of Medicine Committee on the Future of Chimpanzees in Biomedical and Behavioral Research. The Committee published its report in Dec 2011: "Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity." She is also a commentary author in the recently released publication *Principles of Animal Research Ethics* by Tom Beauchamp and David DeGrazia.

CORRINE LUTZ, PHD

Dr. Lutz is the director of Behavioral Services at the Southwest National Primate Research Center (NPRC) and an active member of the NPRCs' Behavioral Management Consortium. She has more than 25 years of experience in the study of NHP behavior. Dr. Lutz's main research interests broadly include the behavior of NHPs, environmental enrichment, stress, alopecia, and the effects of captive housing on behavior. She assesses risk factors for behavioral issues with an aim of prevention and to better understand their impact on animal welfare.

JOHN MORRISON, PHD

Dr. Morrison is currently a UC-Davis distinguished professor, director of the California National Primate Research Center (CNPRC), and professor of neurology in the School of Medicine at UC-Davis. Dr. Morrison earned his bachelor's degree and PhD from Johns Hopkins University and completed postdoctoral studies at the Salk Institute for Biological Studies. He then served as a faculty member at The Scripps Research Institute until he joined the faculty at Mount Sinai in 1989 where he went on to be chair of the Department of Neuroscience and serve as Dean of Basic Sciences and the Graduate School of Biomedical Sciences at Mount Sinai before moving to UC-Davis in 2015. Dr. Morrison's research program focuses primarily on the neurobiology of aging and neurodegenerative disorders, particularly as they relate to cellular and synaptic organization of cerebral cortex. His laboratory is particularly interested in age-related alterations in structural and molecular attributes of the synapse that compromise synaptic health, lead to cognitive decline, and potentially leave the brain vulnerable to Alzheimer's disease (AD). Dr. Morrison is currently developing NHP models of AD. Dr. Morrison has served on Council for the Society for Neuroscience (SfN), as Editor-in-Chief of SfN's public-facing website, BrainFacts.org, and is currently serving as Secretary of SfN. Dr. Morrison is a member of the National Academy of Medicine.

TONY MOVSHON, PHD

Dr. Movshon is a professor in the Center for Neural Science at New York University. Dr. Movshon studies vision and visual perception, using a multidisciplinary approach that combines biology, behavior, and theory. His work explores the way neural networks in the brain compute and represent the form and motion of objects and scenes, the way that these networks contribute to perceptual judgments and to the control of visually guided action, and the way that normal and abnormal visual experience influences brain development in early life. Dr. Movshon received his BA and PhD from Cambridge University. Among his honors are the Young Investigator Award from the Society for Neuroscience, the Rank Prize in Optoelectronics, the António Champalimaud Vision Award, the Karl Spencer Lashley Award from the American Philosophical Society, and the Golden Brain Award from the Minerva Foundation. He is a member of the National Academy of Sciences and a fellow of the American Academy of Arts and Sciences

BILL NEWSOME, PHD
Dr. Newsome is an investigator of the Howard Hughes Medical Institute and professor of neurobiology at the Stanford University School of Medicine. He received a BS in physics from Stetson University and a PhD in biology from the California Institute of Technology. Dr. Newsome is a leading investigator in systems and cognitive neuroscience. He has made fundamental contributions to our understanding of the neural mechanisms underlying visual perception and simple forms of decision making. Among his honors are the Rank Prize in Optoelectronics, the Spencer Award, the Distinguished Scientific Contribution Award of the American Psychological Association, the Dan David Prize of Tel Aviv University, the Karl Spencer Lashley Award of the American Philosophical Society, and the Champalimaud Vision Award. His distinguished lectureships include the 13th Annual Marr Lecture at the University of Cambridge, the 9th Annual Brenda Milner Lecture at McGill University, and, most recently, the Distinguished Visiting Scholar lecture at the Kavli Institute of Brain and Mind, University of California, San Diego. He was elected as a member in the National Academy of Sciences in 2000 and to the American Philosophical Society in 2011. Dr. Newsome recently co-chaired the NIH BRAIN working group, charged with forming a national plan for the coming decade of neuroscience research in the United States.

DAVID O'CONNOR, PHD
Dr. O'Connor is a UW Medical Foundation professor of pathology and laboratory medicine in the University of Wisconsin–Madison School of Medicine and Public Health and was recently a Miegunyah Distinguished Visiting Fellow at the University of Melbourne. His research focuses on the interplay of genetics, immunity, and pathogenesis of HIV and other emerging RNA viruses. He received his BS from the University of Illinois at Urbana Champaign and his PhD from the University of Wisconsin–Madison.

KELLY METCALF PATE, DVM, PHD
Dr. Metcalf Pate is an assistant professor in the Retrovirus Laboratory and associate director for Academic Training in the training programs for veterinarians and veterinary students in the Department of Molecular and Comparative Pathobiology at the Johns Hopkins University. She is also co-director for the Cure Scientific Working Group within the Johns Hopkins University Center for AIDS Research (CFAR). Dr. Metcalf Pate's research focuses on the role of platelets in the immune response to viral infection, and how social stress affects the course of disease and the platelets' response. She also serves as a laboratory animal veterinarian whose specialty is the refinement of animal models of infectious disease and determining how these refinements affect the data from these models. She received her PhD from the Johns Hopkins University School of Medicine, a DVM from Purdue University, and a BA from Boston University.

MARK PRESCOTT, PHD
Dr. Prescott leads the Policy and Outreach Group at the UK's National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs). He provides strategic oversight of NC3Rs' relationships with other research funders and the academic community, peer review and advice service, and programs on animal welfare and experimental design. He ensures that the NC3Rs has a long-term, coordinated, impact-driven, and sustainable strategy for supporting changes in 3Rs policy and practice. Dr. Prescott was trained as a zoologist and primatologist and has more than 25 years of research experience in primatology, animal behavior, and animal welfare science. He serves on several ethics committees and scientific advisory boards at project, institution, journal, and governmental levels.

MARGARET FOSTER RILEY, JD

Ms. Riley is a professor at the University of Virginia (UVA) Law School, has a secondary appointment at the Medical School, and is affiliated with the Batten School of Public Policy. Ms. Riley has written and presented extensively about bioethics, research ethics, health care law, and food and drug law. She directs UVA's animal law program. She serves on a number of UVA's institutional review boards and has served on several National Academies committees devoted to research ethics. Ms. Riley has advised numerous committees of the Institute of Medicine, the National Institutes of Health, the National Science Foundation, the Food and Drug Administration, and the Virginia Bar. She received her bachelor's degree from Duke University and her law degree from Columbia University.

JEFFREY ROGERS, PHD

Dr. Rogers is an associate professor in the Human Genome Sequencing Center and the Department of Molecular and Human Genetics, Baylor College of Medicine. He serves as chair of the NIH National Primate Center's Working Group for Genetics and Genomics and is a core scientist for the Wisconsin National Primate Research Center. Dr. Rogers's research focuses on the genetics and genomics of NHPs, with emphasis on neurogenetics and behavioral genetics in primate models of psychiatric disorders. In collaboration with various colleagues, Dr. Rogers has published numerous articles on the genetics of individual differences in behavior among macaques, baboons, and other primates, targeting risk factors in mental illness. His work also includes genetic analyses of variation in primate neuroanatomy. Dr. Rogers has led or participated in several projects generating whole-genome assemblies for primates used in biomedical research, and he is currently conducting genome sequencing studies to discover and characterize SNPs and other genomic variation across several species, including macaques, baboons, and mouse lemurs. These studies are designed to identify novel genetic models of human disease.

KOEN VAN ROMPAY, DVM, PHD

Dr. Van Rompay obtained his DVM degree at the University of Ghent, Belgium, followed by a PhD in comparative pathology at the University of California, Davis. Since 1990, he has been working at the California National Primate Research Center, where he is currently a core scientist in the Infectious Diseases Unit, and scientific leader of the Primate Assay Laboratory. In the 1990's, he was on the forefront of helping to develop HIV prophylaxis and therapy regimens, in particular using tenofovir. He has also been part of many collaborative teams studying SIV/HIV pathogenesis and testing novel HIV vaccine strategies. In recent years, Dr. Van Rompay has expanded his expertise to other research areas, including the development and use of NHP models of Zika virus, Chikungunya virus, and other emerging infectious diseases to study pathogenesis and test antiviral interventions. He is also founder and CEO of Sahaya International, a volunteer-based 501(c)(3) nonprofit organization that supports grassroots programs in developing countries aimed at improving education, health, and socio-economic conditions of underprivileged communities.

STEVEN J. SCHAPIRO, PHD

Dr. Schapiro is an associate professor of comparative medicine and chief of the Section of Primate Behavior and Environmental Enrichment in the Department of Comparative Medicine of The University of Texas MD Anderson Cancer Center. For more than 30 years, his research group has been conducting studies focused on the behavior and welfare of captive rhesus monkeys and chimpanzees, including collaborative research projects funded by NIH and NSF. An important emphasis of his research program is to provide NHPs with opportunities to voluntarily participate in their own care. He is a co-founder of the Primate Training and Enrichment Workshop and the founder of the Primate Behavioral Management Conference. He is the editor of the *Handbook of Primate Behavioral Management* and co-editor of the *Handbook of Laboratory Animal Science*. He is a past-president and former treasurer of the American

Society of Primatologists, and the former treasurer and vice president for membership of the International Primatological Society. He currently organizes the '3Rs for the CPRC', an effort to revitalize Cayo Santiago after the devastating effects of Hurricane Maria.

CARRIE WOLINETZ, PHD

Dr. Wolinetz is the acting chief of staff, as well as the associate director for science policy and director of the Office of Science Policy (OSP) at the NIH. As leader of OSP, she advises the NIH director on science policy matters of significance to the agency, the research community, and the public, on a wide range of issues including human subjects protections, biosecurity, emerging biotechnologies, data sharing, the organization and management of NIH, and the innovation polices related to NIH-funded research. Prior to joining NIH, Dr. Wolinetz worked on biomedical research policy issues as the deputy director for federal affairs at the Association of American Universities (AAU) and the director of scientific affairs and public relations at the Federation of American Societies for Experimental Biology (FASEB). She also served as the president of United for Medical Research, a leading NIH advocacy coalition. Outside of NIH, Dr. Wolinetz teaches as an adjunct assistant professor at Georgetown University in the School of Foreign Service's program on Science, Technology & International Affairs. She has a BS in animal science from Cornell University, and she received her PhD in animal science from The Pennsylvania State University, where her area of research was reproductive physiology.

TONI ZIEGLER, PHD

Dr. Ziegler is a distinguished scientist at the Wisconsin National Primate Research Center and University of Wisconsin-Madison. She received her PhD from Texas A&M University where she was a part of the first NIH supported marmoset and tamarin colony set up to provide these species for biomedical research. Her research interest is in the neuroendocrine mechanisms promoting positive social bonding. Specifically, her lab is studying what promotes strong paternal-infant bonding. Additional areas of study include social bonding in humans and other NHP species, and developing biomarkers for studies of obesity, metabolic syndrome, and the implications of dietary fats in common marmosets on adolescent psychosocial development.

Appendix 3: Workshop Agenda

February 18-19, 2020
Room 620/630, Porter Neuroscience Research Center
National Institutes of Health, Bethesda Campus

AGENDA

FEBRUARY 18: LESSONS LEARNED FROM NONHUMAN PRIMATE (NHP) RESEARCH

9:00 AM **Opening Keynote**
Steve Hyman, MD – Harvard University and the Broad Institute

9:30 AM **Session I: Ensuring Rigorous Study Design**
Discuss lessons learned and generalizable approaches across scientific disciplines and experimental aims regarding rigorously designed and conducted research studies using NHP models.

Moderator: Bill Newsome, PhD – Stanford University
Panelists:

- Simon Barratt-Boyes, BVSc, PhD, DACVIM – University of Pittsburgh
- Melanie Graham, MPH, PhD – University of Minnesota
- Jeffrey Kordower, PhD – Rush University
- Dave O'Connor, PhD – University of Wisconsin – Madison

10: 15 AM **Session I Discussion**

11:00 AM **Break**

11:15 AM **Session II: Considering External Factors and Interpretation of Findings**
Discuss approaches for addressing the intersection of species selection, ethological considerations, biological outcomes, and other external factors in study design, and how these factors may affect interpretation of study findings when using NHP models.

Moderator: Steven Schapiro, PhD – University of Texas MD Anderson Cancer Center
Panelists:

- Corrine Lutz, PhD – Texas Biomedical Research Institute
- Mark Prescott, PhD – National Centre for the Replacement, Refinement and Reduction of Animals in Research, United Kingdom
- Toni Ziegler, PhD – University of Wisconsin – Madison

11:50 AM **Session II Discussion**

12:35 PM **LUNCH**

1:45 PM **Session III: Reporting Results and Sharing Data**

Identify opportunities for maximizing data/metadata sharing from NHP research studies, including considerations around research culture and infrastructure needed for ensuring data quality and replication of study design.

Moderator: Kelly Metcalf Pate, DVM, PhD – Johns Hopkins University

Panelists:

- Tony Movshon, PhD – New York University
- Jeffrey Rogers, PhD – Baylor College of Medicine
- Koen Van Rompay, DVM, PhD – University of California, Davis

2:20 PM **Session III Discussion**

3:05 PM **BREAK**

3:20 PM **Day 1 Wrap-Up Discussion: Lessons Learned and Generalizable Strategies**

Moderator: Lyric Jorgenson, PhD – National Institutes of Health

Discussants:

- Steve Hyman, MD – Harvard University and the Broad Institute
- Bill Newsome, PhD – Stanford University
- Kelly Metcalf Pate, DVM, PhD – Johns Hopkins University
- Steven Schapiro, PhD – University of Texas MD Anderson Cancer Center

4:45 PM **Summary Remarks and Adjournment**

Carrie Wolinetz, PhD – National Institutes of Health

FEBRUARY 19: LOOKING TO THE FUTURE

9:00 AM **Session IV: Research Opportunities and Challenges in the 21st Century**

Discuss technological advances or transformative breakthroughs on the horizon that may pose new scientific and ethical challenges to the design or conduct of rigorous research studies.

Moderator: Margaret Foster Riley, JD – University of Virginia School of Law

Panelists:

- Don Conrad, PhD – Oregon Health & Science University
- Robert Desimone, PhD – Massachusetts Institute of Technology
- Margaret Landi, VMD, DipACLAM, MBioethics – GlaxoSmithKline

9:20 AM **Session IV Discussion**

10:05 AM **BREAK**

10:20 AM **Session V: Strategies for the Future**

In light of opportunities and challenges on the horizon, discuss generalizable strategies for continuing to maximize the value of NHP studies through preparatory strategies:

- *Designing studies to facilitate translation*
- *Mitigating ethical considerations by incorporating evidence-based approaches*
- *Identifying cross-discipline competencies for ensuring robust research training paths*
- *Ensuring robust animal well-being and welfare science*

Moderator: Eliza Bliss-Moreau, PhD – University of California, Davis

Panelists:

- Kristine Coleman, PhD – Oregon Health & Science University
- Joseph Garner, DPhil – Stanford University
- John Morrison, PhD – University of California, Davis

10:40 AM **Session V Discussion**

11:25 AM **Day 2 Wrap-Up Discussion: Charting the Path Forward**

Moderator: Lyric Jorgenson, PhD – National Institutes of Health

Discussants:

- Eliza Bliss-Moreau, PhD – University of California, Davis
- Margaret Foster Riley, JD – University of Virginia School of Law
- Bill Newsome, PhD – Stanford University
- Kelly Metcalf Pate, DVM, PhD – Johns Hopkins University
- Steven Schapiro, PhD – University of Texas MD Anderson Cancer Center

12:25 PM **Concluding Remarks**

Carrie Wolinetz, PhD – National Institutes of Health

12:30 PM **Adjourn**