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Transgenic Neuroscience Research

Exploring the Scientific Opportunities Afforded by New Nonhuman Primate Models

PROCEEDINGS OF A WORKSHOP

Lisa Bain, Sheena M. Posey Norris, and Clare Stroud,
Rapporteurs

Forum on Neuroscience and
Nervous System Disorders

Board on Health Sciences Policy

Health and Medicine Division

The National Academies of
SCIENCES • ENGINEERING • MEDICINE

THE NATIONAL ACADEMIES PRESS
Washington, DC
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

This activity was supported by contracts between the National Academy of Sciences and the Alzheimer's Association; Cohen Veterans Bioscience; Department of Health and Human Services' Food and Drug Administration (5R13FD005362-02) and National Institutes of Health (NIH) (HHSN26300089 [Under Master Base #DHHS-10002880]) through the National Center for Complementary and Integrative Health, National Eye Institute, National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, National Institute on Aging, National Institute on Alcohol Abuse and Alcoholism, National Institute on Drug Abuse, and NIH Blueprint for Neuroscience Research; Department of Veterans Affairs (VA240-14-C-0057); Eli Lilly and Company; Foundation for the National Institutes of Health; Gatsby Charitable Foundation; George and Anne Ryan Institute for Neuroscience at The University of Rhode Island; Janssen Research & Development, LLC; The Kavli Foundation; Lundbeck Research USA; Merck Research Laboratories; The Michael J. Fox Foundation for Parkinson's Research; National Multiple Sclerosis Society; National Science Foundation (BCS-1064270); One Mind; Sanofi; Society for Neuroscience; Takeda Pharmaceuticals International, Inc.; and Wellcome Trust. Any opinions, findings, conclusions, or recommendations expressed in this publication do not necessarily reflect the views of any organization or agency that provided support for the project.

International Standard Book Number-13: 978-0-309-48873-0
International Standard Book Number-10: 0-309-48873-7
Digital Object Identifier: https://doi.org/10.17226/25362

Additional copies of this publication are available from the National Academies Press, 500 Fifth Street, NW, Keck 360, Washington, DC 20001; (800) 624-6242 or (202) 334-3313; http://www.nap.edu.

Printed in the United States of America

Suggested citation: National Academies of Sciences, Engineering, and Medicine. 2019. *Transgenic neuroscience research: Exploring the scientific opportunities afforded by new nonhuman primate models: Proceedings of a workshop*. Washington, DC: The National Academies Press. doi:https://doi.org/10.17226/25362.

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We thank the following individuals for their review of this proceedings:

ROBERT DESIMONE, Massachusetts Institute of Technology
HENRY T. GREELY, Stanford University
JON HENNEBOLD, Oregon National Primate Research Center
KAREN PARKER, Stanford University

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the content of the proceedings nor did they see the final draft before its release. The review of this proceedings was overseen by **ELI ADASHI,** Brown University. He was responsible for making certain that an independent examination of this proceedings was carried out in accordance with standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the rapporteurs and the National Academies.

Contents

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Introduction and Background[1]

Neurological and psychiatric disorders impose a tremendous human and economic burden on individuals, families, and societies, affecting more than 1 billion people globally and costing approximately $1.5 trillion per year in the United States alone (Feigin et al., 2017; Nager and Atkinson, 2016). Despite the significant unmet medical needs and large market potential, the development of new therapeutics in this area lags behind other diseases, and research and development are often characterized by costly, late-stage clinical trial failures (Hyman, 2012, 2016).

Although a number of factors contribute to this stalled therapeutic development, the challenge of translating scientific discoveries from rodent models to humans has been a major factor in slowing the development of new therapies for brain disorders, said Guoping Feng, the James and Patricia Poitras Professor of Neuroscience at the McGovern Institute for Brain Research, Massachusetts Institute of Technology. While research with rodent models has led to significant medical discoveries and increased fundamental understanding of brain function, pathology, and disease pathogenesis through basic science research, there are limitations to their use in studying human nervous system disorders, including the vast differences in brain structure and their inability to model many aspects of normal human cognition and behavior (IOM, 2013). About 93 percent of

[1]The planning committee's role was limited to planning the workshop, and the Proceedings of a Workshop was prepared by the workshop rapporteurs as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants, and have not been endorsed or verified by the National Academies of Sciences, Engineering, and Medicine. They should not be construed as reflecting any group consensus.

drugs for nervous system disorders that show efficacy in rodent models fail in human clinical trials (Kola and Landis, 2004). Given the many recent failures of rodent-based treatments for neurological disease to translate to humans in clinical trials, neuroscientists are giving increasing thought to approaches to therapeutic development that do not rely on rodent models (see, e.g., IOM, 2014, and NASEM, 2017).

To address this challenge, the field needs to expand its bandwidth and think of new ways to conduct experiments and study disease, said William Newsome, the Vincent V. C. Woo Director of the Stanford Neurosciences Institute. Working with nonhuman primates and using powerful transgenic tools holds great promise in this regard, he added. One might ask whether the use of nonhuman primates for research is essential and necessary in order to better understand and develop therapies for neurological and psychiatric diseases. "We don't know the answer to that fundamentally because we can't predict the future," said Newsome. "But what is essential is that we have a more diverse scientific ecosystem and a more diverse set of approaches to start understanding the biology underlying these diseases and how we might creatively take approaches to treat them." Because nonhuman primates are much closer to humans from an evolutionary perspective—and therefore have more similar cognitive and behavioral functions, social cognition, and neuroanatomical organization (Belmonte et al., 2015; Jennings et al., 2016; Kaas, 2013)—Newsome suggested that increasing the use of animals such as marmosets and macaque monkeys for research is both justified and necessary.

The development of powerful transgenic tool applications—including transgenesis, viral gene delivery, genome editing, and cloning—in nonhuman primate models opens new potential opportunities to significantly advance neuroscience research and therapeutic development (see Okano and Kishi, 2018), and also to introduce scientific and bioethical questions that merit deep consideration.[2] To examine the promise, concerns, and challenges related to neuroscience research using genetically modified nonhuman primates, the Forum on Neuroscience and Nervous System Disorders hosted a public workshop on October 4, 2018, bringing together an international

[2]In September 2016, the National Institutes of Health hosted a congressionally requested workshop on Ensuring the Continued Responsible Oversight of Research with Non-Human Primates to review the appropriateness of research in nonhuman primates, including rationale and guidelines. For more information, go to https://osp.od.nih.gov/pastevent/nih-workshop-on-ensuring-the-continued-responsible-oversight-of-research-with-nonhuman-primates (accessed December 13, 2018).

group of experts and stakeholders representing academia, industry, laboratory animal management, disease-focused foundations, and federal agencies.

WORKSHOP OBJECTIVES

The workshop was designed to explore the current state and future promise of research using genetically modified nonhuman primate models of disease to understand the complex functions of the brain that control behavior, movement, and cognition in both health and disease states, said Frances Jensen, professor and chair of neurology at the Perelman School of Medicine, University of Pennsylvania (see Box 1-1). Many of these complex functions and systems cannot be replicated in a lower species such as a rodent, she said. Yet, the field must still grapple with the question of what merits taking the step of developing nonhuman primate models with genetic modifications, and if these models are deemed to be appropriate and essential, how the field will ensure the appropriate use of this extremely unique resource, said Jensen. For a comprehensive look at the issues, the workshop considered some of the distinct aspects of animal husbandry required to ensure optimal care of genetically modified nonhuman primates; the ethical considerations related to the use of these animals in research and the global regulatory and conduct codes that are in place or that need to be developed; and the infrastructure required worldwide to ensure that research achieves its greatest scientific impact through collaboration and partnerships. Finally, said Jensen, given the fact that the nonhuman primate research community is and will continue to be relatively small and specialized, attention must be given to training the next generation of scientists to continue working with these unique animals (see Figure 1-1). Workshop presentations primarily focused on models using the common marmoset, Cynomolgus macaque, or Rhesus macaque. Debating whether transgenic and chimeric nonhuman primate research should be undertaken was not within the scope of this workshop.

BOX 1-1
Statement of Task

An ad hoc committee will plan and conduct a 1-day public workshop that will bring together experts and key stakeholders from academia, government, industry, and nonprofit organizations to examine the scientific opportunities and challenges, as well as bioethical considerations, of genetically engineered nonhuman primate models for neuroscience research.

Invited presentations and discussions will be designed to:

- Discuss the state of the science of transgenic and chimeric neuroscience research and emerging models for nervous system disorders, and explore the potential usefulness of such models to enhance understanding of higher cortical function and advance therapeutic development.
- Examine current tools and technologies used in rodent models (e.g., transgenesis, chimera, AAVs [adeno-associated viruses], gene therapy, etc.) and explore how they would need to be modified for use in other animal models, such as nonhuman primates.
- Consider bioethical principles and issues related to genetic engineering of animal models for nervous system disorders, and discuss potential metrics for determining the models' readiness for nonhuman primate research.
- Discuss policies and infrastructure needed to advance research in this domain, including, for example, training, recruitment of early-career scientists, and the potential development of specialized research centers and international collaborations.

The committee will develop the agenda for the workshop, select and invite speakers and discussants, and moderate the discussions. A proceedings of the presentations and discussions at the workshop will be prepared by a designated rapporteur in accordance with institutional guidelines.

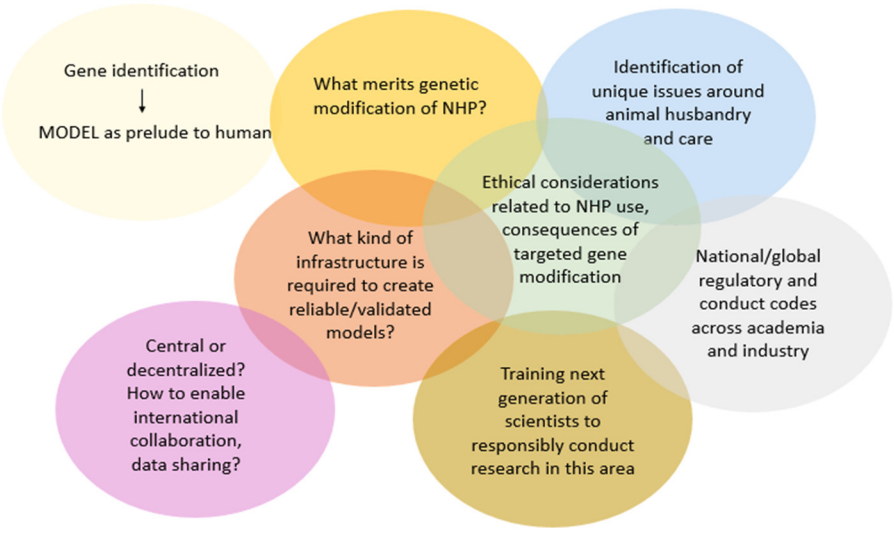

FIGURE 1-1 Workshop scope. The workshop addressed multiple overlapping issues related to the development of genetically modified nonhuman primate models for neuroscience research.
NOTE: NHP = nonhuman primate.
SOURCE: Presented by Frances Jensen, October 4, 2018.

ORGANIZATION OF THE PROCEEDINGS

Chapter 2 explores the rationale for developing genetically modified nonhuman primate models of nervous system disorders and provides an overview of challenges and opportunities associated with this area of research for academia, industry, and the public. Chapter 3 describes the development of several nonhuman primate models of human diseases using genetic modification and highlights a case study of how viral delivery of genes was used to develop the first successful gene therapy of a central nervous system disease. Chapter 4 discusses several approaches that have enabled the translation of discoveries from nonhuman primates to humans. Ethical issues related to nonhuman primate research are explored in Chapter 5. Chapter 6 focuses on the policy, infrastructure, and funding requirements to build and sustain a robust research enterprise, as well as other potential opportunities to move the field forward. Chapter 7 provides the final remarks.

2

Genetically Modified Nonhuman Primate Models for Neuroscience Research: Rationale and Overview of Potential Opportunities and Challenges

Science advances when the right model is available, said Robert Wurtz, scientist emeritus at the National Eye Institute, adding that the monkey is truly such a model for studying complex brain disorders. Humans and monkeys share common brain structures, including a highly developed frontal lobe that controls cognitive functions, and they display similar behaviors, said Wurtz. These commonalities enable scientists to identify circuits, examine how perturbation of those circuits affects behavior, and develop approaches to ameliorate the deficits created by that perturbation, he said.

Marmoset models, for example, have been developed using different technologies for many important human diseases, including brain disorders and diseases such as Parkinson's disease (PD), Alzheimer's disease, amyotrophic lateral sclerosis, stroke, and autism, said Hideyuki Okano, dean of the Keio University Graduate School of Medicine and team leader of the Laboratory for Marmoset Neural Architecture at the RIKEN Center for Brain Science. These models and others in nonhuman primates may also facilitate the development of novel and personalized treatments for neuropsychiatric disorders, said Angela Roberts, professor of behavioral neuroscience at the University of Cambridge. She predicted that in the future, chimeric models that incorporate neurons derived from induced pluripotent stem cells may enable scientists to develop better animal models of bipolar disorder and schizophrenia.

Examples of how these models are being explored in different disease states are discussed in Chapter 3.

OVERCOMING THE LIMITATIONS OF MOUSE MODELS WITH NONHUMAN PRIMATE MODELS

Although transgenic mice have revolutionized biomedical research, said Guoping Feng, they have their limitations. One key limitation is the fact that the prefrontal cortex in rodents is far less developed than in humans, which makes rodents a poor model for studying higher brain functions such as cognition, executive function, and emotion (see Figure 2-1).

The less complex (relative to human) rodent cortex results in a limited repertoire of cognitive function and this makes it difficult to interpret cognitive changes in a way that is translatable to humans, said Yoland Smith, professor of neurology and director of the neuropharmacology and neurological diseases division at the Yerkes National Primate Center of Emory University. The small size of the rodent brain also makes it difficult to translate new therapies from the bench to people, said Smith. In addition, mice have a limited life span, which makes them unsuitable for studying long-term neurodegenerative disorders.

Another limitation of mouse models for studying developmental disorders such as autism is that mice have very different social and communicative characteristics compared with humans and primates, said Feng. An example of how nonhuman primates are being used to better understand, and possibly develop, new diagnostic tests and treatments for autism is discussed in Chapter 3.

The lack of predictive animal models has contributed to numerous high-profile failures in translating preclinical success in rodents to clinical trials in humans, said Feng. Consequently, over the past 60 years, although great advances have been made in developing new treatments in other disease areas such as cardiovascular disease, there has been little progress in developing drugs that target new mechanisms in psychiatry. About 93 percent of drugs for nervous system disorders that show efficacy in rodent models fail in human clinical trials (Kola and Landis, 2004), said Karen Parker, associate professor of psychiatry and behavioral sciences at Stanford University. But rodent models do not just fail to predict human efficacy, they also fail to adequately test human safety. The sedative thalidomide, for example, was prescribed for pregnant women outside the United States in the late 1950s and early 1960s based on safety testing done only in rodents, she said, but limb deformities were seen when the drug was tested in marmosets and rhesus monkeys and the drug never received the Food and Drug Administration's approval.

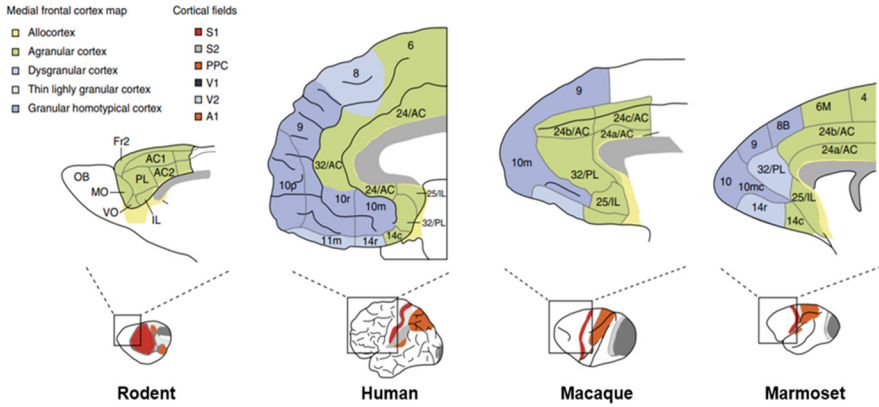

FIGURE 2-1 The change in cortical fields and medial frontal cortex architecture since the last common ancestor of rodents and humans.
NOTE: A1 = primary auditory cortex; AC1 and AC2 = anterior cingulate cortex area 1 and 2; Fr2 = frontal area 2; IL= infralimbic cortex; MO = medial orbital cortex; OB = olfactory bulb; PL = prelimbic cortex; PPC = posterior parietal cortex; S1 and S2 = primary and secondary somatosensory cortex; V1 and V2 = primary and secondary visual cortex; VO = ventro orbital cortex.
SOURCES: Presented by Guoping Feng, October 4, 2018; adapted from Kaiser and Feng, 2015.

OPPORTUNITIES PROVIDED BY NONHUMAN PRIMATE MODELS TO FOSTER NEUROSCIENCE RESEARCH

Nonhuman primate models enable enhanced understanding of behavior and higher cortical function, potentially better model neurodegenerative and neuropsychiatric disorders, and can be used to advance therapeutic development, including developing personalized treatments, testing drugs for safety before their first use in humans, and facilitating biomarker discovery. Feng and many others have focused their efforts on two types of models: the common marmoset and the macaque. The common marmoset has the advantage of a short life span (approximately 14 years in captivity), which can be particularly useful when studying late-onset diseases such as PD, said Feng. In contrast, macaques have brains that are bigger and evolutionarily closer to humans and thus may be useful to study higher brain function and neurodevelopmental disorders, but their

longer life span (approximately 30 years in captivity for Cynomolgus macaques) would necessitate extremely long studies.

Feng said this has all been made possible by the development of CRISPR genome editing technology,[1] which allows researchers to manipulate nearly any cell type or any organism (Sander and Joung, 2014). The most efficient genome editing approach is to make deletion mutants (knock-outs), said Feng, but to understand how genetic mutations or variations contribute to disease requires inserting or modifying genes (knock-ins), which is far less efficient. Tools for knocking-in genes efficiently have long been available in mice, but not in nonhuman primates, said Feng. His lab and many others have been working to develop these technologies. For example, they have recently used CRISPR to insert Cre recombinase into the paravalbumin (PV) gene locus in macaque embryos with about 60–70 percent efficiency. Cre recombinase is an enzyme that enables site-specific recombination events. Because PV neurons have been implicated in many disorders, Feng said this approach would provide a tool that would allow neuroscientists to probe and manipulate PV neuron function in the macaque. His team is also generating opsin knock-in monkeys, which will open the door to optogenetic approaches that have proved so valuable in increasing understanding of neural connectivity and circuit function.

CHALLENGES OF GENETICALLY MODIFIED NONHUMAN PRIMATE MODELS

While there are a number of opportunities afforded by the use of genetically modified nonhuman primate models for neuroscience research, challenges remain related to logistics and feasibility, selecting the appropriate primate species to study a specific disorder or disease, developing tools to bridge translation findings from nonhuman primates to humans, and exploring ethical considerations. These are briefly introduced here, and discussed in greater detail in later chapters.

[1]Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing is a cost-effective, quick, and efficient technique that enables scientists to make targeted changes in the genome of living cells.

Addressing Infrastructure, Logistics, and Feasibility Issues

Generating, housing, and caring for sufficient numbers of nonhuman primates to support the range of research activities anticipated for these models will be difficult, said Smith. Parker noted that nonhuman primate colonies need to be housed in environments that provide naturalistic outdoor settings with species-typical enrichments and native temperatures as well as complex social conditions, including species-typical complex social groupings.[2] The costs to maintain such a colony over a long period could be substantial, said Smith.

Breeding and obtaining sufficient numbers of animals also presents challenges. Cynomolgus macaques are continuous breeders with a 160-day gestation period and take 3.5 years to reach sexual maturity, typically producing singletons with each birth. Marmosets are also continuous breeders with a 140-day gestation period. They mature in only 1 to 1.5 years and give birth twice per year, producing twins or triplets with each birth. Nonseasonal breeders allow scientists to generate genetically modified animal models year-round through assisted reproductive technology.

Homozygous animals, while desirable for many types of studies aimed at understanding gene function, can take a long time to breed. Feng and colleagues have recently developed a new technology that dramatically increases the homozygous rate of knock-ins to about 50–80 percent with only one injection. This approach could dramatically shorten the time required to obtain homozygous animals, he said. If they can get this approach to work in neurons or somatic cells, Feng said he thinks it may have great potential in gene therapy. Infrastructure, logistics, and feasibility issues are discussed further in Chapter 6.

Selecting the Right Model to Study a Disease

The selection of primate species depends on the disease being studied and the questions one seeks to answer, said Smith. Rhesus monkeys, for example, have much larger brains, enabling investigators to target and manipulate sufficient numbers of neurons needed to apply and translate new

[2]For an in-depth discussion about care, use, and welfare issues, see the forthcoming proceedings from the complementary workshop on the Care, Use, and Welfare of Marmosets as Animal Models for Gene Editing–Based Biomedical Research, hosted on October 21–22, 2018, by the National Academies' Institute for Laboratory Animal Research. For more information, see http://nas-sites.org/ilar-roundtable/roundtable-activities/care-use-and-welfare-of-marmosets-as-animal-models-for-gene-editing-based-biomedical-research.

neurogenic, optogenetic, chimeric therapy, and gene therapy techniques. Rhesus monkeys also have a broad and sophisticated cognitive repertoire and can learn extremely complex tasks, said Smith. Feng added that rhesus physiology has been extensively studied and well characterized.

Marmosets also have advantages related to scale, said Smith, because they breed faster, thus enabling the generation of many more animals in a shorter time, and they also have a shorter life span. Marmosets also have emerged as good models of social behavior (Miller et al., 2016) and the marmoset genome has been completely sequenced (Marmoset Genome and Analysis, 2014).

Many factors need to be considered in developing and selecting the appropriate model for studies, said Parker. For example, modeling a neurodevelopmental condition like autism must be done in a younger animal to see onset of the behavioral phenotype, whereas for dementia, the phenotype begins in late adulthood, she said. Prevalence also should be considered, she said. Autism is much more common in males than in females, while depression is more common in females; ideally, the animal model will show a similar sex-biased pattern. Does the animal share the human disease (homolgous modeling) or only a part of the phenotype (endophenotypic modeling)? Is there a similarity in behavior or appearance between the model and human condition (face validity); is there a similarity in the underlying cause of disease (construct validity); and does the animal's response to treatment reflect what will happen in a person (predictive validity)? Are behavioral readouts ecologically informed, that is, does the animal exhibit them under natural circumstances?

These various factors can be leveraged to identify a range of models that may be appropriate, said Parker. For example, to study autism, she and her colleagues have taken three approaches: (1) a behavior first approach using models with face validity in that they exhibit naturally occurring social deficits; (2) a genetics first approach with construct validity that uses homologous pathogenic variants, selective breeding, and/or genome engineering; and (3) a candidate signaling pathway approach with construct validity based on homologous biomarker profiles (see Figure 2-2). Parker's work in autism is discussed in more detail in Chapters 3 and 4.

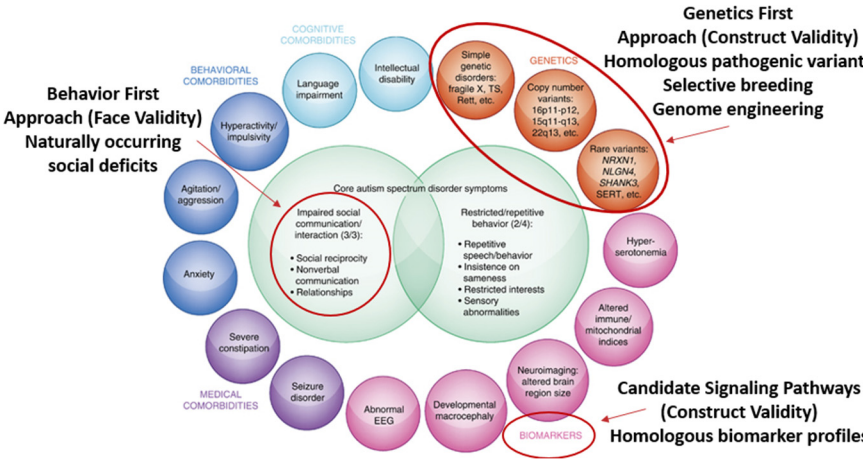

FIGURE 2-2 Points of entry for nonhuman primate models: Autism as an example. Developing primate models of neurodevelopmental disorders can be based on behavior, genetics, or candidate signaling pathways.
NOTE: EEG = electroencephalography.
SOURCES: Presented by Karen Parker, October 4, 2018; adapted from Veenstra-VanderWeele and Blakely, 2012.

Developing Tools to Translate Findings from Nonhuman Primates to Humans

In order to use nonhuman primate models to better understand the underlying psychological and neurobiological causes of psychiatric symptoms and develop new treatments, a range of primate behavioral tests are needed that can assess different aspects of cognition and emotion and translate easily to the human clinical condition, said Roberts. This is discussed in more detail in Chapter 4.

Exploring Ethical Considerations

The justification for using genetically modified nonhuman primate models comes down to the limitations of doing certain kinds of research in humans or in other research models, said Jeffrey Kahn, the Andreas C. Dracopoulos director of the Johns Hopkins Berman Institute of Bioethics. This means, he said, that other models should be considered before using

this "valuable and valued resource." Then, if research on nonhuman primates is determined to be justified, oversight by people with specialized expertise is needed, said Kahn. Ethical issues related to the use of nonhuman primate models are further explored in Chapter 5.

3

State of the Science of Transgenic Nonhuman Primate Models for Nervous System Disorders

Highlights

- Nonhuman primate models enable the study of human cognitive processes and complex behaviors that are essential to understanding neurodevelopmental and psychiatric disorders (Gordon, Okano, Roberts).
- A transgenic marmoset model of Parkinson's disease has enabled the study of mechanisms of sleep dysregulation in humans starting at the prodromal stage of this disorder (Okano).
- A transgenic macaque model of Huntington's disease shows motor, cognitive, behavioral, and pathological similarities to the human disease (Smith).
- Transgenic macaque and marmoset monkeys that express genes associated with autism show behaviors similar to those seen in humans with autism (Feng, Poo).
- Through the use of somatic cell nuclear transfer, macaque monkeys have been cloned to produce genetically uniform offspring (Poo).
- Viral vectors are able to efficiently transfer genes to the brains of mice, but improvements in the technology are needed to achieve similar results in nonhuman primates (Deverman).
- A successful gene therapy using a viral vector achieved for a rare form of genetic blindness was made possible, in part, by safety studies conducted in nonhuman primates (Bennett).

NOTE: These points were made by the individual speakers identified above; they are not intended to reflect a consensus among workshop participants.

Several workshop participants noted that the advantages that nonhuman primates offer to study and develop treatments for nervous system disorders will require adapting a range of research approaches—including transgenesis, chimera, viral vectors, and gene therapy—many of which have been successfully applied in rodent models, and some even in humans. According to Ben Deverman, director of the vector engineering research group at the Stanley Center for Psychiatric Research at the Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard University, tools such as different types of sensors, actuators, optogenetic tools, and gene editors enable scientists to study how specific cells and circuits affect behavior and learning. They are especially powerful, he said, because they are genetically encoded, which enables scientists to perturb or modulate specific cell types and circuits. When combined with genetic modification—either through transgenesis, viral gene delivery, genome editing, or cloning—scientists have a wide array of tools with which to study how gene disruptions affect brain function and explore potential interventions.

Deverman added that in mouse models, the large number of transgenic lines has facilitated the development of these tools by restricting the expression of transgenes to specific cell types and brain regions. However, the Cre technology typically used to generate transgenic mice carries with it some risk (e.g., potential toxicities) when applied to nonhuman primates, he said.

Even without genetic modification, several workshop participants said that nonhuman primate models have been valuable in studying human disease. Joshua Gordon, director of the National Institute of Mental Health (NIMH), noted that primates are needed to study cognitive processes and other complex behaviors. For mental health disorders, the case is even stronger, he said, because at both neurobiological and genetic levels, structures in the brain do not exist in rodents and other lower species. For example, Angela Roberts and colleagues have used a marmoset model to explore the variable sensitivity to selective serotonin reuptake inhibitors (SSRIs) seen in humans with affective disorders such as anxiety and depression. Genetic sequencing of the marmoset genome showed that like humans, marmosets express a range of polymorphisms in the serotonin transporter gene and that a certain polymorphism, similar in function to one seen in humans, is associated with changes in expression of the transporter gene, changes in the anxiety response to a human intruder, and variable responses to SSRIs (Santangelo et al., 2016).

Roberts and colleagues have also used marmosets to test the hypothesis that in people with depression, overactivity in an area of the brain called the subgenual anterior cingulate cortex causes anhedonia, a symptom characterized by loss of pleasure that is commonly associated with depression, schizophrenia, and Parkinson's disease (PD) (Alexander et al., 2018). They showed that overactivation of this brain region in marmosets blunted cardiovascular and behavioral anticipatory arousal and their ability to work for a reward.

GENETICALLY MODIFIED NONHUMAN PRIMATE MODELS OF DISEASE

Throughout the workshop, several participants discussed the state of science of genetically modified nonhuman primate models and their potential usefulness to enhance understanding of nervous system disorders and advance therapeutic development for PD, Huntington's disease (HD), autism spectrum disorder (ASD), and circadian disorders. Workshop participants also explored some of the technological advances that might further enhance development of these types of models, including cloning and gene modification through vector delivery.

Transgenic Marmoset Models to Study Parkinson's Disease

Gene transfer (Sasaki et al., 2009) and genome editing (Sato et al., 2016) have both been used successfully to develop neurodegenerative and neurodevelopmental disease models, said Hideyuki Okano. Among neurodegenerative diseases, nonhuman primate models have been especially helpful in PD to understand the cells and circuits involved and to evaluate potential new therapies (Emborg, 2007). PD is a highly heterogeneous and complex disorder with both motor and non-motor features. It is associated with the degeneration of dopaminergic neurons and other pathologies resulting from a combination of genetic and environmental factors (Kalia and Lang, 2015). Okano and colleagues generated a transgenic marmoset model of PD that overexpresses the A30P mutation in the α-synuclein gene. A30P is one of many mutations associated with familial forms of PD (Kruger et al., 2001). Using a wireless system that simultaneously measures electroencephalography and electromyography in the marmoset, they showed that REM [rapid eye movement] sleep without atonia—which is seen in humans with PD—was also present in the sleep stage in the PD

transgenic marmosets. They also examined longitudinal changes of dopamine neurons using positron-emission tomography imaging with a radioligand that specifically binds the dopamine transporter. This allowed them to track the association of motor and sleep dysregulation symptoms with loss of dopamine neurons. Okano said they also noticed tremors and gait disturbances in the transgenic marmosets similar to what is seen in humans with PD. Using dopamine as a rescue treatment, they showed that the gait symptoms are likely due to dopamine deficit.

Using diffusion imaging in this marmoset model, Okano and colleagues also demonstrated a reduction in the number of nigro-striatal fibers projecting into the striatum. In the future, Okano hopes to use this model to determine which neuronal circuits are damaged at different clinical stages of disease and whether there is a relationship between clinical symptoms and pathogenic protein accumulation. Another group in Japan is currently developing an α-synuclein positron-emission tomography (PET) probe, which Okano and colleagues are evaluating in the PD transgenic marmosets.

Transgenic Rhesus Macaque Models of Huntington's Disease

Anthony Chan and colleagues at the Yerkes National Primate Research Center and the Emory University School of Medicine developed a transgenic rhesus macaque primate model of HD, a complex autosomal dominant neurodegenerative disorder that affects about 40,000 people in North America with motor, cognitive, and psychiatric manifestations (Yang et al., 2008). HD is caused by an expansion of trinucleotide repeats (cytosine-adenine-guanine or CAG) within the huntingtin gene (HTT). Most people have fewer than 26 CAG repeats. The disease manifests when the number of repeats exceeds 36, with disease onset highly correlated with an increasing number of repeats.

According to Yoland Smith, Chan's model was created by transfecting mature rhesus oocytes with a lentivirus carrying a mutant form of the HTT gene, followed by in vitro fertilization of these oocytes and implantation into surrogates. Chan, Smith, and colleagues now have a group of transgenic rhesus monkeys modeling HD that they have been following longitudinally with the same kinds of cognitive, behavioral, and imaging assessments that are used clinically in humans. Smith said these studies indicate that monkeys modeling HD develop slowly progressive motor, cognitive, and pathological changes that closely resemble what is seen in

HD patients. As was hypothesized, the monkeys show progressive worsening on tests of object retrieval at 16 months of age and visuospatial orientation at 36 months of age, said Smith. At 5 years of age, the monkeys also showed increased anxiety, irritability, and aggression in response to an acute stressor (the human intruder task) and they also exhibited elevated levels of pro-inflammatory cytokines and increased induction of immune pathway genes (Chan et al., 2015; Raper et al., 2016). Imaging studies show reductions in brain volume and alterations in white matter connectivity as well as microstructural changes that parallel progressive motor and cognitive decline (Meng et al., 2017).

At necropsy, the brains of the transgenic monkeys also appeared similar to the brains of humans with HD, with widespread deposition of mutant huntingtin aggregates in the striatum and cerebral cortex and significant neuronal loss in the caudate nucleus and putamen (Chan et al., 2015). Smith added that more recent studies indicate other similarities between the HD monkeys and humans with HD, including relative sparing of GABAergic and cholinergic interneurons in the striatum.

Nonhuman Primate Models of Autism Spectrum Disorder

ASD is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and the presence of restricted, repetitive patterns of behaviors (APA, 2013). It is also thought to be etiologically heterogeneous. A literature review conducted in 2011 concluded that defects in more than 100 genes have been implicated in ASD (Sanders et al., 2015). John Spiro, deputy scientific director of the Simons Foundation Autism Research Initiative, said that between 65 and 100 highly penetrant genes are known to be major risk factors for this disorder. Given this large number of genes, nonhuman primate models are likely to play an increasingly important role in autism research. For example, in developing gene therapy approaches, Spiro suggested that a nonhuman primate model might be a critical step before moving to human trials. Gene therapy is an extremely appealing route to pursue because in theory it could allow investigators to bypass decades of basic research needed to understand the biology associated with a particular gene and to proceed directly to seeing if modifying a gene might alter the disorder.

Among the genes associated with ASD are *MECP2*, *SHANK3*, and *CHD8. MECP2* encodes a regulator of neural development called Methyl-CpG-binding protein-2 (Wen et al., 2017). Mu-Ming Poo, director of the Institute of Neuroscience of the Chinese Academy of Sciences (CAS) and

CAS Center for Excellence in Brain Science and Intelligence Technology, and colleagues at the Institute of Neuroscience at CAS generated lentivirus-based transgenic cynomolgus macaque monkeys that express the human *MECP2* gene in the brain, show germline transmission of the transgene, and exhibit behaviors similar to those seen in humans with ASD (Liu et al., 2016a). For example, the transgenic monkeys display repeated circular movements that mimic the stereotypical movements of individuals with autism, said Poo. Like humans with ASD, the monkeys also display anxiety responses, reduce social interactions, and show differences in cognitive function, he added.

Poo and colleagues wanted to produce a second generation of transgenic monkeys, but the relatively slow sexual maturation of macaques meant that it would be 5 or 6 years before these animals would reproduce. To circumvent this problem, they turned to a technique that had been successful in mice, in which the testis tissues from a young monkey were incubated subcutaneously in nude mice for 11 months to speed up sperm maturation. Mature sperm were then injected into oocytes to produce embryos that were transferred to female surrogate monkeys, resulting in eight pregnancies, seven births, and six healthy monkeys, including five that carried the *MECP2* transgene (Liu et al., 2016b). The problem with this approach, said Poo, is that the monkeys are all different because multiple copies of transgenes are randomly inserted in the genome, thus limiting reproducibility.

SHANK3 is a gene that is critical for synaptic development, said Guoping Feng, noting that single-gene mutations in *SHANK3* lead to a severe neurodevelopmental disorder with autism spectrum features in humans (Durand et al., 2007). Feng's lab at MIT has collaborated with a group in China to develop viable cynomolgus macaque monkeys with both homozygous and heterozygous mutations, and has shown that these animals show reduced motor activity, dramatic sleep disruption similar to what is seen in humans, and impaired social interaction, including lack of reciprocal play and vocalizations. Feng and colleagues are examining resting state local connectivity in these animals to try to understand the circuit defects that lead to these problems. The hope, he said, is that they will be able to modulate the affected circuits, correct the problems, and then translate this approach into humans. These studies may also lead to the identification of resting state function biomarkers that will be applicable to humans, said Feng.

Working with collaborators in Japan and at the National Institutes of Health, Feng's group has also established a marmoset genetic engineering

platform and produced two *SHANK3* knock-out marmosets. Next, they plan to generate knock-in models, including models that insert Cre into PV neurons, he said. Because marmosets are more social than macaques, Feng believes they will provide a superior model for studying disorders associated with social dysfunction, although analyzing marmoset behavior is challenging because they move in three dimensions: walking, running, and jumping and leaping. Working with the MIT Computer Science and Artificial Intelligence Lab, Feng and colleagues have also developed software to automatically track marmoset movements and interactions, and they are working to develop tools that will assess sophisticated cognitive functions.

Ablation Model of Circadian Disorders

Poo and colleagues have also been developing a macaque monkey model to investigate the genetics of circadian rhythm disorders, which have been linked to chronic and degenerative diseases of aging. The circadian clock is regulated by multiple genes that regulate metabolism, including *Bmal1* (Bass and Takahashi, 2010). According to Poo, *Bmal1* along with other genes such as *CLOCK*, *PER*, and *CRY1* drives rhythmic gene expression in all cells of the body. Using CRISPR technology, Poo and colleagues have generated complete and partial *Bmal1* knock-out macaque models and are using these to assess how the loss of this gene affects the cycling of enzyme and hormone levels and the behavior phenotype.

Cloning Macaque Monkeys

Poo noted that even though CRISPR avoids the random insertion of multiple gene copies seen with transgenics, the production of these knock-out and knock-in models is inefficient and subject to off-target effects and mosaicism, where progeny have a mixture of edited and unedited cells. The problems and difficulties associated with embryonic gene editing via viral expression of transgenes or CRISPR editing led Poo and colleagues to explore cloning as an alternative. This approach has the additional benefit of producing animals with uniform genetic backgrounds, said Poo. His group is using somatic cell nuclear transfer, where they fuse fetal fibroblast cells with enucleated oocytes to produce embryos expressing the genome of the animal from which the fibroblasts were obtained. These embryos are then implanted into surrogates to produce genetically uniform

offspring (Liu et al., 2018). Poo added that he hopes that future manipulations, screening, and gene editing can be carried out in primary cultures before performing somatic cell nuclear transfer.

Gene Modification via Vector Delivery

Gene modification in primates and other animal models can also be accomplished through delivery of genes with viral or other types of vectors (Nayerossadat et al., 2012). Deverman's lab has been developing vector tools in mice that they hope can be applied to other nontransgenic organisms using viral vectors alone. They developed a method called CREATE (Cre recombination-based adeno-associated virus [AAV] targeted evolution) to select variants of the naturally occurring AAV9, which crosses the blood–brain barrier and transduces various neuron populations in the adult mouse brain with high efficiency after intravenous injection (Deverman et al., 2016). While the variant they identified—AAV-PHP.B—transfers genes 40 times more efficiently than AAV9, large doses of vector are required. For even more efficient transduction, they used CREATE to evolve the virus further and ended up finding a virus they call PHP.eB that, with a 24-fold lower dose, transduced more than 50 percent of the cells in the striatum and nearly 70 percent in the cerebellum (Chan et al., 2017).

Deverman noted that this technology is useful not only for disease modeling or developing potential gene therapies, but also has some relevance to circuit studies. In collaboration with David Anderson's lab at the California Institute of Technology, his group created a Cre-dependent vector that expresses a neuropeptide called Tac2, which is upregulated under stressful conditions in mice. Using this vector, they showed that the symptoms resulting from chronic isolation stress, such as aggressive behaviors and prolonged fear responses, could be mimicked through expression of Tac2. In other words, Tac2 actually drives the response to stress.

Whether these approaches will work in nonhuman primates remains an unanswered question, said Deverman. The viruses seem to work well in rats, but not in marmosets, and there have been mixed studies in macaques, he said. Because of this, his team is trying to develop new vectors that work across species. They are also developing approaches that achieve cell-type specificity in the absence of transgenics. For example, they have developed CREATE 2.0, which uses deep sequencing of the viruses combined with negative selection in silico to find sequences that are broadly transducing and specific for various cell types. Another approach they are

investigating is inserting cell-type specific promoters in their vectors. Using this approach with different promoters, they were able to target dopaminergic, serotonergic, and GABAergic neurons with high efficiency (Chan et al., 2017). They are also exploring techniques to reduce off-target expression as well as intersectional strategies that enable targeting of specific neurons by injecting one virus at axon terminals and another at the soma.

GENE MODIFICATION AS THERAPY

Nonhuman primate models have proven valuable not only as models to advance understanding of human diseases and discover new therapeutic approaches, but also to ensure that treatments can be delivered safely to humans (Friedman et al., 2017). Lisa Stanek, senior principal scientist at Sanofi Genzyme, said that when it comes to gene therapy, a large complex primate brain is needed to understand the delivery and distribution of vectors in the brain. In addition, she said, the genome should be similar to that of humans because some of the off-target effects might not be seen in a rodent model. Viral vector gene therapy also sometimes relies on connections within the brain in order to transport the vectors, said Stanek. Nonhuman primate models would therefore be useful to study whether those connections exist even in the diseased brain, she said. Nonhuman primate models may also be useful for biomarker development, added Stanek.

A recent example of the potential of gene therapy was realized in December 2017, when the Food and Drug Administration approved Luxturna® (voretigene) as the first directly administered gene therapy that targets a specific disease-causing gene mutation, according to Jean Bennett, F. M. Kirby Professor of Ophthalmology at the Perelman School of Medicine, University of Pennsylvania. The therapy is now available to patients with a rare form of blindness caused by mutations in the *RPE65* gene, which encodes a protein that converts light to an electrical signal in the retina. Mutations in the gene result in gradual loss of vision and eventually complete blindness. Bennett developed the reagent that is now known as Luxturna® in collaboration with her colleagues at the University of Pennsylvania.

Proof of concept for the treatment was performed in a naturally occurring animal model, said Bennett. Briards are herding dogs that develop a form of progressive retinal degeneration caused by mutations in *RPE65*. In 2001, Bennett and colleagues at the University of Pennsylvania, Cornell

University, and the University of Florida, Gainesville, published a paper showing that gene therapy with a recombinant AAV restored vision in blind Briards within a few months of receiving a single injection (Acland et al., 2001). Bennett said the first dog treated showed robust improvements on electroretinogram assessments with waveforms similar to what is seen in normal dogs, and that these improvements persisted over his life.

To gain assurance that the treatment was safe and effective in humans, Bennett and colleagues next tested the therapy in nonhuman primates. It was not only because the structure and vasculature of the human eye are different from the canine eye, but also because among species other than human, only primates have a macula, the region in the eye that provides humans with fine visual acuity, said Bennett (Kostic and Arsenijevic, 2016). The monkey studies indicated that the treatment was safe and effective, providing the evidence they needed to move forward into human studies. Bennett showed videos of one child who received the treatment. Before the treatment he walked with a blind cane and needed assistance to get around. A year and a half later, he was able to ride his bike to a friend's house, see the blackboard at school, play sports, and do puzzles. Ten years later, he reports that he is hunting and hitting targets, said Bennett.

Bennett said the question from all subjects enrolled in the clinical trial is "When can I have my second eye injected?" Readministration raises concerns about immune responses, and according to Bennett, efficacy after readministration of AAV in humans has been described in only one other clinical trial. However, in a study in dogs and subsequently in nonhuman primates, readministration in the contralateral eye was shown to be safe and effective (Amado et al., 2010). These results led to U.S. and European Union approval of the reagent as a drug, said Bennett.

Bennett and colleagues have tried to develop treatments for other eye diseases, such as choroideremia—an X-linked degenerative retinal disease that leads to loss of night vision, peripheral vision, and eventually blindness. Lacking a naturally occurring animal model of this disease, her team turned to induced pluripotent stem cells for proof-of-concept studies (Vasireddy et al., 2013), then demonstrated that the treatment was safe in nonhuman primates before proceeding to a clinical trial, which has also shown a high degree of safety.

While the nonhuman primate models have been predictive of safety, Bennett noted that few retinal diseases occur naturally in nonhuman primates, perhaps because they are selected against in the wild. One exception is that male squirrel monkeys have an incidence of color blindness similar to that of the X-linked form of red–green color blindness seen in

human men. A gene therapy approach to treating red–green color blindness has been developed in the monkeys (Mancuso et al., 2009), which may lead to clinical trials in humans, said Bennett.

4

Translating Research from Nonhuman Primates to Humans

Highlights

- Translating discoveries from nonhuman primates to humans may be possible because of the similar size and complexity of their brains (Smith).
- Gene-edited monkeys provide an approach to understanding the different phenotypes of autism (Amaral).
- Sophisticated nonhuman primate models of diseases such as autism will be useful to identify therapeutic targets and biomarkers, and possibly develop new treatments (Parker).
- Behavioral assays in monkeys that replicate the kinds of assays used in humans have been developed to demonstrate that the monkey model accurately reflects features of human autism (Parker, Poo, Roberts).
- Tools that demonstrate similar cognitive processes and neurobiological correlates in humans and nonhuman primates enable both forward and back translation of discoveries (Roberts).
- Marmosets are useful for longitudinal imaging studies of brain development because they reach maturity in only 2 years (Roberts).
- The marmoset brain is ideal for mapping brain circuits involved in cognition and other behaviors because although small, it is structurally similar to humans (Okano).
- Calcium imaging in deep layers of the marmoset cortex is possible in freely moving animals, enabling the monitoring and decoding of complex behaviors (Okano).

NOTE: These points were made by the individual speakers identified above; they are not intended to reflect a consensus among workshop participants.

The hope embedded in the nonhuman primate research taking place around the world is that discoveries made in these animals will translate to humans in ways that research in other animal models has not. Yoland Smith said that making the leap from nonhuman primates to humans will be neither trivial nor easy, but the size and complexity of the primate brain will make it easier to target a sufficient number of neurons to facilitate the diffusion of vectors or achieve other forms of genetic modification.

A TRANSLATIONAL RESEARCH ROADMAP IN AUTISM

David G. Amaral, distinguished professor of psychiatry and neuroscience and Beneto Foundation Chair of the MIND [Medical Investigations of Neurodevelopmental Disorders] Institute at the University of California, Davis, has been studying nonhuman primates for many years, and in the past two decades has also been studying human children with autism. He believes that gene-edited monkeys offer a way to understand the different phenotypes of autism and parse this heterogeneous disorder into meaningful subtypes that have clinical and possibly therapeutic implications.

For example, Amaral was involved in a study published in 2014 that investigated the role of *CHD8* [chromodomain helicase DNA binding protein 8] mutations in children with autism (Bernier et al., 2014). What they found, according to Amaral, was that of the 15 children in the study that had loss-of-function truncations in the *CHD8* gene, 13 had autism. Most of these children also had macrocephaly, or large brains. Amaral said that about 15 percent of boys with autism have large brains, and that this subgroup also has more severe symptoms and a worse prognosis (Amaral et al., 2017). To investigate the neurobiology of this large-brain form of autism, an animal model was needed, but according to Amaral, disrupting *CHD8* in rodents produces macroencephaly, but not the behavioral characteristics typical of autism. He suggested that manipulating the gene in nonhuman primates could provide not only a model for understanding the neurobiology of this form of autism, but also for testing new therapeutic interventions.

Karen Parker described what she called a translational research roadmap for autism that begins by developing sophisticated animal models to identify biomarkers and targets; translates those monkey findings to autism patients; and then uses that model to test novel treatments.

Earlier work in twins had demonstrated that autistic traits and the polygenic burden or risk underlying these traits is distributed across populations (Constantino and Todd, 2003), suggesting it would be valuable to model autism polygenically in monkeys, said Parker. She started with an extremes group approach in a population of 5,000 rhesus monkeys at the California National Primate Research Center. These animals are bred to preserve genetic heterogeneity and housed in ecologically valid environments (outdoors in mixed male and female groups with access to play gyms and swinging perches). Parker said that like humans, these animals exhibit stable, individual differences in social behavior. At the extremes are individuals who can be identified as "low social" or "high social." Low-social animals, like humans with autism, initiate and receive fewer affiliative interactions, spend less time grooming and playing, display more inappropriate social behaviors, and show diminished interest and less social competence than their typical peers (Sclafani et al., 2016).

Parker asked a group of clinicians to identify the kinds of behavioral assays that would provide face validity for her monkey model, that is, that would convince them that the monkeys were accurately modeling human autism. She and her colleagues then used this information to develop a monkey social behavior test battery that would allow them to interrogate the core symptoms seen in patients. Criteria for these tests included ecological relevance, prior successful use in macaques or related macaque species, the ability to generate variation in performance, and no need for extensive training prior to test administration.

For example, Parker said there is evidence that children with autism have face recognition but not object recognition problems, so her team created a monkey version to test these two domains. Other tests included in the battery test social competence, joint attention, ability to read social cues, social motivation, and intellectual disabilities, all using species-specific behaviors, such as lip smacking and vocalizations.

To validate this model from a biological perspective, Parker and colleagues turned to biomarkers. Because reproducibility problems have plagued much of the work done in humans on blood biomarkers, Parker thought it might be better to look at cerebrospinal fluid (CSF), particularly at some of the signaling pathways that have been implicated in regulating prosocial behavior in mammals and those dysregulated in syndromic forms of autism. They discovered that CSF vasopressin levels could, with high accuracy, predict whether an animal was high or low social (Parker et al., 2018). To see if this is replicated in human children, they piggy-

backed on clinical indications where children were getting lumbar puncture for standard of care reasons. In their first study, they found that 13 of 14 children could be classified with high accuracy based on their CSF vasopressin level alone. They have since replicated the study and shown that CSF vasopressin levels correlate with social deficits (Oztan et al., 2018).

Based on these results, Parker and colleagues have also conducted a small clinical trial of vasopressin as a possible treatment for autism.

ASSESSMENT TOOLS FOR NONHUMAN PRIMATES

In addition to developing these new disease models, tools will be needed to assess behavior and cognition in nonhuman primates in a manner that translates to human conditions, said Angela Roberts. For example, more than 20 years ago, Roberts and colleagues developed a battery of tests for use in nonhuman primates to assess various aspects of cognitive function, including working memory, visual recognition memory, visual spatial processing, planning, and behavioral flexibility.

Their lab also demonstrated that damage to two distinct regions of the prefrontal cortex affected different aspects of behavioral flexibility. They developed a test to assess these two forms of behavioral flexibility in monkeys and were able to demonstrate that damage to the orbito-frontal cortex impaired a monkey's ability, having learned that one of two stimuli was associated with reward, to switch responding to the other stimulus as the association between stimuli and rewards changed (reversal learning). On the other hand, damage to the ventrolateral prefrontal cortex disrupted higher-order attentional set shifting, the ability to switch attention from one aspect of a stimulus (e.g., shape) to another (e.g., color) (Dias et al., 1996). Roberts said this was subsequently forward translated into humans preclinically by showing a similar dissociation using functional imaging, and clinically by showing that a deficit in shifting attention may be a vulnerability marker for obsessive-compulsive disorder (Chamberlain et al., 2006) and possibly an early marker of Huntington's disease.

Roberts and colleagues have also developed a test to measure threat-driven behaviors in the marmoset that relate to fear and anxiety. They used this test to fractionate out the different forms of anxiety produced by lesions to different areas of the prefrontal cortex (Agustin-Pavon et al., 2012; Shiba et al., 2017). They showed that animals with lesions in the ventrolateral prefrontal cortex are anxious because they have problems shifting attention, while those with lesions in the anterior orbitofrontal cortex

are anxious because they have problems tracking punishment and reward in their environment. The human analog, said Roberts, would be two patients with a similar anxiety phenotype but different underlying causes, who thus should receive different treatment.

Another human characteristic that demonstrates high cognitive function is self-recognition. Mu-Ming Poo and colleagues have developed a test of mirror self-recognition in macaques that replicates the self-recognition test used as an indicator of self-consciousness and body awareness in humans and apes. Because some people with psychiatric conditions or autism demonstrate impairments in this test, Poo reasoned that if macaques could be trained to perform mirror self-recognition, their brains could be examined with brain imaging to study the neural processes underlying this ability. Although macaques do not spontaneously demonstrate mirror self-recognition, Poo and colleagues were able to train them to recognize face marks through the use of visual-somatosensory training where face marks seen in the mirror were simultaneously associated with laser-light stimulation-induced sensation of the corresponding area of the face (Chang et al., 2015). Subsequently, they also trained the monkeys to learn precise visual-proprioceptive association for mirror images, and that this ability could be generalized to novel situations where the monkeys demonstrated self-directed behaviors after seeing mirror images of themselves (Chang et al., 2017).

BRAIN MAPPING AND IMAGING IN GENETICALLY MODIFIED NONHUMAN PRIMATES

Marmosets are an ideal model for longitudinal imaging studies of brain development because they are fully developed by 2 years of age, said Roberts. She and her colleagues imaged a group of marmosets every 3 months between the ages of 3 months and 2 years to measure growth trajectories within 53 cortical regions. Thus, they were able to document substantial variability in the prefrontal cortex during development, which may relate to different symptoms that emerge during childhood and adolescence, she said (Sawiak et al., 2018). She suggested that transgenic models may help to show how stress interacts temporally with the genome in order to affect prefrontal circuits.

The marmoset brain is also ideal for mapping brain circuits of cognitive behaviors because it is small but with primate structures, said Hideyuki Okano (see Figure 4-1). He and his colleagues recently performed calcium imaging

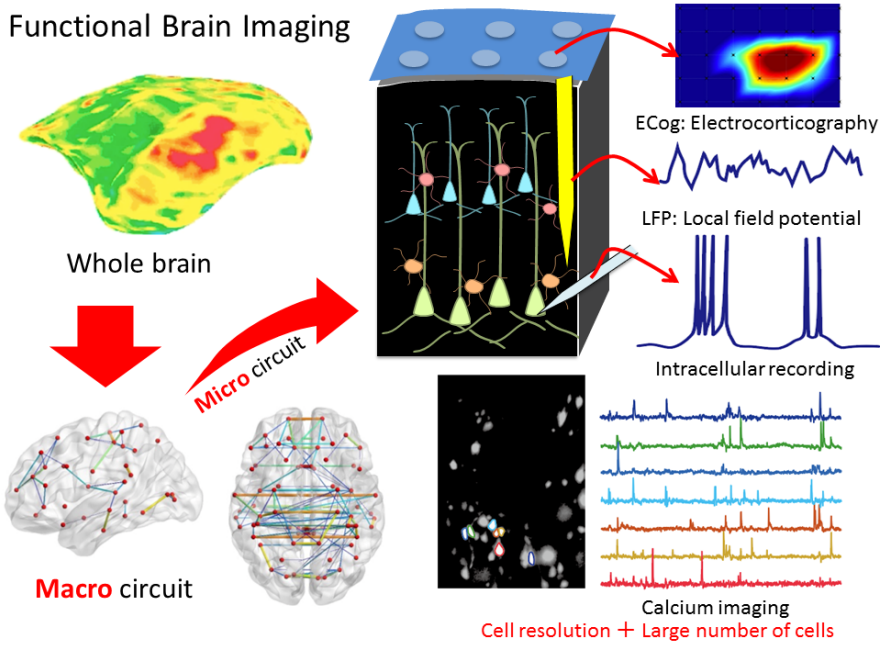

FIGURE 4-1 Functional brain imaging. Technologies such as electrocorticography, local field potential, intracellular recording, and calcium imaging have made it possible to functionally map deep-brain microcircuits in both humans and nonhuman primates.
SOURCE: Presented by Hideyuki Okano, October 4, 2018.

in deep layers of the marmoset cerebral cortex using a microendoscope—a miniaturized fluorescent microscope mounted on the skull to enable deep imaging of freely behaving animals (Ghosh et al., 2011) combined with adeno-associated virus (AAV)-delivered GCaMP (Park et al., 2016) to measure calcium influx into cells as a marker of neuronal excitation (Broussard et al., 2014). GCaMP is a molecule created by fusing a calcium-binding protein with green fluorescent protein (Akerboom et al., 2012). This approach enabled them to monitor and decode the activity of 100 to 1,000 neurons in the motor cortex during a complex behavior such as pellet reaching (Kondo et al., 2018). Okano said this technology using free-moving marmosets will make it possible to dissect large-scale neural circuits during human-relevant behaviors under natural conditions, enabling the study of complex behaviors such as social interactions, fear,

anxiety, and complex motor tasks. In combination with transgenic technologies, this has the potential to transform our understanding, diagnosis, and treatment of human diseases, he said.

Okano is now developing techniques to monitor the activity of neurons in control and disease model marmosets. He predicted that the microendoscope will also make possible other imaging advances, including imaging of deeper brain areas such as the basal ganglia, pathway-specific and cell-type-specific imaging using Cre lines and in vivo genome editing, and imaging multiple sites simultaneously. It may also be used in combination with optogenetics, Okano added.

5

Bioethical Considerations for Transgenic Nonhuman Primate Models in Neuroscience Research

Highlights

- Genetically modified nonhuman primates provide models that closely resemble human diseases, but raise ethical issues related to benefits and harms and the justifications for using these unique resources (Gordon, Greely).
- "Humanized" animal models are designed to better model human disease, not to make animals that phenotypically resemble humans (Treue).
- Nonhuman primate research is ethically justifiable when monkeys are uniquely suited to study a certain system (e.g., vision) and when there is an important health problem at stake and a clear route to translation (Morrison).
- The ethical principles of replacement, refinement, and reduction (the 3Rs) hold particular relevance for nonhuman primate neuroscience research (Jensen, Landi).
- Nonhuman primate studies are ethical only if they are adequately designed and powered (Emborg, Hyman, Landi).
- There are many questions to be addressed related to unintended and intended consequences of transgenic nonhuman primate models, including does it matter what disease is being modeled, how are the symptoms associated with the disease managed, and is "humanization" in animal models different in nonhuman primates compared with other species (Kahn)?
- Oversight of nonhuman primate studies should go above and beyond that required for animal studies in lower species (Landi).
- Researchers have a duty to communicate with the public and other stakeholders about the associated ethical challenges (Kahn, Treue).

NOTE: These points were made by the individual speakers identified above; they are not intended to reflect a consensus among workshop participants.

The best way to study a human brain is in a living human body, but because there are ethical limits to what can be done experimentally with humans, scientists are left with looking at surrogates, including genetically modified and chimeric nonhuman primates, said Henry Greely, the Deane F. and Kate Edelman Johnson Professor of Law and professor, by courtesy, of genetics at Stanford University. The dilemma, he said, is that as these animals are engineered to more closely resemble humans, the same ethical issues that prevent studies in humans begin to arise. These ethical issues relate to determination of the relationship between benefits and harms, the justification for using nonhuman primates as animal models for research, and the justification for genetically modifying nonhuman primates in ways that may cause pain and distress. Greely added that the public may have unrealistic fears about the humanization of these nonhuman primates and that even scientifically unrealistic fears can have significant consequences for research, and therefore, need attention.

John Morrison, director of the California National Primate Research Center and professor of neurology at University of California, Davis, cited four principles to follow when choosing to work with nonhuman primates: (1) the nonhuman primate should be uniquely well suited to the problem being studied; (2) there should be a critically important health problem closely aligned with the research; (3) the research should be attractive to funding agencies and industry; and (4) there should be a clear route to translation. Joshua Gordon added that studies also should not be conducted in nonhuman primates if they could be done more easily and cheaply in humans, and that nonhuman primate models should not be used for "fishing expeditions," but for targeted studies where other research in rodent or other models has already provided information on what to seek.

3R PRINCIPLES AND HARM–BENEFIT

Ethical principles known as the 3Rs (replacement, refinement, and reduction) were articulated 60 years ago (Russell and Burch, 1959) and are now widely applied to animal research throughout the world (Kirk, 2018). They are of particular relevance for research on nonhuman primates (Prescott et al., 2017), especially neuroscience research (Lemon, 2018).

These principles reflect the recognition that nonhuman primates are a unique but limited resource, said Frances Jensen, as well as the fact that like humans, they are sentient, social, and have high cognitive abilities, said Margaret Landi, chief of animal welfare, ethics, and strategy for GlaxoSmithKline.

In terms of harms and benefits, Landi said the 3Rs address only potential harms and direct investigators either to replace an animal with a nonanimal or a lower phylogenetic species, reduce the number of animals, or refine the studies to decrease or eliminate pain or distress. Benefits such as increasing understanding of the disease in question or finding new treatments are generally accepted, she said, adding that benefits potentially could be increased if the translational fidelity of the animal model was increased.

Different Ethical Considerations for Nonhuman Primates Versus Rodents and for Genetically Modified Animals Versus Wild Type

Stefan Treue, director of the German Primate Center in Goettingen, said that regardless of the species, and whether genetically modified or not, animal welfare principles should apply to all animals. However, he said, particular challenges arise with transgenic animals because of the potential harm inflicted by genetic modification and the need to maintain a healthy breeding colony of these purpose-bred disease models. This requires development of new assessment techniques not only in terms of outcome measures, but also to understand the consequences of the genetic manipulation, said Treue.

Some genetically modified animal models are referred to as "humanized" models, although Treue noted that the purpose of genetic modification is usually not to make them phenotypically more like humans, but to build disease models that come closer to the human disease. From an ethics perspective, he said, this is an important and relevant distinction. The main concern, said Treue, is whether the genetic modifications result in unintended (beyond those associated with the disease) consequences that are detrimental for the animal.

Intended consequences are also a concern, said Jeffrey Kahn. If we are concerned about the effects of symptoms in humans, he asked, should we not also be concerned about those effects in the animals that are modeling those symptoms? Moreover, does it matter what disease is being modeled, for example, whether the disease is life threatening or not? He mentioned that similar questions about what counts as sufficiently important research

also arose in the context of gene therapy when the National Institutes of Health's (NIH's) recombinant advisory committee considered whether it is appropriate to expose somebody to the risk of gene therapy for a non-life-threatening disease. Regarding humanization, Kahn said questions need to be answered about which capacities matter; how those capacities are assessed as animals are manipulated to act, look, and feel more like humans in their symptomology; and whether humanization in animal models is qualitatively different in nonhuman primates compared with other species.

Xenotransplantation of neural tissue raises other concerns, said Treue, because this involves interfering with exactly the organ that underlies the critical species differences between human and nonhuman primates.

Nonhuman primate research should be held to an especially high standard, said Steven Hyman, Harvard University Distinguished Professor of Stem Cell and Regenerative Biology and director of the Stanley Center for Psychiatric Research. Nonhuman primate studies are ethical only if they are adequately designed and powered, which may require large numbers of animals, he said. Marina Emborg, professor of medical physics at the University of Wisconsin–Madison and director of the Preclinical Parkinson's Research Program at the Wisconsin National Primate Research Center, suggested that it is too early to use transgenic nonhuman primates for efficacy studies. She agreed with Hyman that unless such studies are properly powered, they are premature, which is the reason that the infrastructure for raising these animals and conducting the studies needs to be increased. Kahn agreed, stating, "It is not ethical to use too few animals and have underpowered research."

Landi added that lack of robust study designs further compromises the propriety of a study. Unblinded or nonrandomized studies, she said, have a high likelihood of producing a biased outcome that will not translate to people. Guoping Feng added that it is important to realize that monkeys are not human. Researchers need to determine which human characteristics are important to model and recognize that models can only reflect certain aspects of a disease, not the human disease itself, he said. Genetic models may be generated using human mutations—either monogenic or combinations of mutations—that help elucidate the biology of the disease, replicate the circuits implicated in human disease, and thus advance translation of this knowledge to humans.

Emborg reminded workshop participants than an investigator's idea for the use of an animal model is vetted several times, starting with the call for proposals by federal and private funding agencies, the NIH Blueprint

for Neuroscience Research,[1] and peer review. The justification for animal use continues through the review of the vertebrate sections of the proposal and Institutional Animal Care and Use Committee (IACUC) protocols. This multistep process provides guidelines about how to prioritize research and ensure that scarce resources are being allocated in the most efficacious manner. Treue argued that while peer review works for assessing individual proposals, it is ultimately a political or science policy decision where funding lines are established that allow for approaches that break the general mold, including the large, multicenter projects needed for nonhuman primate research.

Feng added that if researchers know of possible approaches that might lead to a treatment and do not pursue them, that also may be unethical. However, the more human-like a model is, and the closer it comes to replicating human disease, the more concerns arise about the ethical implications of making animals sick in order to study them, said William Newsome. He suggested that guidance from those who have already done research with nonhuman primates using transgenic approaches may be helpful. For example, by looking at early studies with *SHANK3* mutant animals, investigators may be able to determine whether the mutation made the animals more fragile or difficult to care for, or if they needed special housing or social arrangements.

Regarding the question of whether the care of transgenic and chimeric animals needs to be different, Emborg said that there were many references highlighted throughout the workshop to the use of these types of models that had contributed to human and animal health. In order for those studies to provide valid data, she added, the animals had to receive appropriate care. Emborg said in her MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) studies, they were prepared to treat the animals with Sinemet if needed, which is the standard of care for patients with Parkinson's disease (PD). She also used intracarotid rather than systemic MPTP, which produced hemi- rather than bilateral parkinsonism, a milder form of the disease that extends the animals' lives and allows them to care for themselves.

She also pointed to the dedicated team of veterinarians and animal caretakers that keep close watch on the animals and are prepared to intervene if there are any problems (e.g., if they are not eating or drinking enough, not moving normally, need extra food or warming lamps). They

[1]To learn more about the NIH Blueprint for Neuroscience Research, go to https://neuroscienceblueprint.nih.gov (accessed December 22, 2018).

also go back to the clinic and talk with clinicians who care for people with PD, asking them what their patients are experiencing and what they suggest in terms of caring for the monkeys.

Regulatory Oversight of Nonhuman Primate Research

In the United States, research involving nonhuman primates, whether they are genetically manipulated or not, must adhere to the same rules and regulations that guide all animal research and that are enforced by each institution's IACUC, according to Landi. She maintained, however, that these rules and regulations should be viewed as the minimum required, and that scientists working with these animals are obligated to go beyond these laws when assessing benefits and harms. While there are no special rules for nonhuman primates, their use raises additional ethical concerns.

Landi outlined principles or commitments that are central to the management of animal facilities, including access to species-appropriate food, water, and housing. If animals have been genetically manipulated, meeting these basic needs may change how they are delivered. For example, modifications to the animals' environment, feeding, and care may be needed if the manipulations have affected the animal's ability to chew or swallow, caused mobility or balance problems, or resulted in abnormal behaviors, she said. Ensuring that environments are designed appropriately and that animals receive humane and appropriate care requires a program of veterinarians, behaviorists, and technicians who know how to work not only with the nonhuman primate species, but with how the potential disease or phenotypic change may affect that species, said Landi.

Landi said that looking at the human disease that the animal model is supposed to replicate may help predict what kind of environmental changes will be needed for the animals. However, she added that the species is also important because, for example, macaques are different from marmosets. Although they are social animals, they are also predators who when sick or distressed will hide their symptoms to avoid becoming prey or being rejected by their group.

European animal welfare guidelines overlap those applied in the United States, said Treue. 3R principles are an explicit part of the legal framework in Europe. They were established with the passage in 2010 of a European Union-wide directive on the protection of animals used for scientific purposes. Specific to the use of nonhuman primates is a prohibition against the use of great apes. Every institution is required to have an

animal welfare body roughly comparable to an IACUC, said Treue; however, these committees provide advice and recommendations only. Approval of animal experiments rests with national or regional "competent authorities."

Hyman suggested that to further align regulatory and ethical guidelines, it might be useful to establish an advisory committee similar to the the consensus committee on the use of chimpanzees in research established by the Institute of Medicine and the National Research Council at the request of NIH in 2010 (IOM and NRC, 2011). The chimpanzee committee established a set of criteria to guide research in an ethical manner, said Kahn, who chaired the committee. Kahn identified several questions that a similar committee focused on nonhuman primates could address in a systematic way:

- What are the relevant reasons to create and use these models (justification)?
- If research on nonhuman primates is justified, what oversight will be needed in order to implement this research?
- What criteria will be used and by whom regarding whether the scientific rationale for a certain study merits approval for that study?

Kahn added that the scientific community should take the lead in developing these guidelines, and that it would be important to invite relevant stakeholders to participate.

IMPORTANCE OF PROACTIVE COMMUNICATION WITH THE PUBLIC

Given that the public funds most of the research involving nonhuman primates (other than the use of nonhuman primates for regulatory testing), Treue stressed that researchers have a duty to communicate with the public about the ethical challenges of working with animals in general and more specifically with nonhuman primates. He advocated for a proactive approach, particularly with new technologies in development that may increase the proportion and number of nonhuman primates in research. "We should not view this as a nuisance, but rather an opportunity to do scientific communication and bring the public onboard with why we think this is not only ethically justified, but also scientifically important," he said.

Gordon added that some members of the public may be concerned about using nonhuman primates for purely fundamental science purposes. However, he argued that a side effect of basic science is learning unexpected things that turn out to be helpful.

Kahn added that improved communication is needed across stakeholder groups to guide research and help refine and articulate the arguments about justification so that any criteria crafted assure that research moves forward in an ethically acceptable way. These communications need to go beyond simplistic justifications, said Treue. It is not enough to say only that scientists want to cure these terrible diseases; further explanations are needed to convey to the public and other stakeholders that research across many labs is needed, he said. Moreover, while no single study will be sufficient, every study if done well can be argued to be necessary, he said.

6

Understanding the Policy, Infrastructure, and Funding Needed to Advance Neuroscience Research

Highlights

- China, Japan, and the United States are pursuing different but complementary approaches to brain mapping, including mapping of the marmoset and macaque brains (Gordon).
- Seven National Primate Research Centers (NPRCs) in the United States breed and care for most nonhuman primates used in U.S. research; however, these centers are underfunded (Levine, Morrison, Parker).
- In Germany, as in the United States, nonhuman primate research is distributed across primate centers and academic laboratories (Treue).
- NPRCs facilitate convergent research on nonhuman primates that enables efficient translation to human disease (Morrison).
- Research involving primates has been slowed by limitations in the number of animals that can be generated in a reasonable time frame (Feng, Smith).
- Centralizing resources for nonhuman primate breeding and research has both advantages and disadvantages (Buhring, Gordon, Smith).
- Sharing animals around the world would advance research, but is limited by restrictions on transporting monkeys (Okano).
- Sharing of information and data from nonhuman primate research studies is essential to ensure reproducibility of results and to minimize duplicative studies (Emborg, Frasier, Jensen).
- To sustain a robust nonhuman primate research enterprise, training, educating, and incentivizing the next generation of researchers needs to be a priority (Desimone, Hyman, Poo).

- There is an urgent need to craft a strategy to develop genetically modified nonhuman primate models (Feng).
- A global consortium representing industry, academia, funders, and other stakeholders could ensure efficient use of resources (Amara, Hyman), although a large consortium may also lack the agility to answer disease-focused questions (Frasier).

NOTE: These points were made by the individual speakers identified above; they are not intended to reflect a consensus among workshop participants.

Brain initiatives in different countries are taking on different, but complementary, approaches to brain mapping, noted Joshua Gordon. In Japan, the Brain/MINDS (Mapping by Integrated Neurotechnologies for Disease Studies) project is focused on mapping the marmoset brain; China has taken on mapping and cataloging the cell types in the macaque brain; and the United States has launched an initiative to discover and catalog human cell types. Likewise, he said, both the U.S. BRAIN Initiative and the European Brain Initiative include significant theory components. Mu-Ming Poo, director of the China Brain Project, said that within the next 10 to 15 years, they hope to map the cell-type-specific connectome of important brain regions in the macaque as an intermediate step toward mapping all the connections in the human brain using platforms for neuromodulation, neural information processing, and transgenic nonhuman primate development. They are also working on technologies for single-neuron gene expression profiling and labeling, circuit tracing, mesoscopic and microscopic brain imaging, and in vivo electrophysiological and electrochemical recording (Poo et al., 2016).

PRIMATE RESEARCH CENTERS

As noted earlier, advancing primate research will require breeding and husbandry facilities for large numbers of animals. The United States has seven National Primate Research Centers (NPRCs)[1] supported, in part, by National Institutes of Health (NIH) base grants, said John Morrison. Each center houses approximately 4,000 to 5,000 monkeys, participates in 80 to

[1]To learn more about the National Primate Research Centers, go to https://nprcresearch.org/primate (accessed December 22, 2018).

100 experimental protocols, and forms collaborative partnerships with affiliates, he added. Several centers have large outdoor corrals that provide a naturalistic setting excellent for breeding. In addition, they enable scientists to work with monkeys—including those that have been genetically manipulated—across the life span, from embryos through to geriatric colonies. The centers also provide imaging facilities and animal husbandry teams, said Morrison. Having highly skilled husbandry professionals is particularly important when working with monkey models of schizophrenia or other conditions with complex phenotypes, he said.

Morrison provided an example of how the California NPRC advanced translational vision research moved quickly and efficiently. After the Center's animal health technicians noticed that some of the animals were avoiding sunlight, the vision scientist group tested the animals and found they had no cone function. They went on to identify the gene associated with this phenotype and have now obtained a grant to breed more of these animals and move forward with developing gene therapy.

This is just one example of how the large teams at NPRCs facilitate convergent research in nonhuman primates, said Morrison. When there are very complex phenotypes, it is not enough to record from one part of the frontal cortex; there is also a need to study metabolism and cardiovascular and respiratory function, to get a full phenotype, and to find biomarkers, he said.

According to Karen Parker, the NPRC P51 base grants have not increased, or have modestly increased, in the past 5–10 years. While all NPRCs have undergone extensive construction and renovation projects in the past decade, she added that some facilities within the NPRCs have not been updated since the 1960s or 1970s. Jon Levine, director of the Wisconsin NPRC, noted that the lack of increased funding means a decrease in real dollars over this time. These resource limitations will make it difficult for the Centers to add emerging technologies, including genome editing, said Parker; they also will be unable to expand to meet user demand.

Parker called for a national investment to support and advance nonhuman primate models of complex brain disorders, including scaling emerging genetic technologies and sophisticated behavioral tools for use at NPRCs. She suggested that to advance transgenic work, this may mean distributing efforts among the seven NPRCs or relying on academic institutions to provide the financial backstops for this research. Levine offered some good news regarding funding for nonhuman primate research. He said the NIH's Office of Research Infrastructure Programs recently completed Phase I of a nonhuman primate needs assessment and is in the midst

of preparing a report from a Phase II expert panel discussion. This report will identify the desperate need to have a national strategy to develop marmosets as a resource and to support the development of these tools, he said.

The German Primate Center in Goettingen is the only publicly funded center in Germany doing both research and breeding for the academic community, said Stefan Treue. In Europe, nonhuman primates comprise only about 0.05 percent of all animals used in research, he said, yet their small proportion belies their scientific importance. Only about 10 percent of these are used for basic research studies; most are used for toxicology studies and other aspects of product development and safety assessment. As in the United States, noncommercial, nonhuman primate research in Europe is distributed across national primate centers and academic laboratories, said Treue. He suggested that such a dual approach is critical to optimizing the role of nonhuman primate research.

Developing Nonhuman Primate Resources: To Centralize or Not?

Research involving primates has been slowed by limitations in the number of animals that can be generated in a reasonable time frame, said Yoland Smith. What is needed, he said, is a resource that provides scientists with access to nonhuman primates, but that does not require each lab or each academic institution to establish its own breeding facility. Guoping Feng concurred, noting that it is not feasible or cost-effective for every university to house a transgenic primate facility. He advocated a centralized facility shared by several universities. Gordon commented that a large-scale, Jackson Lab-type approach (which breeds genetically modified mice for labs around the world) to maintaining and sharing nonhuman primate lines might not be feasible, though it would be important to develop infrastructure and policies to promote sharing. While centralized breeding facilities offer many advantages, Bettina Buhring of the National Institute of Mental Health (NIMH) also encouraged individual laboratories to continue working on animal models that are nearly ready. It would be unethical, she said, to withhold such tools simply because the infrastructure is not yet ready.

Steven Hyman noted that vigilance will be needed to keep these animals healthy. He added that this will require animals to be outbred, which necessitates a different way of interpreting results than is used with inbred mouse strains where there is a totally isogenic background. A chip that

enables fast whole genome sequencing of marmosets and macaques might be a useful tool, he said.

Hideyuki Okano reiterated the importance of sharing animals around the world, but noted that most airlines will not transport monkeys. A solution, he said, is to transport frozen sperm. Sperm from genetically modified marmosets is available from his institute, the RIKEN Center for Brain Science, he said. Feng added that once the first male founder is generated, in vitro fertilization can be done quickly. Another potential solution mentioned by Gordon is to develop the infrastructure for observatories where scientists from various institutions could conduct their research.

John Spiro agreed that coordinated efforts are needed to move forward, adding that this will require a cultural acceptance that these models must be shared among other groups. However, he added that in the case of disorders like autism where even people with the same driver mutation may look very different, a large number of monkeys may be needed to accurately model the human condition.

Nonhuman primate research centers could also facilitate breeding of animals when researchers identify unique pathologies mimicking human diseases, said Jean Bennett. For example, macular degeneration, which is responsible for about 8 million cases of blindness in the United States, would be a great target for gene therapy if there were good models, she said. After performing ophthalmoscopy on more than 100 nonhuman primates over the past 20 years, her team found one animal with white spots on the retina that resembled drusen, the hallmark pathology seen in people with macular degeneration. Genetic analysis of this animal showed biallelic mutations in the gene for an inherited form of macular degeneration called Stargardt disease, suggesting a possible path toward breeding an animal model of macular degeneration. She said it would be important for the field to consider strategies about what could be done when such unique animals are identified so others can study them.

SHARING INFORMATION AND DATA

Reproducibility of results has dogged many areas of biomedical research, said Mark Frasier, senior vice president of research programs at The Michael J. Fox Foundation (MJFF) for Parkinson's Research. To address this need, MJFF funds academic investigators to bring their expertise, knowledge, and sophisticated tools to characterizing models in a

standardized way, and then to make those models accessible through different vendors for both academic and industry research. Although MJFF has not done this with nonhuman primate models yet, the model has been successfully implemented with other tools such as viral vectors, he said. Frasier added that they are also supporting more natural history work to understand disease at the human level in a multidimensional way, and that the data from these studies are shared across the globe.

Sharing of other information is also critical to minimize duplications, said Marina Emborg. Frances Jensen agreed, adding that sharing of negative as well as positive data is needed within the nonhuman primate research community, especially data from unique transgenic animals. Given the limited resources available for nonhuman primate work, Frasier wondered how to balance innovation in developing new methodologies with the need for standardization. Parker suggested that funders might make sharing of resources an expectation of all grant recipients.

TRAINING AND EDUCATING THE NEXT GENERATION OF RESEARCHERS

One of the lessons learned from efforts to develop genetic models in mice is that going from a gene mutation to a mechanistic causal pathway and then to a druggable target is difficult, according to Robert Desimone, director of the McGovern Institute and the Doris and Don Berkey Professor of Brain and Cognitive Sciences at the Massachusetts Institute of Technology. Moreover, manipulating genes in the brain markedly increases the difficulty, he said. These issues will not be solved by any one lab, one center, or even one country, he said; many laboratories working on many different aspects of the problem will be needed. The challenges involve money, facilities, and people. Given the impracticality of every institution having its own primate facility, he advocated finding ways to share genetic material and establish centralized breeding colonies that can make animals available to investigators at many universities. To accomplish all of this, he said, more trained researchers will be needed.

Poo said the China Brain Project envisions a national brain health training and education center to promote sustainable research in primate biology. As part of this effort, they plan to establish a permanent summer program to train students who are interested in working with nonhuman primates, said Poo. A few workshop speakers added that other changes will also be needed in academic institutions to incentivize young scientists

to pursue careers that involve working with nonhuman primates. For example, Hyman pointed to the need for career development pathways that credit scientists for their participation in large consortia.

ORGANIZING EFFORTS TO MEET INFRASTRUCTURE AND SCIENTIFIC NEEDS

Feng agreed about the urgency of crafting a strategy to develop genetically modified nonhuman primate models. In 2 or 3 years, he said, many genetically modified lines will be available, yet the infrastructure to breed and distribute them has not kept up. Gordon said NIMH (mostly through the U.S. BRAIN Initiative, but also the NIH Blueprint for Neuroscience Research) has identified the two highest priorities: (1) obtaining and expanding marmoset breeding colonies in the United States, and (2) establishing centers or expanding existing centers that will continuously breed marmosets and make them available. Morrison said there are currently only two primate centers, Wisconsin and Southwest, that breed marmosets. The expertise, resources, and space are available to establish a breeding colony at the California NPRC, he said; however, funds are lacking. Gordon said there is agreement among the neuroscience institutes that collaborations and pooling of resources across multiple NIH institutes should be supported.

Given the precious nature of these resources and the ethical pressure to limit their use, Susan Amara, director of the Division of Intramural Research Programs at NIMH, wondered if a larger, global consortium representing industry, academia, federal and nonprofit funders, and other stakeholder groups could coordinate efforts on generating transgenic nonhuman primate models to ensure that resources are well used, and to minimize the potential for duplication of efforts. Morrison agreed, and suggested that in setting resources and priorities, the research community should be guided not by what has been done in rodent models, but by what the human literature suggests could be gained by research in monkeys. Hyman added that the Foundation for the National Institutes of Health might be the entity that could bring industry partners onboard.

Lisa Stanek and Morrison noted that collaborations between industry and academia are becoming more common, but they suggested that more could be done. Stanek said that many academics may not appreciate that industry partners are ready, willing, and waiting to work with them. She said that a collaboration between Sanofi and Ben Deverman's laboratory in developing novel adeno-associated virus (AAV) capsids for nonhuman

primate research was a perfect marriage of his skills in the academic setting and their infrastructure that enabled them to execute nonhuman primate studies relatively rapidly.

However, Frasier countered that a large consortium can be effective if there is a shared common goal, but it may lack the agility to answer some disease-focused questions. For example, he said MJFF has partnered with other organizations, including NIH and other nonprofit funders, to focus on a common but more targeted question related to Parkinson's disease. Stanek also pointed to a model advanced by the CHDI Foundation, a nonprofit, private, disease-specific foundation focused on Huntington's disease that brings researchers or organizations together to address specific questions and avoid duplicating efforts.

Story Landis, director emeritus of the National Institute of Neurological Disorders and Stroke and co-chair of the Forum on Neuroscience and Nervous System Disorders, suggested that it might also be useful to engage organizations such as the Allen Brain Institute that already have a robust pipeline of gene expression and single-cell technologies. She said they have ways of mapping connectivity that would take years for another institution to put together. All they need are the marmosets, she said. Hyman suggested that a neutral convener might be able to bring these organizations into the discussion. He added that because many of the important tools for nonhuman primate research are being developed in China and Japan, there may be value in convening some of these meetings in Asia.

FINAL REMARKS

In closing, Steven Hyman highlighted some of the important points that emerged during the workshop. One of the key messages, he said, is that nonhuman primate research complements studies in humans and other organisms. Researchers have the opportunity to select a model system—ranging from cell culture to drosophila, zebrafish, mice, and now nonhuman primates—that is most likely to be able to answer the research questions at hand. The workshop made clear that translational research involves a new set of constraints that does not exist in basic research, he said, especially the need for conservation across all components that contribute to an outcome: molecular targets, cell synapses, and circuits. Indeed, it is that need for conservation that makes selecting the right model so important, said Hyman.

As an example, he noted that in humans, anxiety disorders and addictions are as much the result of top-down control failures as with the bottom-up functions of reward and fear circuits. Nonhuman primates enable scientists both to study failures of top-down control in realistic contexts as well as to flag reward circuits and possibly treat addiction, he said. Depending on the question being asked, genetic modification with either transgenesis or viral vectors may add further utility to nonhuman primate models, said Hyman. For example, as described in Chapter 3 by Hideyuki Okano, a transgenic marmoset model of Parkinson's disease (PD) carrying an α-synuclein mutation enabled his team to model sleep disturbances that are a common complaint of patients with PD, but that were not seen in the mouse MPTP model that had been so useful in developing deep-brain stimulation as a treatment for the motor symptoms of PD. Hyman added that antisense oligonucleotides and gene therapy have taken the value of these nonhuman primate models another step further.

Hyman predicted that in the case of autism, gene-modified monkeys will be needed for every 1 of the 100 genes identified as strong drivers of autism that John Spiro mentioned in Chapter 3. Each of these models will enable different questions to be asked and answered, including questions related to intervention, off-target effects, biomarkers, and safety, he said.

To address the scientific and practical challenges identified throughout the workshop, Guoping Feng highlighted the urgency of crafting a strategy to develop genetically modified nonhuman primate models and the needed infrastructure to support this promising area of research.

A

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B

Workshop Agenda

Transgenic and Chimeric Neuroscience Research: Exploring the Scientific Opportunities Afforded by New Nonhuman Primate Models—A Workshop

October 4, 2018
Keck Center of the National Academies
500 Fifth Street, NW, Washington, DC

Background:

The translational disconnect from preclinical studies with predominantly rodent animal models to human clinical trials remains a key challenge associated with lagging development of therapies for brain disorders. Since 2012, the Forum on Neuroscience and Nervous System Disorders has hosted a series of workshops examining different aspects of this challenge, including maximizing the translation of effective therapies from animal models to clinical practice and exploring the evidence needed to bring compounds that appear to be safe into human efficacy trials. While no animal model will fully recapitulate human nervous system disorders, nonhuman primates (NHPs)—such as marmosets and macaques—have shown promise in their ability to serve as models for complex brain disorders, given the phylogenetic proximity and genetic similarity to humans, similarity of neuroanatomical organization (e.g., a well-developed prefrontal cortex) and associated cognitive and behavioral functions, social cognition, and the ability to study developmental phenotypes and prodromal disease states.

Workshop Objectives:

This 1-day public workshop will bring together experts and key stakeholders from academia, government, industry, and nonprofit organizations to examine the scientific opportunities and challenges, as well as bioethical considerations, of genetically engineered nonhuman primate models for neuroscience research.

Invited presentations and discussions will be designed to:

- Discuss the state of the science of transgenic and chimeric neuroscience research and emerging models for nervous system disorders, and explore the potential usefulness of such models to enhance understanding of behavior and higher cortical function and advance therapeutic development.
- Examine current tools and technologies used in rodent models (e.g., transgenesis, chimera, adeno-associated viruses [AAVs], gene therapy, etc.) and explore how they would need to be modified for use in other animal models, such as nonhuman primates.
- Consider bioethical principles and issues related to genetic engineering of animal models for nervous system disorders, and discuss potential metrics for determining the models' readiness for nonhuman primate research.
- Discuss policies and infrastructure needed to advance research in this domain including, for example, training, recruitment of early career scientists, and the potential development of specialized research centers and international collaborations.

DAY ONE: October 4, 2018

8:30 a.m. Welcome and Overview of Workshop
FRANCES JENSEN, Perelman School of Medicine, University of Pennsylvania (*Chair*)

Session I: Emerging Transgenic and Chimeric Nonhuman Primate Models for Neuroscience Research and Therapeutic Development for Nervous System Disorders

Objective:

- Discuss the state of the science of transgenic and chimeric nonhuman primate models for nervous system disorders and explore the potential usefulness of such models to enhance understanding of behavior and higher cortical function and in translational science to advance therapeutic development.

8:45 a.m. Session Overview
SARAH CADDICK, Thalamic (Moderator)

8:55 a.m. Speakers
GUOPING FENG, Massachusetts Institute of Technology
HIDEYUKI OKANO, Keio University School of Medicine; RIKEN Brain Science Institute
YOLAND SMITH, Yerkes National Primate Research Center; Emory University
ANGELA ROBERTS, University of Cambridge

9:55 a.m. Discussion
Discussant: WILLIAM NEWSOME, Stanford University

10:30 a.m. BREAK

Session II: Technology, Research Methodology, and Assessment Tools for Transgenic and Chimeric Nonhuman Primate Models

Objectives:

- Examine how current tools and technologies developed in rodent models (e.g., transgenesis, chimera, AAVs, gene therapy, in vitro fertilization, etc.) through the BRAIN Initiative and elsewhere might be modified for use in nonhuman primates.
- Consider potential logistical and feasibility issues unique to nonhuman primate models (e.g., cost).

10:45 a.m. Session Overview
ROBERT WURTZ, National Eye Institute (Scientist Emeritus) (Moderator)

11:00 a.m. Speakers
MU-MING POO, Chinese Academy of Sciences
BEN DEVERMAN, Broad Institute of Massachusetts Institute of Technology and Harvard University
JEAN BENNETT, University of Pennsylvania
KAREN PARKER, Stanford University

12:00 p.m. Discussion
- Why and how do you make that leap from rodents to nonhuman primates technically?
- What are the logistical and feasibility issues in using genetic and chimeric technologies in nonhuman primate models for neuroscience research (e.g., cost)?
- What tools and technologies are currently being used or are needed to create these models?
- What measures and assessment tools are needed (i.e., behavioral assessments)?

Discussants: DAVID AMARAL, University of California, Davis
ROBERT DESIMONE, Massachusetts Institute of Technology

12:30 p.m. LUNCH

Session III: Bioethical Considerations for Transgenic and Chimeric Nonhuman Primate Models in Neuroscience Research

Objectives:

- Explore bioethical principles and issues related to the genetic engineering of nonhuman primate models or the creation of chimeric nonhuman primate models for neuroscience research.
- Consider key questions that will necessitate nonhuman primate models for basic and translational research.
- Discuss potential safeguards needed for transgenic and chimeric nonhuman primate models of nervous system disorders to ensure proper animal welfare.

1:30 p.m. Session Overview
HENRY T. GREELY, Stanford University (Moderator)

1:40 p.m. Speakers
MARGARET LANDI, GlaxoSmithKline
STEFAN TREUE, German Primate Center; Georg-August University
JEFFREY KAHN, Johns Hopkins University

2:25 p.m. Discussion

- As NHPs deserve greater or different consideration than other nonhuman animal species used in research generally, should there be particular considerations about their use in transgenic and chimeric neuroscience research? How should that be reflected in which research is carried out, and in the care of NHPs in research settings?
- What criteria must be met in order to justify the use of nonhuman primates in transgenic and chimeric neuroscience research, that is, type and importance of research questions; unique aspects of nonhuman primates; data from other research models; clinical testing that cannot be performed in human subjects, etc.?
- What are the possibilities that transgenic and chimeric neuroscience research in NHPs could confer some qualitatively different aspect of cognition on the NHP? How

could that be assessed? What would be the significance if that were to happen?

Discussant: MARINA EMBORG, Wisconsin National Primate Research Center; University of Wisconsin–Madison

3:00 p.m. BREAK

Session IV: Moving Forward: Policy and Infrastructure Needs to Advance Research

Objectives:

- Synthesize and discuss key highlights from the workshop presentations and discussions, including identifying next steps and promising areas for future action and research.
- Discuss policies and infrastructure needed to advance research in this domain, including, for example, training, recruitment of early career scientists, and the potential development of specialized research centers and international collaborations.
- Consider the roles of national primate research centers, governments, private philanthropy, and other key stakeholders to advance this research.

3:15 p.m. Session Overview
FRANCES JENSEN, Perelman School of Medicine, University of Pennsylvania (*Chair*)

3:25 p.m. Keynote
MU-MING POO, Chinese Academy of Sciences

3:40 p.m. Panel Discussion
JOHN MORRISON, California National Primate Research Center, University of California, Davis
HIDEYUKI OKANO, Keio University School of Medicine; RIKEN Brain Science Institute
JOSHUA GORDON, National Institute of Mental Health
MARK FRASIER, The Michael J. Fox Foundation for Parkinson's Research

JOHN SPIRO, Simons Foundation Autism Research Initiative
LISA STANEK, Sanofi

4:25 p.m. Discussion

5:15 p.m. Closing Remarks
STEVEN HYMAN, Broad Institute of Massachusetts Institute of Technology and Harvard University

5:30 p.m. Adjourn Workshop

C

Registered Attendees

Neeraj Agarwal
National Eye Institute

Afomeya Agonafer
National Center for Complementary and Integrative Health

Diaa Ahmed
Utrecht University

Bernadette Alisantosa
George Mason University

Susan Amara
National Institute of Mental Health

David Amaral
University of California, Davis; MIND Institute

Bryan Ampey
National Institutes of Health

Kathleen Anderson
National Institute of Mental Health

Alexander Arguello
National Institutes of Health

Karyn Armstrong
U.S. Army

Ayodeji Asuni
Lundbeck

Shelli Avenevoli
National Institute of Mental Health

Lisa Bain
Science and medical writer

Amy Bany Adams
National Institute of Neurological Disorders and Stroke

Andrea Beckel-Mitchener
National Institute of Mental Health

Jean Bennett
Perelman School of Medicine, University of Pennsylvania

Adam Berger
National Institutes of Health

Jan Bernal
Pfizer Inc.

Juliet Beverly
Society for Neuroscience

Kishan Bhatt
National Institutes of Health

Lizbet Boroughs
Association of American Universities

Linda Brady
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Jeffrey Braff
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Patricia Brown
National Institutes of Health

Tyler Brown
Broad Institute of Massachusetts Institute of Technology and Harvard University

Bettina Buhring
National Institute of Mental Health

Teresa Burke
Gallaudet University

Laura Cabrera
Michigan State University

Sarah Caddick
Gatsby Charitable Foundation

Erin Cadwalader
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Daofen Chen
National Institute of Neurological Disorders and Stroke

Huei-Ying Chen
Lieber Institute

Jiu-Chiuan (J. C.) Chen
Keck School of Medicine, University of Southern California

Qiang Chen
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Food and Drug Administration

Lisa Chiu
Society for Neuroscience

Randy Contreras
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Emily Cukier
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Bernard Dardzinski
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Bianca De Paulis
Fordham University School of Law

John Dennis
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Robert Desimone
McGovern Institute, Massachusetts Institute of Technology

Ben Deverman
Broad Institute of Massachusetts Institute of Technology and Harvard University

C. J. Doane
University of Arizona

Emmeline Edwards
National Center for Complementary and Integrative Health

Jennifer Elam
Washington University School of Medicine

Marina Emborg
University of Wisconsin–Madison

Richard Esposito
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Margaret Fabiszak
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Keith Fargo
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Lynnie Fein-Schaffer
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Guoping Feng
Massachusetts Institute of Technology

Mark Frasier
The Michael J. Fox Foundation for Parkinson's Research

Ellen Gadbois
National Institutes of Health

John Gardenier
Independent

Turkan Gardenier
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Miri Godin
Hadassah Medical Center

Anna Goodroe
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Neera Gopee
National Institutes of Health

Joshua Gordon
National Institute of Mental Health

Meg Grabb
National Institutes of Health

Steven Grant
National Institute on Drug Abuse

Henry Greely
Stanford University

Amelie Gubitz
National Institute of Neurological Disorders and Stroke

Yan Guo
Pacific Biosciences

Yueqi Guo
Johns Hopkins University

Stacie Gutowski
Food and Drug Administration

James Harris
Johns Hopkins University

John Hasenau
Lab Animal Consultants

Jim Hendrix
Alzheimer's Association

Meena Hiremath
National Institute of Mental Health

Richard Hodes
National Institute on Aging

Gregg Homanics
University of Pittsburgh

Dea Houser
Drs. J. B. and D. D. Houser

Juan Huang
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