PERSPECTIVE

Opportunities and limitations of genetically modified nonhuman primate models for neuroscience research

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The recently developed new genome-editing technologies, such as the CRISPR/Cas system, have opened the door for generating genetically modified nonhuman primate (NHP) models for basic neuroscience and brain disorders research. The complex circuit formation and experience-dependent refinement of the human brain are very difficult to model in vitro, and thus require use of in vivo whole-animal models. For many neurodevelopmental and psychiatric disorders, abnormal circuit formation and refinement might be at the center of their pathophysiology. Importantly, many of the critical circuits and regional cell populations implicated in higher human cognitive function and in many psychiatric disorders are not present in lower mammalian brains, while these analogous areas are replicated in NHP brains. Indeed, neuropsychiatric disorders represent a tremendous health and economic burden globally. The emerging field of genetically modified NHP models has the potential to transform our study of higher brain function and dramatically facilitate the development of effective treatment for human brain disorders. In this paper, we discuss the importance of developing such models, the infrastructure and training needed to maximize the impact of such models, and ethical standards required for using these models.

nonhuman primate | primates | genetic engineering | CRISPR | disease models

The application of genetic engineering technologies, from basic research in animal models to clinical applications in cancer therapy, has revolutionized biomedical research, including neuroscience research. Until recently, the use of these technologies has been limited mostly to rodents and other lower model organisms. While studies using a variety of animal model systems have dramatically enriched our knowledge of molecular, cellular, and systems neuroscience, there has been limited impact on our understanding of higher human brain function, such as emotional states, cognitive function, and social interaction, partially due to structural and functional differences between rodent and human brains. This is also reflected in our modest progress on understanding pathological mechanisms of brain disorders affecting higher brain function, such as psychiatric disorders, autism, and dementia, which in turn has

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contributed to a failure in translating preclinical research in animal models to effective treatment.

The recently developed, highly efficient new genome-editing technologies, such as the CRISPR/Cas system, now make it feasible to expand genetic engineering to many other species (1–3), thus opening the door for generating genetically modified nonhuman primate (NHP) models for basic neuroscience and brain disorders research. Such models are urgently needed if we are to make progress in understanding higher brain function and related disorders in humans. The human brain contains about 86 billion neurons, similar numbers of glial cells (4), and roughly 100 trillion synaptic connections. Unlike cells in many other organs, neurons do not perform their function autonomously, and the nervous system requires numerous local and long-distance connections to form massively complex circuits to process external information, to generate internal states, and to reach decisions and drive actions. Equally important, these circuits are extensively refined by sensory experience during early postnatal life, and certain regions retain extensive plasticity into adulthood that allows for learning and memory formation. Such complex circuit formation and experience-dependent refinement are very difficult to model in vitro, and thus require use of in vivo whole-animal models. For many neurodevelopmental and psychiatric disorders, abnormal circuit formation and refinement are increasingly emerging as central to their pathophysiology (5–9). For example, degeneration of key higher-order circuits underlies disorders such as Parkinson's disease (PD) and dementia (10). Importantly, many of the circuits and even some cell populations implicated in higher human cognitive function and in many neuropsychiatric disorders do not exist in lower mammalian brains, while analogous areas are indeed replicated in NHP brains (11–13).

Genetic engineering can be performed at multiple developmental stages and with a variety of approaches. Germline manipulations are likely the most valuable in modeling human genetic mutations. However, due to the current low efficiency in generating large numbers of mutant founder animals, combined with the long waiting time for sexually mature NHPs to produce offspring, genetic engineering in somatic cells offers useful approaches. For example, regional genetic manipulations in utero or postnatally can be achieved by local injection of adeno-associated virus (AAV) to deliver the CRISPR/Cas system or transgenes (14). The continued improvement of blood–brain barrier penetrant AAV vectors will greatly facilitate the systemic delivery of the CRISPR/Cas system for somatic cell genetic manipulations in the whole brain (15). In addition, ongoing efforts to identify cell type-specific promoters and enhancers will add another layer of sophistication for somatic genetic manipulations (16).

The ability to genetically modify the genome in NHPs to generate cell type-specific tools and disease models has the potential to transform our study of higher brain function and dramatically facilitate the development of effective treatment for human brain disorders. To facilitate the realization of this potential, the Forum on Neuroscience and Nervous System Disorders at the National Academies of Sciences, Engineering, and Medicine organized a workshop in October 2018 titled "Transgenic Neuroscience Research: Exploring the Scientific Opportunities Afforded by New Nonhuman Primate Models." Many of the authors were participants and many of the topics discussed and expanded in this paper were considered at that workshop (17). The workshop discussions highlighted the need for guidelines, and mechanisms to sustain guidelines, for the NHP research community as it transitions increasingly to the use of genetically modified animals to address many complex conditions of the nervous system that have high global societal and economic significance.

The Need for Genetically Modified NHPs in Neuroscience Research

NHPs have long been used in neuroscience research, and some of our most impactful discoveries have been made in NHP research. For example, prior to the emergence of genetic modification technology, NHP studies have led to the development of deep brain stimulation for the treatment of PD (18). In addition, NHP research currently plays a critical role in the development of a promising cell replacement therapy involving implantation of dopaminergic neurons derived from patient skin cells into monkey models of PD (19–21). This approach may provide long-term relief or even a cure for patients suffering from PD. Another example is research into neural prosthetics, which uses neural signals from the brain to control movement of robotic arms (22–25). This type of brain–machine interface research, which is dependent on NHPs, will one day help people who are paralyzed due to brain injury or disease to walk or manipulate their environment again. Moreover, a new Food and Drug Administration-approved gene therapy for retinal degeneration was based on extensive work in macaques (26, 27). Similar approaches are on the horizon for age-related macular degeneration, which is the cause of 8 million cases of blindness in the United States, and affects a component of the retina unique to primates. In addition, research into the neuropharmacology of dorsolateral prefrontal function in macaques contributed to the use of guanfacine to treat a number of disorders in humans, including children with attention-deficit hyperactivity disorder (28–30).

In the past decade, genomics has revealed a wide array of genetic and epigenetic mutations implicated in disorders of the human nervous system, and as a result there is now the opportunity to more specifically model these disorders in NHPs to replicate the complex consequences of neurodegenerative and neurodevelopmental disorders (31, 32). First and foremost, the cerebral cortex, including the prefrontal cortex (PFC), exhibits an enormous expansion and organizational change in NHPs and humans (11–13). Many higher-order brain functions are mediated by circuits involving the PFC, which is of critical relevance to several disabling brain disorders. For example, the dorsolateral PFC (DLPFC), a recently evolved frontal cortex region in humans and NHPs that connects anatomically with many other cortical areas and subcortical regions, plays a critical role in higher brain functions, including the executive control functions of working memory, cognitive flexibility, planning, and goal-directed behavior (33). Circuits such as these are severely affected in a range of psychiatric and neurodegenerative disorders, including schizophrenia and Alzheimer's disease (AD), as well as in aging (34–37). Although traditional techniques, such as lesion studies, have advanced our knowledge of PFC circuits and function, to fully understand its role in higher brain function and its dysfunction in brain disorders, genetic models and tools that allow cell type-specific manipulations (such as cell type-specific Cre lines and promoters) are required. Moreover, research defining the diversity of cells and circuits within the brain is expanding rapidly, in part due to significant programmatic funding from the NIH BRAIN initiative (38). The ability to use this information to generate genetic models and tools will allow neuroscientists to map cortico–cortical and cortico–subcortical connectivity with cell-type specificity and to dissect the function and behavioral output of these circuits. In particular, the use of genetic models with human

mutations along with optogenetic and chemogenetic approaches appears to be promising for identification of pathological changes, but such studies of complex brain circuitry have been and remain limited in nonprimate species.

While comparative studies highlight that many molecular and cellular nervous system components are evolutionarily conserved across species, recent large-scale single-cell transcriptomic and connectomic analyses have also revealed evolutionarily divergent features. Thus, although a variety of model systems, rodents in particular, have played and will continue to play key roles in advancing neuroscience research, a focus on these divergent features will benefit from research in NHPs. Evolutionary divergence at molecular and cellular levels is multifaceted, including changes in the abundance of conserved cell types, changes in the genetic programs of conserved cell types, differential allocation of conserved cell types across locations, and evolution of new cell types (39–43). With these changes come alterations in localized circuit motifs: For example, the divergent structure but analogous function of cholinergic cortical circuits between macaques and rats (44) and the unique glutamatergic layer III microcircuits in the DLPFC involving (NMDAR) NR2B-dependent recurrent excitation in macaques, likely providing the basis for working memory and contributing to our understanding of the vulnerability of this region in schizophrenia (45). Moreover, a recent study (43) identified an abundant striatal interneuron type in primates that has no molecularly homologous cell population in rodents. This cell type constitutes almost 30% of striatal interneurons in marmosets and humans and expresses a unique combination of transcription factors, receptors, and neuropeptides. Rodent studies using cell type-specific Cre lines have revealed the importance of striatal interneurons in regulating motor and action planning, decision making, motivation, reinforcement, and habit formation (46–51). Genetic models and tools are required to manipulate this group of primate-specific interneurons in the striatum in order to fully understand the function of the primate striatum and its dysfunction in brain disorders.

Another critical need for genetically modified NHP models is in research on brain development and neurodevelopmental disorders, such as autism. While many aspects of mammalian brain development are conserved, the primate brain has unique features. For example, during evolution, primates follow different scaling rules, resulting in larger numbers of neurons per unit volume in the mature brain, a feature that is likely important for the superior cognitive function of primates (52). Another distinct feature is the appearance of the massively expanded outer region of the subventricular zone that is critical for the cortical size and complexity in primates (53–56). Since many of the risk genes for neurodevelopmental and psychiatric disorders are expressed during cortical development (39, 57, 58), NHP models will be critical for elucidating pathogenic mechanisms, and rodent models are insufficient as they lack these primate-specific structures.

As gene therapies for monogenic neurodevelopmental disorders are now gaining US Federal Drug Administration and European Commission approval (59–61), it is critical to know whether, and at what stages, the pathogenic process and symptoms are reversible. Compared to rodents, the developmental duration of NHPs is much closer to humans (5 mo of gestation and 12 to 18 mo to sexual maturity for marmosets; 5.5 mo of gestation and 3 to 4 y to sexual maturity for macaques). Rodent models are well-suited for initial studies of these questions, but there are likely to be significant differences in critical periods and adult plasticity between rodents and primates. Indeed, cortical inhibitory interneurons, thought to be critical in higher-order processing and plasticity, have an additional site of origin and distinct morphological and molecular characteristics in primates compared to rodents (13, 43). Already, recent neuroimaging studies in marmosets have revealed marked heterogeneity in the development trajectories of functionally distinct regions of prefrontal and anterior cingulate cortices (62). The ability to answer these important questions in genetically modified NHP disease models will further define developmental windows best-suited for effective intervention. At the other end of the age spectrum, there are similar advantages in genetically modified NHP models (63–65). Studies of aged NHPs have shown that the DLPFC is uniquely vulnerable to aging, and synaptic pathology in this region leads to precisely the same kinds of cognitive decline seen in aged humans (33). This region is also highly vulnerable in AD. In order to understand how AD risk genes contribute to vulnerability of this region in human aging, NHP models with risk alleles and causative familial mutations for AD will be essential.

Studying neuropsychiatric disorders in animal models presents a different level of challenges. Many of these disorders involve cognitive impairment, emotional dysregulation, and social deficits, all of which have primate-specific features and heavily involve unique functions of the PFC. For example, in humans and other primates, visual cues have paramount importance in shaping social behavior, attention, memory, and many other aspects of cognition. In contrast, rodents primarily use olfaction and somatosensory cues, making translation to humans much more difficult. Unfortunately, current clinical diagnosis is based purely on assessment of symptom clusters due to the lack of biomarkers. Numerous studies in various animal models have significantly advanced our understanding of the biological function of risk genes for psychiatric disorders and the circuits involved in different aspects of abnormal behaviors. However, these advances have not been successfully translated into effective treatment. There are many possible reasons that may contribute to these translational failures, including the lack of biomarkers for objective efficacy assessment and a focus in animals on subcortical networks, despite the fact that major alterations in cortical function are found in neuropsychiatric disorders. Since the neocortex is proportionally far larger in humans and contains neurons with primate-specific molecular and cellular properties (39, 43, 66), the lack of animal models that have cortical development, specialization, structure, and physiology similar to humans is considered a key bottleneck for dissecting neuropathology and developing effective treatment for these brain disorders (67). Lesion studies are constrained spatially and temporally, and can only represent a small component of a system-wide genetic brain disorder. Recent large-scale genetic studies have identified many risk genes for various psychiatric disorders (68–72). Genetically modified NHP models, with much closer brain structure, function, and behavior to humans than rodents, may significantly improve translatability of preclinical studies as well as facilitate the development of biomarkers that accurately mirror biomarkers available from human studies. With respect to the growing field of gene-modification therapy in the clinical neurosciences, there is increased demand for accurate and more precise reproduction of genetic disorders, so that short- and long-term consequences can be identified to ensure safety for human translation.

The Responsible Use of Genetically Modified NHP Models

While it is clear that genetically modified NHP models have great potential for utilization in translational neuroscience, these unique

animal models must be well-justified for each application, both by grant review panels and by local veterinary leadership and Institutional Animal Care and Use Committees in the United States or equivalent bodies internationally. We suggest that projects must meet at least one of the following criteria:

1) Have a clear scientific understanding and justification that an NHP model is the best way to address an important question with respect to neural specialization, such as studying retinal disorders, coordinated reaching and grasping movements of the arm and hand, or PFC mechanisms related to executive function.
2) Have clear evidence of failure using other models to address important basic or translational questions. For example, rodent models of the neurodevelopmental disorder fragile X syndrome do not conserve the CGG repeats-mediated DNA methylation and gene silencing present in humans (73), and already several clinical trials based on rodent studies have failed (73, 74).
3) Have a history of success with other models, suggesting that an important basic or translational question might be answered, but there are clear reasons to think that they need to be validated in the NHP model to more effectively guide human applications. Examples include defining critical developmental windows or gene-therapy doses for effective treatment of neurodevelopmental disorders.

When at least one of these criteria have been met, another important aspect of NHP research is to minimize the number of animals through collaboration and data sharing. Collaboration should rise to the level of convergence on the animal model from multiple disciplinary perspectives, such as when endocrinologists, immunologists, imaging scientists, behavioral neuroscientists, electrophysiologists, and cellular neurobiologists all obtain data from the same cohorts of monkeys. Each dataset can inform the others such that an integrated scientific understanding may become superior to what could emerge through targeted investigations alone.

Increasing reproducibility is critical for all areas of biological science and can contribute toward decreasing the number of animals used. This is particularly important for NHP behavioral studies due to the heterogeneity in genetic background, which may lead to more variability in behaviors. Developing robust, automated systems for tracking and analyzing behavior in genetically modified NHPs will be important for objectively assessing species-specific normal behavior and disease phenotypes (75), and for sharing data across laboratories as well. Finally, it will be important to use sufficient numbers of animals in each experiment to ensure adequate power for a sound conclusion. Underpowered studies will inevitably lead to ambiguous results and the necessity to repeat experiments. Biostatistical paradigms currently used in human clinical research may prove especially useful when working with small numbers and a high degree of individual variation also present in genetically modified NHP studies (76, 77).

Ethical Considerations with the Use of Genetically Modified NHPs

Responsible use also requires ethical use. Ethical issues are important in the creation and use of genetically modified NHPs for research purposes, as they have long been with all research with nonhuman animals. Research with nonhuman animals is justified when one can reasonably expect sufficient benefits to humans or benefits to science—the latter often having unforeseen benefits for humans and potentially other animals—to justify the risks or harms to the nonhuman animals. But not all nonhuman animals are the same. NHPs hold a unique position, both because of their close phylogenetic relationship to humans and from their often sophisticated and complex behaviors and cognition, one that has generated much discussion (78), as well as standards proposed by primatologists (79, 80). Correspondingly, animal welfare laws and regulations worldwide weigh research risks or harms to NHPs more heavily than those to mice, let alone simpler organisms, such as *Caenorhabditis elegans* or *Drosophila*.

Research with genetically modified NHPs will, at least in some cases, harm, or risk harm, to the animals. This is especially likely to be the case in modeling some of the most burdensome human neurodegenerative and neurodevelopmental disorders that remain without a cure or even a disease modifier. For example, models of human diseases such as AD and related dementias, autism spectrum disorder, or PD, would be expected to have similar courses to the human patient populations when replicated in the NHP brain. It is precisely this ability to replicate a more complete lifespan manifestation of the human disease that is the unique contribution of genetically modified NHPs. Not dissimilar to research in wild-type NHPs, modern medical science acknowledges the justification of treatment discovery in nonhumans as a reasonable effort to alleviate the suffering of humans. Brain disease causes enormous suffering among humans, and is one of the highest causes of morbidity and mortality worldwide. Not only is the toll enormous—not just on the patients but on their families and friends—but progress in ameliorating, let alone preventing or "curing," these conditions has been frustratingly slow for the last few decades. Rodent models, human ex vivo tissues, human experiments, and computer modeling have each failed to create sufficient progress in many of these conditions. It is in this context of continuing limitations of existing research approaches that research with genetically modified NHPs must be considered. Given the societal burden of neurological and psychiatric diseases worldwide, it is arguably unethical to refrain from performing research that holds promise for understanding and treating these tragic disorders (81).

As indicated above, research with genetically modified NHPs is not fundamentally different from earlier, and existing, research with wild-type NHPs, but the consequences of the modifications to the NHPs will need to be monitored carefully, whether the animals were modified to be disease models, to carry a common human genetic variation, or to have another genetic modification. In the case of novel phenotypes, the researchers, veterinarians, and animal caretakers may need to be especially alert to the presence, and absence, of evidence of suffering by these somewhat novel animals.

While these welfare concerns must be kept front and center, genetically modified NHPs clearly have potential to provide much improved models of human disease. A major advantage of a given manipulation in NHPs is that it will be more similar to humans than the same manipulation performed in rodents or other phylogenetically more distant animal models, not only due to similarities in brain structure and function across human and NHPs, but also due to similarities in bodily systems that interact with the brain, such as the endocrine and immune systems.

Institutions that engage in research with genetically modified NHPs will need an institutional culture that emphasizes the responsibility of ensuring a high level of welfare, the highest scientific standards, appropriate staff training, as well as a robust regulatory system overseeing such research. National primate research centers will be critical in providing training and country-specific regulatory guidance for academic and industry-based

research centers. Given the potential importance of genetically modified NHPs for biomedical research, an international effort is needed to harmonize ethical, legal, and regulatory approaches to ensure responsible application of these technological advances globally, and promote free international dissemination of scientific and methodological advances, as well as of genetically modified animal lines. Depending upon the country, the optimal setting for this kind of research will vary based on expertise in transgenic techniques, as well as the assessment, care, and treatment of the relevant species of NHPs (see Resources and Infrastructure Required for Neuroscientific Study of Genetically Modified NHPs). Regardless of setting, it will be essential that similar ethical and regulatory codes be adopted internationally as research moves forward with genetically modified NHPs.

It should be noted that the "three Rs"—replace, reduce, and refine—which are the guiding principles in research with nonhuman animals, will need to be applied carefully in this context of genetically modified NHPs. The possibilities opened by genetic modification of NHPs may in some specific instances actually lead to more, not less, use of NHPs in research. Any short-term increase needs to be justified by greater potential benefits to humans or science, but the goals of minimizing the numbers of animals used and the risks and harms to which they are exposed will still apply.

Public Communications Issues

Research such as genetic modification of NHPs raises a sensitive issue involving public communication. While there is compelling demand for cures and new model systems from patient advocacy groups around many complex brain disorders, communication about the reality of genetically modified NHP research must also take into account the fact that there are numerous fictional references in classical and scientific fiction literature and film regarding chimeras between humans and other nonhuman primates. Public education needs to be more extensive about brain biology in general, including the major differences in brain structure and function across the animal kingdom, and the important homologies between human and NHP brains. Greater public awareness of the genetic basis of many brain disorders will assist in conveying the critical role that genetically modified NHPs can play in the search for cures for some of the most burdensome disorders of humanity.

The kinds of focused research platforms currently envisioned have effectively no chance of producing an NHP with anything resembling human consciousness. In fact, transfer of human gene mutations to nonhuman species (almost exclusively rodents) has been performed without substantial controversy for more than 20 y to enable mechanistic studies of human biology and disease that is not possible in living humans for ethical reasons. Similar experiments are now being contemplated and performed in NHPs, not to create human-like creatures, but to take advantage of similarities that naturally exist between the cell populations and circuits in monkey and human brains, enabling more precise modeling of human disease and understanding of human biology.

The general public may not be aware of this background. Popular concerns involving fictional accounts of human–nonhuman chimeras (e.g., *The Island of Dr. Moreau*, *Planet of the Apes*), even when unjustified, may slow or even stop potentially beneficial research. Individual researchers, scientific institutions, and "science" more generally must be open, transparent, and clear about what they are, and are not, doing with these NHPs, and why. Fortunately, extensive efforts toward a transparent and fact-based communication on NHP-research in Europe (www.labanimaltour.org; www.dpz.eu/en/unit/about-experimental-animal-research/animal-experimental-research.html; http://www.tierversuche-verstehen.de/affen/), and more recently in the United States (https://nprcresearch.org/primate/; https://www.brainfacts.org/In-the-Lab/Animals-in-Research) and in Japan (https://www.pri.kyoto-u.ac.jp/research/sisin2010/guideline_ver3_20170817.pdf), provide best-practice examples that await more widespread adoption and hopefully help to overcome all-too-frequent hesitancy from institutional leadership. Statements from patient advocacy groups as well as veterinary experts will be essential complements to public educational materials on the topic.

Resources and Infrastructure Required for Neuroscientific Study of Genetically Modified NHPs

Generating genetically modified NHP models requires significant resources and expertise above and beyond the already rigorous conditions required for wild-type NHP research. Therefore, it is critical for scientists to share these models nationally and internationally to maximize their value and to avoid unnecessary duplications. A consortium focusing on planning and coordinating the efforts in this important frontier could provide regular guidance to the community internationally. In the United States, there are seven National Primate Research Centers, all of which receive funding from the NIH and breed NHPs. These facilities have a mandate from the NIH to reach out to veterinarians and researchers throughout the country and facilitate their efforts to move into NHP research. There are similar centers in other countries, such as the Germany Primate Center in Göttingen, Germany; the Kunming Primate Research Center of the Chinese Academy of Sciences in Kunming, China; the Shanghai International Primate Research Center, China; the Primate Research Institute of Kyoto University in Inuyama, Japan; and the Central Institute of Experimental Animal in Kawasaki, Japan. All of these primate research centers have great expertise in primate neurobiology, as well as specialized veterinary care and housing for complex phenotypes. Some of these centers also have the required expertise in reproductive biology and developmental biology to help generate such models. While some gene-editing approaches will be directed at the germ cell line to develop a reproductive line of affected NHPs, other approaches will be directed at postnatal animals (e.g., somatic cell editing, vector delivery of mutated genes), and in both cases phenotypes will emerge that require highly specialized and expensive housing and health care that will not be available at most university settings. For example, some of the primate research centers also have highly naturalistic group housing for monkeys, which may be important for certain phenotypes.

While several of the primate centers house neuroscience laboratories with cutting-edge resources, many of the pioneering laboratories that contribute to the field by developing genome-editing technologies, artificial intelligence-supported behavioral tracking systems, and novel optogenetic and chemogenetic tools for circuit manipulations may not be available at a primate research center but are distributed across dozens of universities and research institutions. Thus, we envision a future that enhances close collaboration between universities and primate research centers to move the field forward. The larger primate centers can play a key role in the development and phenotypic analysis of the complex models likely to emerge from these approaches. These centers could also facilitate access to these unique models, as well as NHP expertise to the whole neuroscience community through breeding, distributing, data-sharing, training, and collaboration.

Once an NHP model is developed, establishing a breeding colony at a primate research center for distribution is likely to be critical, since few university laboratories will have the resources and expertise to breed and distribute them. In some cases, it may be more practical to move the genetically modified NHPs to primate centers, whereas in other cases sperm could be provided and the models would then be developed on site (e.g., international collaborations).

Once a transgenic colony is established, researchers could use a combination of transporting the NHPs to laboratories and transporting investigators to primate research centers to pursue particular projects. Funding will need to be identified to develop large-scale mechanisms for such broad collaborative efforts. Under pressure from animal rights organizations, many national and international airlines have banned transport of research animals on their flights. These policies are deeply misguided because they delay critical disease-related research as described in this paper, and because they place research animals at greater risk from long land journeys by truck in contrast to safe, rapid transport by air. International scientific organizations should work with major airlines to reverse the destructive effects of these policies.

On the international level, China and Japan have identified the development of genetically modified NHPs as a national research priority and have increased their investment in similar centers, with marmosets a particular focus in Japan (82) and macaque monkeys in China (83). Both China and Japan are highly committed to applying gene-editing and other genetic tools to NHPs to develop animal models of disease (84–87). In Japan, marmoset models and research are a major part of Japan's Brain/MINDS Initiative and are provided with initial funding for 10 y to ensure long-term planning and execution. This sustained long-term funding allowed the establishment of a highly successful platform for genetic engineering (88–90), an MRI-based, 3D digital atlas of the marmoset brain (91, 92), which will provide a framework for collating and registering marmoset data across the entire Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) project (https://www.brainminds.riken.jp/atlas-package-download-main-page/reference-atlas-data).

China has invested significantly in developing technologies in genome-editing in NHPs and has made dramatic progress in the past few years (93–102). The China Brain Project is expected to provide 15 y of generous funding to focus on the neurobiological basis of cognition and brain disorders with emphasis on the use of genetically modified macaque monkeys, clinical research, and application of artificial intelligence technologies (84). In the United States and Europe, there have not yet been concerted efforts to specifically fund the development of genetically modified NHPs, and correspondingly, the efforts to develop genetically modified NHPs have been on a local scale without strategic funding on a national level.

To take best advantage of powerful NHP models of disease as they emerge, an international consortium will be required. In particular, where restrictions exist on the international sharing of materials and animals, a governmental commitment is needed. Part of such an effort should also be centered on training the next generation of investigators, veterinarians, and technicians that will be required to develop and sustain the large-scale effort needed to reap maximal translational impact from genetically modified NHP models of human brain disorders. Furthermore, funding mechanisms need to recognize the slow pace of this line of research and commit long-term funding with intermediate milestones for measuring success.

Training the Workforce

Biomedical research with NHPs is practiced by a relatively small number of highly trained specialists, both within major primate centers and distributed among specific universities that are home to primate laboratories. NHPs comprise less than 0.5% of the research animals used in the United States, for example, and the number of researchers involved in these studies is correspondingly small. The modest use of NHPs reflects the expense and technical challenges of working with these animals, along with the heightened regulatory scrutiny associated with these species. In addition, research with NHPs—especially research involving genetic manipulations—is much slower than with other species because of long gestation and reproductive maturity times, leading to fewer total offspring born over the lifetime of a typical female.

In light of the foundational role that NHP studies are projected to play in basic neuroscience research, and the promise of genetically modified NHP models to address neurological and psychiatric disorders, it is imperative to maintain the vitality of a skilled workforce for NHP research at all levels, from animal husbandry and technical support staff to doctor of philosophy- (PhD), doctor of medicine- (MD), and doctor of veterinary medicine (DVM)-level researchers. Specialized skills are necessary at each of these levels (103), and good workforce training is thus a high priority for any research enterprise involving NHPs.

Training needs for neuroscience research involving genetically modified NHPs are highly diverse, reflecting the diverse use of NHPs in laboratories. These training needs can be usefully grouped into six areas. Only the fifth of these areas is a direct result of moving to research with genetically modified NHPs, but all six will be essential to the success of that research.

1) Colony maintenance: Research institutions must meet federal and institutional standards for housing, general health maintenance, and psychological welfare of each animal. Training related to care and welfare of some (but not all) lines of genetically modified NHPs may require special effort due to behavioral phenotypes and special needs that mimic human neurological or psychiatric disease.
2) Noninvasive behavioral studies: Skilled research staff must be carefully trained in quantitative measurements of behaviors as they play a critical role in neuroscience research with NHPs, both for readout of circuit function and dysfunction and for measurement of disease-relevant phenotypes. This must also include ethologically appropriate behavioral management of NHPs as well as specific behavioral measurements relevant to a particular research program. Research staff must be well trained in statistical and modeling techniques that are necessary to extract maximum insight from experimental measurements of behavior in order to assess the similarities and differences between human patients and genetically modified NHP models of human neurological and psychiatric disorders.
3) Non- or minimally invasive physiological studies: As in human subject research, noninvasive techniques include magnetic resonance imaging for neuroanatomical and functional studies. Minimally invasive techniques include positron emission tomography, electroencephalogram, electromyography, and evoked potentials for assessing physiological function. Similar to clinical applications, these techniques require months or years of specialized training of personnel who make the measurements and analyze the resulting data. This category is important because these measurements—both in normal and

genetically modified NHPs—can be compared directly to human data obtained by the same techniques.

4) Invasive physiological studies: A great deal of incisive and innovative neuroscience research occurs in this category because new technologies are enabling researchers to gain unprecedented insight into neural circuit function by monitoring and manipulating cellular-level activity of many hundreds or thousands of neurons simultaneously. These studies are likely to play key roles in dissecting circuit mechanisms of disease-relevant abnormal behaviors in genetically modified NHP models. Optical imaging, multielectrode recording, and optogenetic and chemogenetic manipulation are but a few examples. Studies in this category require years of training for skilled experimentalists who surgically implant animals with optical or electrophysiological devices, make measurements, and analyze the massive datasets that are now being obtained for the first time. These experimentalists are typically PhD, DVM, and MD researchers or trainees, often with additional years of postdoctoral training.
5) Transgenic technologies: Creating genetically modified NHP lines is technically demanding and labor intensive. Although TALEN and CRISPR technologies are enabling more efficient modification of the germline genome, each transgenic embryo must still be injected with reagents and implanted in a female's uterus and carried to term through the normal gestational period, ~5 mo in marmosets and 5.5 mo in macaques. Each stage of this process—genome modification, surgical implantation of embryos, assessment and care of pregnant females, and assiduous husbandry and further breeding of precious transgenic offspring—requires highly skilled scientific staff. Few institutions can commit the financial, space, equipment, and staff resources necessary to run a high-quality genetically modified NHP program, and it is nearly inevitable that regional or national facilities will be needed to support the need for genetically modified NHPs for basic and disease model research (see Resources and Infrastructure Required for Neuroscientific Study of Genetically Modified NHPs).
6) Ethical use of genetically modified NHPs: Genetically modified NHPs raise somewhat different ethical concerns than those of most laboratory animals, including wild-type NHPs. Those who use or care for genetically modified NHPs in research must be trained to be sensitive to those issues, and adhere to ethical principles around their care and use.

In the United States, the National Primate Research Centers are ideally positioned to sponsor the training and to set new standards as new technologies are developed. To do this, the primate centers will require additional financial resources and expertise. Even after lines of genetically modified NHPs are established at central locations, there remains the further hurdle of disseminating genetically modified animals to laboratories in universities and other research institutions for state-of-the-art experimental measurements described above, and the National Primate Research Centers could also play a key role in this effort.

Concluding Remarks

The high level of similarity between NHPs and humans makes research with genetically modified NHPs a vital, albeit small, component of neuroscience research and fulfils the increasing need for research of more direct relevance to human health. Genetically modified NHPs will likely occupy a prominent position as we try to make sense of the growing knowledge of the genetic and molecular underpinnings of disorders that result in wide dysregulation of higher-order brain circuits. They offer unique promise for elucidating normal and diseased nervous system functioning in primates, creating new opportunities for understanding, diagnosing, and treating human neurological and psychiatric diseases that represent one of the major human health burdens worldwide. Because of the special nature of NHPs compared to other laboratory animals, and the likely higher levels of public concern about this work, researchers must both adhere to high ethical standards in the creation and use of genetically modified NHPs and must clearly communicate the reasons for, and the nature of, this research to the public.

Neuropsychiatric disorders affect one in five humans at some point in their lifetime (104, 105). While information relevant to future treatments and potential cures for many disorders is emerging from rodent and even brain organoid models, substantial progress in the more challenging areas of human cognitive, neurodevelopmental, and neurodegenerative disorders is likely to require research with genetically modified NHP models. Advanced functional monitoring of the human brain is revealing numerous intricately organized, widely distributed networks whose activity correlates with human behavioral, emotional, and cognitive states (106, 107). Traditional experimental approaches, even in the NHP brain, are inadequate (by themselves) for understanding these networks and how they are dysregulated in disease. Genetic modification allows for a more precise replication of the human disorders, and NHPs offer the substantial advantage that behavioral, anatomical, and physiological metrics of brain function can most closely resemble the actual clinical diagnostic tests performed on humans. In addition, biomarkers in serum and cerebrospinal fluid of NHPs can be obtained that most closely mirror such measurements in humans. Indeed, human clinical research designs, paradigms, and biostatistical analyses may be increasingly useful in the future to accommodate the high level of individual variation and small sample sizes in the field of genetically modified NHP research (76, 77).

Since NHP work is long-term by its nature, especially when creation of genetically modified NHP lines is involved, its success will require durations of financial support that are longer than that typically available, because of the fact that lifespan outcome measures will be essential to assure greatest value. New models of long-term financial support are needed from both government and private sources in order to secure career paths for talented scientists who embark on this voyage of discovery. In addition, complex, distributed collaborative teams will be needed to extract multiple interactive datasets from these valuable animals, and this will likely require new funding mechanisms.

Despite the challenges discussed here, we see genetically modified NHP models as essential for the future of neuroscience research. Without NHP models, our hopes for understanding higher brain function and for developing preventative measures, treatments, and cures for brain disorders will be severely delayed and some may be dashed.

Data Availability. There are no data underlying this work.

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