Disease Models & Mechanisms 3, 15-19 (2010) doi:10.1242/dmm.004861
Published by The Company of Biologists 2010

Primates as a model for research

Despite the political, ethical and financial complexities, there are some human diseases that researchers feel can only be understood by working with non-human primates. **Nicole Garbarini** investigates.

When choosing a model organism to study disease and disease mechanisms, scientists rarely base their selection on basic biology alone. External influences such as the availability of collaborators, facilities and staff all factor into this decision, as do the personal preferences of the investigator. Several, small, cost-effective animal models are available that can provide robust and meaningful data that are relevant to human disease phenomena. Organisms with short reproductive cycles, a rapid turnover of generations, and exquisite genetic tractability can rapidly provide statistically significant results. However, one animal model that has absolutely none of these qualities remains a mainstay in human disease research: the non-human primate. Working with non-human primates not only presents many technical challenges, but also unique financial and ethical challenges. Weighing these unique challenges against the benefits for human disease research presents a paradox for scientists and research institutions: whether to embrace, or abandon, primate research. However, several aspects of primate biology keep researchers persevering through financial and political challenges.

Research under pressure

All animal research receives some level of criticism from animal rights advocates, with protests escalating the further one climbs the phylogenetic or evolutionary tree. However, in the last few years, heightened threats and assaults from animal rights activists have waged a war between 'primate liberation' groups and the scientists who perform primate research. In 2007 and 2008, acts of violence against non-human primate researchers most notably included a string of attacks on UCLA (University of California, Los Angeles) and UC (University of California) Santa Cruz professors, who were targeted at their homes with fire-starting devices and other threatening actions and materials. In the wake of such incidents, the scientific community has increasingly responded to the mounting attacks on scientists and their families, calling for an end to the violence and for increased protection [for examples, see 'When less is not more' in *Nature Medicine* in August 2008 (Nature Publishing Group, 2008); 'It is time to take a stand for medical research and against terrorism targeting medical scientists' in *Biological Psychiatry* in 2008 (Krystal et al., 2008); and 'Against vicious activism' in *Nature* in February 2009 (Nature Publishing Group, 2009)].

Other animal rights supporters have taken non-criminal and legislatively savvy methods of fighting against primate research. For example, in the USA, the well-known animal rights group PETA (People for the Ethical Treatment of Animals), which opposes all medical research on animals, offers clerkships in animal law – a law specialty that is now taught in many law schools worldwide. PETA hires undercover investigators to work in medical research labs and record lab experiments through journals, photographs and videos in order to expose 'the shocking reality of animal suffering', and to find violations of animal care protocols. One such investigation at the Oregon National Primate Research Center gathered enough evidence to result in a United States Department of Agriculture (USDA)-led investigation and an official USDA warning against the facility.

Many signs point to the fact that such pressures, combined, have been effectively influencing researchers, research institutions and legislators to limit primate research. In the USA, Indiana University, which only had two primate labs but boasts a robust medical research program, ended all primate research campus-wide in 2008. In an e-mail interview, researcher Preston Garraghty stated, 'The decision here destroyed my research career on this campus.' Garraghty's work on synaptic plasticity, learning and memory uses squirrel monkeys as a model system. 'The President of this institution, in rendering his unilateral decision, encouraged me to find an alternative model system', Garraghty stated. 'That's like asking a developmental psychologist interested in, say, child language acquisition, to use song birds. The President's ignorance, naiveté and stupidity is astounding.' As a well-established researcher, Garraghty describes switching models in the late stage of his career as 'verging on impossible.' 'It is hard enough to secure external funding to support research for which one is well known', Garraghty said, and he plans on continuing his research through collaborations at other universities.

In Europe, limits on non-human primate research have been continually contested in government. In late 2008, European Parliament draft legislation proposed new restrictions that scientists say would effectively ban most non-human primate studies. However, in May 2009, the directive was revised to allow greater flexibility for researchers, in that basic science research can be performed on monkeys, and not just studies of 'life-threatening' disease.

Another pressure facing primate research is the cost of animal care. In an interview with Jon Kaas, a Vanderbilt University researcher, Kaas describes primate research as not inherently more costly than rat or mouse research, since primate researchers keep relatively few individual animals because fewer animals are usually used in experiments. However, high expectations are set for primate care, and most universities want facilities that go above and beyond the minimum care requirements. For example, primate centers have vastly increased their staff, including full-time vets and enrichment officers/coordinators (to make sure that primates get adequate socialization and stimulation). Kaas notes that primate facilities employ

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five times more staff members than a decade ago. Although official regulations have not changed much, the perceived requirements are for an increasingly higher standard of facility. Asked about why universities may be increasingly critical of facility conditions, Kaas suggests that this increased attention helps to prevent any devastating interruptions in research. '[Universities want] to prevent any mistakes or violations so that the institution won't receive a severe fine or, at the worst case, suspend all research. Research is a multimillion dollar enterprise for a university.'

Kathy Grant, a researcher at the Oregon National Primate Research Center, whose own research career has utilized both primates and rodents, echoes this sentiment. 'I think a lot of universities now do look at their research portfolio and wonder at what level is this research no longer viable, if you will, from the financial point of view,' Grant noted. She also adds that animal rights is a big issue for public relations of colleges and universities, given how it may affect financial contributions and public opinion. 'There is concern as to whether or not alumni associations are comfortable or not with particular research programs. This isn't only about monkey research but other potentially controversial science, such as stem cell research, too.'

Likewise, Kaas notes that activist interference with research can add to the cost of primate research, both in terms of money and time. 'For a tenured professor, it might not matter as much since he/she won't lose his/her job, but for a younger faculty member who is just getting started, interruptions in research can result in not getting tenure.' Citing the case of a colleague in Iowa, he noted that, if such a situation affects the research of an investigator towards the start of their career, some institutions would give an extension of time before tenure review.

Fig. 1. A saddleback tamarin foraging for artificial fruits. Figure reproduced with permission from *J. Exp. Biol.* (Smith et al., 2003).

Reasons to continue using primates as animal models

Regardless of political and financial pressures, researchers continue to perform research on non-human primates because, as model organisms for studying disease, non-human primates provide unique insights that cannot be studied in lower-order organisms (Fig. 1). Additionally, non-human primates offer opportunities for disease research that cannot be provided by examining disease in humans. Several areas of medicine benefit from non-human primate research, and range from cardiovascular and metabolic disease to infectious diseases, autoimmune disease or pulmonary disorders.

The field of neuroscience is one such area of biomedical research that utilizes non-human primates for both basic science research as well as disease research. Intelligence, cognition and emotion – the same characteristics that raise ethical concerns for primate research – are the same faculties that are destroyed in some of the most puzzling diseases of our time, such as Alzheimer's disease, and drug and alcohol addiction.

Marina Emborg, Assistant Professor of Medical Physics at the University of Wisconsin-Madison, co-authored a 2008 *Lancet* article discussing the contributions of non-human primates to translational and basic science research in neuroscience (Capitanio and Emborg, 2008). Emborg and her co-author, John P. Capitanio, describe how non-human primate studies help advance basic disease pathology studies. Primate studies have had an important role in the development of clinical treatments, such as stem cell and gene transfer therapies, before Phase I patient trials. Additionally, Emborg and Capitanio cite studies on primates that have contributed to understanding neurobehavioral outcomes that result from gene-environment interactions. The authors focus on Parkinson's disease, Alzheimer's disease, neuroAIDS and stress-related disease as key neurobiological illnesses that have benefited from primate research.

Emborg's own work focuses on modeling Parkinson's disease in aged monkeys. In an interview, Emborg added that neurotoxin models of Parkinson's disease have contributed greatly to the development of therapies for humans. Deep brain stimulation, for instance, was tested on animals that were administered the drug MPTP, which causes parkinsonian symptoms in both animals and humans.

'One reason that we use primates is because their behavior is much more complex, so the answers we are going to find are much closer than those we will find in humans', Emborg notes. She also describes how the bigger volume of primate brains, as well as the complexity of their brain structures, is another key factor that supports the use of primates as model organisms, particularly when studying the basal ganglia, which are the structures that are affected by Parkinson's disease. 'In the human or monkey, the caudate and putamen are well delineated and separated by the internal capsule. If you look into the brain of a rodent, the clear delineation is not

there. When you are testing therapies, for instance using stem cell transplants or gene therapy as an example, you have to take into account the volume of the space, as well as the internal capsule, as it might prevent you from administering the therapeutic molecules or cells in one injection. In primates and humans, you will probably have to target the caudate and putamen separately.'

Kathy Grant has been using several different animal models throughout her research career to study the brain and behavior in regard to addiction disorders. Her graduate school research utilized rat models of alcoholism, and her postdoctoral work at the University of Chicago used monkeys to study drug abuse. From there, she has been at several different private and government institutions researching, and consulting on using, mice, rats and monkeys as disease models. Her current work focuses on behavioral pharmacology; drug and alcohol abuse; and addiction studies. Now located at the Oregon National Primate Research Center, she not only works with monkeys, but also has a rat and mouse lab as well.

'The animal that I use is dependent on the question that I'm asking,' says Grant. 'I consider which species is going to give me the best answer to the question that I am investigating.'

In studying alcohol abuse, she says that the differences between rodents and primates are large enough to necessitate using non-human primates to accurately study human drinking behaviors. 'The amount of alcohol consumed by some individual monkeys rivals the amount that human alcoholics will drink, and we have trouble showing that kind of intake in mice and rats. You can show it, but you have to take measures such as selectively breeding for high alcohol preference, depriving the rodents of food, making them dependent on alcohol over repeated cycles, and increase intake over time,' says Grant. 'The topography of being able to gulp down your drinks might be one signature of a primate. We can fill our buccal cavities with a lot of fluid and swallow it right down, whereas rodents are like dogs in that they lap at water to drink.'

A large part of Grant's work involves interactions between alcohol and the endocrine and reproductive systems, for instance in investigating fetal alcohol syndrome. Her work also examines the impact of drinking or abusing alcohol at different stages at life, for example, while children and teenagers are developing through puberty. The high similarity between humans and primates is a key component to these studies, not only because of the neurological correlates, but also because of the similarity between hypothalamic-pituitary-adrenal (HPA) axis responses, and menstruation and pregnancy. The fact that the longevity of non-human primates is comparable to humans is also important.

'Neurosteroids are really important in sleep regulation, and we know that sleep is really dysregulated with drinking [alcohol], especially heavy drinking,' says Grant. 'We also know [sleep] remains dysregulated even when the alcoholic or alcohol abuser is abstinent, and that it is one of the more prolonged withdrawal symptoms. So this is a whole area of research where [scientists] would want an animal model that is long-lived.'

When asked why human epidemiological studies were not feasible for this work, Grant used examples from the alcohol field to discuss the types of problems that are commonly encountered when studying substance abuse in human subjects.

'Humans are really, really lousy at being accurate about telling you how much they had to drink. That could be because they don't remember, because it's in a social setting and there are many things going on and they just don't know, or because they are trying to hide their drinking. They may even be in trouble with the law and trying not to drink, so there are a lot of reasons why humans are not accurate,' said Grant, '[Using primates,] we know exactly how much these animals not only drank yesterday, but also last week and last year. We know exactly what their nutritional status is, which is another really difficult thing to track in humans. Also, human alcoholics have a high comorbidity with smoking. Trying to separate the effects of alcohol in studying markers like cognitive performance, and to separate out other factors like cancer, is very difficult.' Furthermore, using primates allows researchers to investigate the true risk of alcoholic drinking and drinking during pregnancy. 'In addressing the question of adolescent drinking leading to a four times, lifelong higher risk of being diagnosed with alcohol abuse or alcoholism, we can't randomize these subjects because it is ethically wrong.' Grant also cited the issue of self-selection in human studies. 'A Wayne State study carried out about 20 years ago says that if a woman stops drinking in her third trimester, there will be a better outcome for her child. Again, this isn't randomized. We want to know what the factors are that determines who can stop drinking before the third trimester, and who cannot.'

In discussing the larger impact of these studies, beyond simply learning about the biology of disease, Grant commented, 'All of this we do, is not just because we want to know, but it is so that we can have accurate public health information out there about what is safe and not safe.'

Jon Kaas at Vanderbilt University is participating in studies of spinal cord injury using primate models. He points out that some studies can utilize rats and mice first, in the early stages of research, but comments, 'Rats won't get us where we need to be in order to understand spinal cord injury in patients.' One key difference in the spinal cord connections is that, unlike in primates, the pyramidal tract, which conducts motor control, and the ascending sensory pathway, which relays sensory information to the brain, are mixed in rats, so you cannot study these pathways in isolation of one another.

Another difference that Kaas notes is detectable on a very macroscopic level, 'Rats don't have hands.' Since his spinal cord injury research includes analysis of hand control, using primates is crucial to determine whether axon regeneration is sufficient to establish hand movements, or whether a brain-directed hand or arm prosthesis will work. Small improvements in movement make a large impact for some spinal cord injury patients. 'Working with the Christopher Reeve Foundation, you see people with different levels of spinal cord injury. Even if [quadriplegic] patients can get a little bit more hand movement, they can do most jobs,' Kaas explained. 'Regaining executive use of the hands and arms is a big accomplishment, allowing patients to do desk jobs, work at computers, and get where they need to go using a wheelchair, which is better than only being able to use/move their mouths. Even if they can get a little bit of recovery, it will make a huge difference in their lives.'

Reproductive biology is another research area with compelling reasons to use primates for human disease studies.

Significant differences exist between primate and rodent reproductive biology, as well as between primates and larger animals that are used in the laboratory, such as dogs and pigs. Many of the key differences revolve around the female reproductive system, including organ shape, the length of gestation, ovulation cycles, and the number of live births (litters versus individuals). In addition, there are many human diseases that are specific to the female reproductive system, such as ovarian cancer. Furthermore, much research revolves around identifying environmental factors that affect oocyte development, or that affect the fetus during gestation.

'I always said that I would never work with anything higher [in the phylogenetic tree] than a mouse,' says Pat Hunt, a researcher of reproductive biology at Washington State University in the USA. Throughout her research career, Hunt combined human studies with mouse model work in order to study oocyte development and genetic quality, specifically the chromosomal changes that occur with advanced maternal age and that lead to an increased incidence of developmental abnormalities. A few years ago, her work took an unexpected twist when her mice were inadvertently exposed to a chemical leaching from newly acquired water bottles. The estrogen-like chemical, bisphenol A (BPA), started to cause abnormalities in the mice – a particularly concerning effect, considering the prevalence of BPA in food and drink packaging such as baby bottles, water bottles, and the interior coating of food and beverage cans.

From this initial observation, Hunt began to pursue a number of different studies to understand the influence of BPA on the reproductive system. 'Everything we do convinces me that this chemical is something we need to be concerned about,' Hunt says. Although she found that the mouse was an excellent model in many respects, she found herself at a critical point where, in order to emphasize the impact of BPA on human health, she would need to expand her studies to other systems. 'We are doing human studies, but we realized that there are a lot of basic questions that we can't address either in a mouse model or by doing studies in humans.'

Hunt explained that such basic questions include understanding how BPA is metabolized, and what specific dosages damage the oocytes. Early data suggest that humans metabolize BPA differently than mice, and that pregnant individuals may metabolize BPA differently compared with non-pregnant individuals. Also, the fundamental differences in the reproductive systems of primates versus mice may influence how BPA affects oocytes, since mice are litter-bearing animals, ovulate multiple eggs at once, and can experience pseudopregnancy.

Some corollary studies have been performed to compare maternal serum BPA levels, and the effects on fetal tissues. However, Hunt points out that these studies only provide a small snapshot of the issue, when in fact it is likely to be a lifelong chemical accumulation process. Since BPA is widely present, it is difficult to calculate chemical exposure based on self-reporting. Additionally, since oocytes are generated during fetal development, it is difficult to directly assess the influence of BPA exposure on oocyte defects because the effect of BPA exposure during fetal development can only be assessed after the subject reaches sexual maturity.

Thus, Hunt is working with colleagues in primate research centers to look at the effects of BPA in rhesus monkeys; different chemical dosages and periods of exposure; exposure during different stages of life; and to study the effects of BPA on the developing ovary and oocytes.

Hunt asserts that this type of research is necessary for commanding serious attention to BPA toxicity. 'We think [mice are] an excellent model in many respects, but critics say that these are only mice, and that we have no evidence that [humans] would respond the same way,' said Hunt. 'I think these studies are really important and need to be done, otherwise I wouldn't undertake them.'

Primate research technology moves ahead

In addition to these inherent biological factors that encourage work in non-human primates, primate animal models are also gaining advantages that formerly only worms, flies and mice could boast. Research on the genetic manipulation of primates continues to advance, demonstrating their potential for genetic tractability in order to model human disease.

In May 2008, Anthony Chan and colleagues at the Yerkes National Primate Research Center in Atlanta, GA, reported another exciting advance toward creating a more human-like animal model of disease. Their paper, published in *Nature*, describes the creation of transgenic monkeys as a model for Huntington's disease, a severe neurodegenerative disorder that is characterized by symptoms such as motor disturbances and cognitive decline (Yang et al., 2008). The report describing the first non-human transgenic primate was published in *Science* in 2001. Chan was also a member of this research group, led by Gerald Schatten, which successfully generated a transgenic monkey (named 'ANDi', a reversed abbreviation of 'inserted DNA') carrying a green fluorescent protein reporter gene (Chan et al., 2001). This initial demonstration of technical ability, and the Huntington's disease model monkeys that were reported in 2008, are the only reports of transgenic non-human primates during the last 7 years. Although it is clear that these are major accomplishments, it is also clear that there are several limitations in the generation of transgenic primates.

In an interview, Chan said that one such factor is the limits on resources. Monkeys require more space and a more enriched environment than rodents, so housing and daily expenses add to the cost. He also noted that coordination with people and facilities can be 'very demanding'.

Chan's group has worked on optimizing techniques to reduce the number of animals used in his research. The initial work that produced ANDi used retroviral vectors for gene insertion, but this newer study used lentiviral vectors that, Chan says, produce close to 100% transgenic offspring. 'With that efficiency, the number of animals involved will be minimized. That's very important to reduce the number of animals and the cost.'

Other limiting factors include finding a surrogate female. Rather than using hormonal synchronization, which may further lessen the chance of a successful pregnancy, the hormonal cycles of females are monitored individually to find a surrogate when transgenic embryos are ready for transfer. 'Sometimes we have embryos that are ready, but we don't have a matching surrogate,' Chan described, 'we are not just dealing with the technique, but also the physiology of the animals.'

In describing precise time differences between mouse and primate reproductive cycles, Chan describes, '[monkeys] don't reach puberty in 4 weeks, they reach

puberty in 3 to 4 years, and the gestation time is 150 to 160 days instead of 21 days [as in the mouse]. These are factors that we cannot change; instead we look for other ways to accelerate the process.'

One such method that Chan's group uses is in vitro fertilization and he noted that, in the future, sperm and eggs from transgenic monkeys can be harvested for assisted reproductive techniques, which will also help to speed up the generation of new transgenic animals for continued research.

Similarly, other researchers are working on new techniques to help make transgenic animal generation more efficient, in terms of both speed and cost. For example, Subeer Majumdar of the National Institute of Immunology in New Delhi, India, studies disease and reproduction in primates, and is developing new methods of transgene insertion with the ultimate goal of using such technology in primate models of human disease.

Majumdar explains that using the same approach in monkeys and mice does not take into account the differences in oocyte production between these animals. In mice, he explains, one sacrificed female mouse can provide up to 30 oocytes, so merely ten mice can provide the 200-300 oocytes that are needed to begin an experiment. From here, approximately 40 to 50 survive pronuclear injection with the transgenic construct, and even fewer zygotes implant successfully in the surrogate female. Furthermore, only a subset of the mouse pups born will be transgenic.

In monkeys, however, Majumdar explains that the oocyte harvesting method is very different. Anesthetized female monkeys are subject to survival surgeries using laproscopic techniques. Only four or five oocytes are available for retrieval, and not all of them survive. Considering the post-injection survival rate in mouse oocytes, Majumdar estimates that up to 80 animals may be needed to generate a starting pool of 300 oocytes.

In order to switch the odds in their favor, Majumdar and colleagues are investigating ways to use males to carry the transgene, rather than injecting DNA into an embryo. His team published a paper in *Nature Methods* describing how they introduce the transgenic construct into the testicular stem cells of anesthetized male mice using electroporation of the testes. Stem cells that successfully take up the transgene produce sperm cells that contain the DNA construct (Dhup and Majumdar, 2008). Majumdar commented that this technique takes less time, does not involve sacrificing any animals, and involves fewer animals because there is no need for a surrogate female. 'Besides the electroporation, the rest of the procedure is natural,' Majumdar commented, 'the mice mate naturally and there is no surrogate mother. The female naturally produces babies, so there is only one place where you interfere – during the insertion of the gene.'

For these reasons, Majumdar sees this new method as very advantageous for making transgenic primates, because it might circumvent the limiting factors that make them difficult to produce. Chan agrees that the result 'sounds promising,' also adding, 'I think it'll be great if it works, but in the meantime we are focusing on the technology that we have in hand, which we believe will be more reliable.'

The road ahead

The future of primate research is moving forward, with new tools to enhance genetic tractability. However, the use of primates in medical research remains a hotly contested issue. Besides the ethics of this research, justifying the costs for staff and enhanced animal care will be important when competing for funding in a tight economy.

Young researchers will have to weigh the costs between the scientific benefits of using non-human primate models and the issues of time, money, ethics and societal pressures. In discussing the careers of trainees who have left his lab, Kaas says that he does see young scientists quit, or become intimidated. Some researchers from his lab never perform research in non-human primates, but prefer to switch to mouse or rat studies. Others do extended postdocs or fellowships to remain in primate research. And even more, Kaas notes with a laugh, scientists are taking another alternative to essentially stay in primate research – by working with humans. 'Fewer places are doing [non-human] primate research, but more places are doing research on humans through imaging centers, because there are facilities for that everywhere.'

REFERENCES

Capitanio, J. P. and Emborg, M. E. (2008). Contributions of non-human primates to neuroscience research. *Lancet* **371**, 1126-1135.

Chan, A. W., Chong, K. Y., Martinovich, C., Simerly, C. and Schatten, G. (2001). Transgenic monkeys produced by retroviral gene transfer into mature oocytes. *Science* **291**, 309-312.

Dhup, S. and Majumdar, S. S. Transgenesis via permanent integration of genes in repopulating spermatogonial cells in vivo. *Nat. Methods* **5**, 601-603.

Krystal, J. H., Carter, C. S., Geschwind, D., Manji, H. K., March, J. S., Nestler, E. J., Zubieta, J. K., Charney, D. S., Goldman, D., Gur, R. E. et al. (2008). It is time to take a stand for medical research and against terrorism targeting medical scientists. *Biol. Psychiatry* **63**, 725-727.

Nature Publishing Group (2008). When less is not more. *Nat. Med.* **14**, 791-792.

Nature Publishing Group (2009). Against vicious activism. *Nature* **457**, 636.

Smith, A. C., Buchanan-Smith, H. M., Surridge, A. K., Osorio, D. and Mundy, N. I. (2003). The effect of colour vision status on the detection and selection of fruits by tamarins (Saguinus spp.). *J. Exp. Biol.* **206**, 3159-3165.

Yang, S. H., Cheng, P. H., Banta, H., Piotrowska-Nitsche, K., Yang, J. J., Cheng, E. C., Snyder, B., Larkin, K., Liu, J., Orkin, J. et al. (2008). Towards a transgenic model of Huntington's disease in a non-human primate. *Nature* **453**, 921-924.