Basic Neuroscience Research with Nonhuman Primates: A Small but Indispensable Component of Biomedical Research

Pieter R. Roelfsema[1,2,3,*] **and Stefan Treue**[4,5,6,*]
[1]Department of Vision and Cognition, Netherlands Institute for Neuroscience, an institute of the Royal Netherlands Academy of Arts and Sciences, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands
[2]Department of Integrative Neurophysiology, Centre for Neurogenomics and Cognitive Research, VU University, 1081 HV Amsterdam, The Netherlands
[3]Psychiatry Department, Academic Medical Center, 1105 AZ Amsterdam, The Netherlands
[4]Cognitive Neuroscience Laboratory, German Primate Centre, 37077 Goettingen, Germany
[5]Bernstein Center for Computational Neuroscience Goettingen, 37077 Goettingen, Germany
[6]Faculty of Biology and Psychology, Goettingen University, 37073 Goettingen, Germany
*Correspondence: p.roelfsema@nin.knaw.nl (P.R.R.), treue@gwdg.de (S.T.)
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Research with nonhuman primates represents a small component of neuroscience with far-reaching relevance that is irreplaceable for essential insights into cognitive functions, brain disease, and therapy. Transparency and widespread information about this research and its importance is central to ensure the support of politicians and the general public.

Two main fields of human endeavor that have propelled mankind forward over the last centuries are engineering and, more recently, biomedical research. Neither our standards of living nor the quality and length of our lives would have been possible without these efforts. Biomedical research has provided us with deep insights into the physiology and anatomy of organisms. But despite the progress, we are far from a complete understanding of humans and other animals in health and in disease. While the likelihood to survive cancer has increased tremendously and patients with AIDS now have a chance for many years of survival after their diagnosis, we still lack a complete understanding of these and many other diseases that would allow for their prevention or a cure. This is even more true for the complex illnesses of the human brain.

A multitude of techniques developed in the last decades underpin the progress that has been made, allowing new insights into the most challenging biomedical questions. Among this tool chest of methods are studies in animals. Here the ethical challenges of weighing the intrusion into the lives and wellbeing of animals against the benefits derived for human patients are complex. Our knowledge-based societies have addressed this conflict by implementing legal and regulatory frameworks, such as the recent Directive of the European Union on the protection of animals used for scientific purposes (http://eur-lex.europa.eu/procedure/EN/197584) and the resulting national animal protection laws, which are built on the broad consensus across science, politics, and society that a certain amount of research on animals is necessary and justifiable. This consensus includes the 3R principles (Russel and Burch, 1959) of Replace, Refine, and Reduce as the shorthand for the three core requirements for animal research, namely to replace animal experiments with alternatives whenever possible, to continuously refine the methodology to make experiments as efficient and of the least possible impact on the animals, and to reduce research using animals as far as possible. Accordingly, animal research represents a minute fraction of the animals used and killed, voluntarily and involuntarily, by human societies. For example, in European countries for every single research animal, about 200–300 animals are killed for human consumption. Of the research animals used, more than 80% are rodents and less than 0.1% are nonhuman primates. Thus, we consume about 500,000 animals for every nonhuman primate in research.

Because of the broad public agreement that some animal research is necessary to ensure human health and medical progress, groups opposed to any animal research have refocused their broad assault onto just "basic" (as opposed to "applied") research and on nonhuman primates (as opposed to the vast majority of other species used). We will therefore focus here on basic neuroscience research with monkeys (nonhuman primates [NHPs]) as a relatively small but essential part of biomedical research. It has provided the basis for groundbreaking discoveries and progress but has also been the focus of very vocal and sometimes violent opposition from well-funded groups waging a campaign against animal-based biomedical research.

Research in Nonhuman Primates Has Elucidated Many Basic Mechanisms Underlying Cognitive Functions

Within neuroscience, the consensus is that research using NHPs has led to a greater understanding of the mechanisms of brain function and many of the processes that underlie brain diseases. One of the fields of neuroscience that has benefitted from NHP research is cognitive neuroscience. Its goal is to understand the causal relationship between neuronal activity and cognitive functions. An important advantage of NHPs as a model for

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Table 1. Example Domains in Cognitive Neuroscience where Research with NHP Provided Decisive Insights

Perception and perceptual organization
Recognition of objects and faces
Attentional modulation of sensory information processing
Storage of information in working memory
Decision making
Sensorimotor transformations
Coding of categories and numerical information
Neuronal representations of reward, punishments, and reinforcement
Motor control (including readout for neuroprosthetic devices)
Mirror neurons
Fate of information that reaches consciousness and that does not

such research is that they can be trained to carry out relatively complex sensory discriminations and motor tasks and can learn to make new associations. Researchers have developed sophisticated techniques that allow recording of the activity of single neurons or small groups of them in various brain structures so that the brain mechanisms for these cognitive functions can be understood. Research with NHPs has identified the functional role of individual nerve cells and brain areas and then taught us that many cognitive functions rely on networks of such areas in the cerebral cortex and subcortical structures (Moore and Armstrong, 2003; Roelfsema, 2006). These networks have evolved differently in animal species. Of all animal models used in neuroscience, the monkey brain is most similar to the human brain (Mantini et al., 2013). Let us consider, for example, the well-studied visual system with its intricate hierarchical structure. Much progress has been made since the groundbreaking work of Hubel and Wiesel, who started the systematic study of neural information coding in visual cortex, leading to their Nobel Prize in 1981. Since their work, we have learned many aspects of the function and connectivity between the many cortical and subcortical areas of the primate brain (Felleman and Van Essen, 1991). This information has been an important guide for our understanding of how sensory brain regions interact with higher brain regions in the parietal, temporal, and frontal cortices and with the thalamus, basal ganglia, and cerebellum.

Researchers take advantage of the rich anatomical information amassed about the macaque brain to reveal the mechanisms underlying many cognitive functions. One such function that has been studied extensively in NHPs is visual attention, our ability to focus onto those aspects of the visual world that are relevant for a task, so that we can ignore distracting aspects. An early breakthrough in attention research was the demonstration that it is possible to measure the effects of allocating spatial attention by recording from single neurons in the visual cortex of rhesus monkeys (Moran and Desimone, 1985). This and many following studies showed that the activity of neurons that code attended visual stimuli increases, whereas the activity of neurons that code unattended information is suppressed. This finding was important because it was among the first to demonstrate that it is possible to study internal mental states at the single-cell level, in a controlled manner. Later studies revealed how the selection by attention comes in many forms and how it influences neuronal activity across many brain structures (Reynolds and Chelazzi, 2004), in accordance with the correspondence between cognitive functions and brain networks mentioned above.

Attention is but one example. There are many domains of cognitive neuroscience where the study of the NHP brain provided decisive insights into the underlying mechanisms. These domains include, but are not limited to, the neuronal mechanisms for object recognition, working memory, decision making, the guidance of motor behavior by sensory information, the neural coding of categories and numerical quantities, the processing of reward and reward expectations for reinforcement and learning, and the difference between information that does and does not reach consciousness (Table 1). A recent, particularly exciting contribution has been the discovery of mirror neurons (Rizzolatti and Craighero, 2004). These neurons code for the intentions of other individuals, and their discovery had a strong impact on theories for social cognition and theory of mind, i.e., theories about how the nervous system of one individual can code for thought processes and emotions of others.

NHP Research Has Provided Insight into Many Brain Diseases

Although the main aim of many of these studies is to gain fundamental insight into the neuronal underpinnings of our mental world, they have also impacted on our understanding of brain disease. It is therefore a flawed approach to define a divide between studies that address the fundamental neuronal processes for cognition and those that apply this knowledge to understand brain disease and to develop new treatments. Let us give a few examples to illustrate this point (see Capitanio and Emborg, 2008 for a more complete review of contributions of NHP research to so-called translational neuroscience).

First, an early and important example has been the development of deep brain stimulation (DBS), a medical technique that has provided relief to more than a hundred thousand people with Parkinson's disease. The development of DBS was inspired by the observation that a number of users of a drug produced in a clandestine home lab developed Parkinson's disease (Capitanio and Emborg, 2008). Research in NHPs ultimately led to the discovery that electrical stimulation of subcortical structures, such as the subthalamic nucleus, alleviate many of the symptoms of Parkinson's disease when they can no longer be controlled with drugs (Kringelbach et al., 2007). Studies of the functional properties of neurons in the monkey brain led to more accurate targeting of deep brain structures in humans and have been decisive in the development of this new treatment.

Second, the study of brain structures involved in reward prediction has provided crucial insights into various psychiatric diseases, including depression, obsessive-compulsive disorder, and addiction (Howell and Murnane, 2008). These studies paved the way for clinical trials that target brain structures with DBS to improve the condition of patients with treatment-resistant psychiatric diseases (Mayberg et al., 2005). Third, insights into the brain mechanisms for attention have proven to be important for our understanding and treatment of the attentional functions compromised in attention-deficit/hyperactivity disorder (ADHD; Volkow, 2012) and the effects of brain lesions in patients with the neglect syndrome (Corbetta and Shulman, 2011). Fourth, the study of mirror neurons inspired new approaches to autism and schizophrenia (McCormick et al., 2012; Vivanti and Rogers, 2014). A final example is provided by several promising approaches for prosthetic devices, which capitalize on our understanding of the NHP's nervous system. One of the aims is to develop prostheses so that paralyzed patients can control an artificial limb with their thoughts. Proof of principle has been demonstrated in monkeys that learned to control a prosthetic arm based on neuronal activity in cortical areas involved in motor control (Velliste et al., 2008). Other aims include the development of vestibular implants to improve the balance of patients suffering from peripheral vestibular disorders and of visual prosthesis for the blind. Thus, basic research in NHPs contributes to our understanding and to the treatment of brain diseases, and the fundamental knowledge that has been acquired will enable future advances.

Research in Humans and Nonhuman Primates Is Complementary

NHP research helps with the interpretation of findings obtained with neuroimaging techniques in humans, and, vice versa, findings in humans aid in the interpretation of the results obtained in NHPs. fMRI is an important technique that helps identify the neuronal structures underlying cognitive functions in healthy human volunteers and patients. Yet, the relationship between the fMRI signals and spiking and synaptic activity is remarkably complex, the spatial resolution of the fMRI signal does not allow to monitor neural activity at a finer scale than across thousands of nerve cells, and the temporal resolution is in the range of seconds, whereas many cognitive functions unfold on a much shorter timescale. Imaging and recording studies in NHPs are therefore necessary to aid in the interpretation of these signals, because they allow the direct comparison between fMRI signals and spiking activity as well as other electrophysiological markers of neuronal activity (Logothetis et al., 2001). Other imaging methods such as EEG and MEG have a better temporal resolution but also a limited spatial resolution because they rely on the synchronized activity of vast numbers of neurons. These methods provide an important, yet indirect way to study the mechanisms by which brain cells encode and decode information and control behavior. Advances in the field require complementary studies with high temporal and spatial resolution during cognitive functioning. In exceptional cases, it is possible to record the activity of single neurons in the human brain, such as during neurosurgical interventions in patients with epilepsy. These studies are restricted to those brain regions that are implicated in the generation of the individual patient's epileptic seizures so that studies in experimental animals remain necessary for systematic explorations of brain functions. This is particularly true if we want to understand how processes in the healthy brain are disrupted by disease, so that we can interpret data from human patients.

An important advantage of NHP research is the possibility of causal approaches. If studies demonstrate that nerve cells in a particular brain region change their activity during perception, action planning, or other types of mental activity, they do not necessarily address the question of whether this activity plays a causal role or is an indirect consequence of activity changes in another brain region. Causal studies directly test the involvement of brain regions in cognitive functions. Take, for example, area V5/MT, an area of visual cortex where neurons code for the direction of moving visual stimuli. Do the V5/MT neurons really cause motion perception? This is precisely the question that Salzman et al. (1990) asked in a groundbreaking study. They showed that activating cells that code for motion to the right with weak electrical pulses biases the monkey toward perceiving rightward motion, thus providing direct evidence for the involvement of these nerve cells in motion perception. Other causal methods include the introduction of well-defined inactivations of brain areas and neuropharmacological interventions (Herrero et al., 2008). Such local or systemic application of drugs allows researchers to investigate the effects of specific neurotransmitters or their receptors on neurons in different brain areas and their influence on the animals' behavioral performance. With some exceptions, these methods cannot be used in humans and they complement noninvasive techniques that interfere with activity in the human brain, such as transcranial magnetic stimulation and transcranial direct current stimulation. The specificity of these noninvasive techniques is more limited than intracortical stimulation, and the mechanisms by which they influence neuronal activity are not yet well understood. As a consequence, NHP research will remain important for causal approaches to understanding brain function.

The set of techniques to influence neuronal activity has recently been expanded by the introduction of optogenetics into NHP research (Diester et al., 2011), allowing researchers to achieve even more control over the activity of specific neurons. Yet, the available methods for optogenetics in NHPs are still more limited than those for rodents, in particular for mice, where the methods for the cell-specific expression of light-sensitive proteins are much more advanced due to the availability of a large diversity of transgenic animals.

Complementary Approaches in Rodents and Nonhuman Primates

The impact of neuroscience studies with rodents has increased in recent years, mostly due to the availability of transgenic rodent models for neurological or neurodegenerative disease but also because of the many new methods for manipulating and monitoring neuronal activity such as optogenetics and genetically encoded markers for neuronal activity (e.g., Chen et al., 2013; Fenno et al., 2011).

Mouse studies are providing insights into brain structures by, for example, elucidating the different role of the various types of interneurons. It is an exciting prospect that researchers will increasingly use these techniques to elucidate cognitive functions.

However, some important aspects of human brain function are difficult to address in rodents. Take, for example, the forward-looking eyes of NHPs, which allows for the binocular processing that gives rise to the perception of depth and the similarity of color perception between humans and NHPs, which is different in rodents. The motor system in NHPs is also radically different from that in rodents, especially in terms of advanced hand function and control of many different types of grip (Courtine et al., 2007). Moreover, some higher cognitive functions are too complex and evolutionarily recent to be meaningfully studied in rodents.

Thus, the many important insights that are generated in rodents permit insight into mechanisms that are difficult to address with NHPs and, vice versa, results in the NHP will complement them for processes and cognitive functions that are hard to study in rodents. An interesting development in the study of cognitive functions is the search for NHPs other than the macaque monkey, such as the marmoset (Mitchell et al., 2014), which breed faster and thereby more readily permit the introduction of genetic manipulations.

Informing the Public about the Necessity of Research Involving NHPs and the Efforts to Minimize Harm

As documented above, basic neuroscience research with nonhuman primates has been and continues to be of paramount importance for past and future medical progress. This does not release researchers studying nonhuman primates (or other species) from the great responsibility they have in ensuring the best possible science with the least possible harm to their animals. The awareness of this responsibility is visible in initiatives such as the international Basel Declaration (http://www.basel-declaration.org) and the recent UK Concordat on Openness on Animal Research (http://www.understandinganimalresearch.org.uk/policy/concordat-on-openness-on-animal-research/). In addition, scientific associations are increasingly recognizing their responsibility in informing the public about the importance of animal research and the efforts made to ensure that animal experiments are of the highest quality and of the least possible impact on the animals. Impressive examples of such information platforms are the UK's Understanding Animal Research (http://www.understandinganimalresearch.org.uk), France's Gircor (http://www.recherche-animale.org), and the BrainFacts website of the U.S. Society for Neuroscience (http://www.brainfacts.org). Noticeably absent from this list is a corresponding centralized source of high-quality information in Germany where the large research organizations have not yet been able to agree on the best approach. Similarly patchy has been the support in some of the larger research nations for ensuring the personal safety of researchers and providing the support for the best possible circumstances for the research animals. Most recently, this has been apparent in the silence of most European and American governments in the face of current tactics of antiresearch organizations to prevent responsible animal research by pressuring airline companies to stop transporting research animals. A notable exception was the statement by David Willetts, the UK Minister of State for Universities and Science, who spoke out to support the airlines transporting NHPs. Air transportation is in many cases beneficial for the monkeys, preventing longer and more stressful journeys by truck.

In summary, human societies have managed to develop a set of laws and regulations ensuring medical and scientific progress with the least possible harm to animals, resulting in a standard of human health and wellbeing that would have been unimaginable just a few generations ago. To ensure the public acceptance of this consensus, animal researchers need to embrace their responsibilities and communicate about the importance of animal research and the care taken in research with animals. Similarly society, through its policies and politicians, needs to protect and support responsible animal research that secures the scientific progress ensuring our standards of living as well as the quality and lengths of our lives.

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