The Application and Challenge of Non-Human Primate Experimental Animals in the Field of Neuroscience

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Abstract:

Non-human primates (NHPs) are highly valued in the field of neuroscience due to their similarity to humans in terms of brain structure and function. They exhibit complex social behaviors and cognitive abilities, and their use in research has led to significant breakthroughs in the understanding of brain development, neurodegenerative diseases, and psychiatric disorders. In recent years, the breeding and use of NHPs has expanded, making them increasingly important as experimental animals. However, with this expansion comes the need for researchers to address important biosafety and animal ethics concerns. It is critical that animal welfare is ensured and that scientific objectives are achieved while observing ethical guidelines. This paper aims to provide an overview of NHPs in neuroscience, their applications in research, and the ethical considerations that must be taken into account. It highlights the various breeding and handling practices that are employed to ensure the health and welfare of NHPs in experimental settings. Additionally, the paper summarizes the challenges associated with the use of NHPs in research and the benefits that have been realized from their use. Overall, this paper underscores the importance of recognizing the role of NHPs in neuroscience, and the responsibility that researchers have to ensure their welfare and to address ethical considerations. By doing so, researchers can continue to advance our understanding of the brain and develop new treatments for neurological and psychiatric disorders.

Keywords: Non-human primates (NHPs); Neuroscience; Experimental animals; Animal ethics

1. Introduction

Non-human primates (NHPs) are a group of mammals belonging to the order primates. They can be further divided into monkeys and apes, with apes being further classified as New World monkeys and Old World monkeys. Among NHPs, Old World monkeys, particularly rhesus macaques, are the most commonly used in research, along with crab-eating macaques and vervet monkeys.[1] NHPs are important in translational medicine and preclinical drug research because they share similarities with humans in terms of pathophysiology, with genetic similarities ranging from 75.0% to 98.5%[2]. They are especially useful for studying complex metabolism, neuroscience, and infectious diseases, where they are often the most suitable or only available animal models. While rodents, such as rats and mice, remain the most widely used experimental animals in biology and medical research, they have limitations due to significant physiological, morphological, size, metabolic, and immune system differences from humans. Certain pathogens, like HIV and novel coronaviruses, have high specificity to their respective hosts and receptors, making rodents ineffective for constructing infectious disease models. Thus, animal models closer to humans are needed.[3] In neuroscience and behavioral research, small rodents differ significantly from humans in brain structure and functional complexity. When it comes to modeling social behavior, psychological disorders, and mental illnesses, rodents often struggle to accurately replicate complex animal models[4]. This disparity prevents them from accurately reflecting human behavior and disease manifestations. In contrast, NHPs have brains that are closer and more complex to those of humans, and they possess high learning abilities, making them suitable for modeling and constructing complex behavioral and mental illness models.

2. The Application of NHPs as Experimental Animals in the Field of Neuroscience

Non-human primates (NHPs) are extensively used in various animal models of diseases, including cancer, infection, aging, and neurological diseases[5]. During the outbreak of the novel coronavirus pneumonia in 2019, NHPs were particularly crucial as the primary animal model for researching the pathophysiology and vaccines of the virus. They played a vital role in combating the epidemic[6]. This highlights the essentiality of NHP experimental animals in preventing, controlling, and researching newly emerging infectious diseases in humans. NHPs are also invaluable in human aging research. While other animal models have been used to study aging, such as yeast, nematodes, fruit flies, and rats, they have limitations in replicating the human aging process due to species differences, morphological and physiological constraints, particularly in the brain and neurons. In contrast, NHPs closely resemble humans in development and can naturally develop diseases similar to those of humans during the aging process. Additionally, they have a relatively moderate lifespan. Consequently, NHPs are considered the primary model for studying human aging. Furthermore, NHPs are preferred in studying human neurological diseases, especially those related to the brain. They possess greater similarity to humans in terms of brain volume, structure, and the ability to perform complex behavioral analysis. These similarities make NHPs crucial in studying the pathogenesis of neurological diseases and developing potential drug targets.[7]

2.1. NHP Models of Neurodegenerative Diseases

While rodents or other small animals remain classic model organisms for studying neuroscience, NHPs have become increasingly employed in neurodegenerative disease research. One such disease is Parkinson's disease (PD). NHP models have been utilized in PD studies, specifically by inducing MPTP to construct an NHP-PD model, which serves as the standard. This NHP-PD model displays symptoms akin to humans, including tremors and paralysis resulting from dopamine deficiency. It also effectively expresses and detects α-synuclein, a promising biomarker for PD diagnosis and treatment. NHPs are crucial models for investigating PD drugs and treatment methods. Tremor animal models in NHPs can be used for screening anti-tremor medications, and NHP models can validate the effectiveness and safety of PD stem cell therapy.

In addition to PD, NHPs are also critical models for Alzheimer's disease (AD) research. AD is the world's leading neurodegenerative disease, and there is still no effective drug available for it. Most drug applications have ended in phase II clinical trials, and the few that have advanced to phase III ultimately failed due to a lack of efficacy. Although the exact mechanisms and causes of AD are still not fully understood, multiple hypotheses have been proposed, and various genes have been found to be involved in the disease's development. Therefore, better and more representative AD animal models are needed to accelerate drug discovery, identify new biomarkers, and study disease mechanisms. NHPs have a natural advantage over rodents in some hypotheses, such as the Free Radical Hypothesis, which suggests that aging mitochondria in the elderly produce excessive reactive oxygen species (ROS) that cause a lot of free radical damage to cell membranes, ultimately leading to cell apoptosis[8]. In aging animals, natural cognitive impairment occurs in models older than two years in rats or above 16 years in monkeys. NHP models have high value in developing AD drugs. Some candidate drugs have effectively and to varying degrees delayed disease progression in rodent models, but their efficacy cannot be replicated in humans. This phenomenon may be due to the differences between rodents and humans. While the genetic differences between rodents and humans are not significant, they differ significantly in physiology, tissue structure, and cognitive abilities. These differences may have contributed to the failure of AD drug clinical trials. NHPs are more similar to humans in physiology and tissue structure, and, due to their advanced vision and brain function, they can complete more complex AD clinical assessments and are capable of using more tests.

Table 1: Establishment and performance of a sporadic NHP-AD model

Method	Performance
Intracerebral injection of Aβ42 (1 mg/mL) and thiophene (2 mg/mL) in rhesus monkeys aged 16~17 years	Intracellular Aβ accumulation, neuronal deformation, glial cell hyperplasia and no difference in behavioural performance between the group receiving the same surgery (blank) and the experimental group[10].
Intracerebral co-injection of lipopolysaccharide (LPS) with Aβ protofibrils in 13~14-year-old marmosets	Chronic inflammation and plaque developed in two-thirds of co-injected marmosets, while no plaque developed in those injected with Aβ only[11].
Young rhesus monkeys (5~8 years old, no AD-related mutations) were given intracerebroventricular injections of formaldehyde for 12 consecutive months	Amyloid β positivity, neuritis-like plaques, neurogenic fibrillary tangle (NFT) formation, Tau protein hyperphosphorylation, neuronal loss and glial cell proliferation[12].

2.2. Models of Mental Illness in NHPs

Animal models are very important for understanding the causes of mental illnesses, which have long been difficult for researchers to unravel. Mental illnesses are now known to involve molecular changes in the brain, including neurotransmitters and certain genes, in addition to psychological factors. Non-human primates (NHPs), which have more complex social behaviors and can show psychological symptoms similar to humans, are especially valuable for studying disorders like schizophrenia, depression, and autism. Some researchers have created a model of adolescent depression-like behavior in cynomolgus monkeys through chronic stress caused by space restriction, intimidation, prolonged illumination, and fasting[13]. This research shows how early stressful experiences can have lasting effects on the nervous system and increase the risk of developing depressive symptoms later in life. Additionally, about half of people with depression have a genetic basis for their illness, so creating animal models through techniques like transgenesis or gene editing is a promising approach for studying mental illnesses and deserves further exploration.

2.3. NHP Addiction Models

Non-human primates (NHPs) possess certain similarities to humans when it comes to drug metabolism, making them a valuable model for studying drug abuse, addictive behavior, and safety evaluations. One significant advantage of NHPs is their physiological and genetic resemblance to humans, which enables researchers to better understand the effects of drugs on the human body. These animals have similar drug-absorption, distribution, metabolism, and excretion processes as humans, allowing for more accurate predictions of drug effects and toxicity.

Furthermore, NHPs' long lifespans offer researchers an extended window to observe the long-term effects of drug use and potential addiction. This is particularly important for investigating chronic drug abuse and the development of drug dependence, which cannot be adequately studied in shorter-lived animal models. By studying NHPs, scientists can gain insights into the neurobiological mechanisms underlying addiction, explore potential therapeutic interventions, and evaluate the safety and efficacy of drug treatments. Despite the advantages, the use of NHPs in research also raises ethical considerations and requires stringent oversight to ensure the well-being and ethical treatment of these animals. Guidelines and regulations are in place to minimize any potential harm and to ensure the responsible and ethical usage of NHPs in scientific investigations. In summary, NHPs serve as a crucial model for researching drug abuse, addictive behavior, and safety evaluations due to their similarities with humans in drug metabolism and their long lifespans, which allow for comprehensive and in-depth observations. However, ethical guidelines must be followed to ensure the welfare of these animals in research settings. Through responsible and ethical utilization, NHPs can contribute significantly to advancing our understanding of drug-related issues and ultimately improving human health and well-being[14].

Non-human primates (NHPs) exhibit a dependency on sedatives and anesthetics similar to that seen in humans. Additionally, their withdrawal symptoms are more evident and easily observed, making them a valuable model for studying addiction mechanisms and assessing the effectiveness of therapeutic drugs. This is particularly relevant in preclinical studies, where new sedatives are frequently tested on NHPs to determine safer dosages for potential use in future clinical trials involving human subjects. One of the key advantages of using NHPs in research is their physiological and genetic similarity to humans. This similarity enables researchers to better understand the effects of sedatives and anesthetics on the human body, including potential drug-induced damage caused by substances like opioids and alcohol. By studying NHPs, scientists can gain insights into the mechanisms underlying addiction and investigate the effectiveness of therapeutic drugs in combating substance abuse and dependence. Moreover, NHPs' withdrawal symptoms can be more easily observed and monitored, allowing researchers to study the progression and severity of withdrawal syndromes. This information is crucial for assessing the potential risks and benefits of new sedatives and anesthetics, as well as evaluating their tolerability and safety in human subjects. Furthermore, NHPs provide a valuable opportunity to investigate drug-induced damage, such as the impact of opioids and alcohol on the brain and other organs. By studying the physiological and behavioral changes in NHPs, researchers can explore the short-term and long-term effects of these substances, aiding in the development of strategies for prevention and treatment. It is essential to note that while NHPs offer significant insights into addiction mechanisms and drug-induced damage, the ethical considerations surrounding their use in research must be carefully addressed. Appropriate guidelines and regulations are in place to ensure the responsible and ethical use of NHPs, minimizing any potential harm and ensuring their welfare during experimental procedures.

In summary, NHPs serve as a valuable model for studying addiction mechanisms, drug-induced damage (including opioids and alcohol), and evaluating the effectiveness of therapeutic drugs. Through their resemblance to humans in terms of sedative and anesthetic dependency, these animals provide insights into withdrawal syndromes and aid in determining safer dosages for potential use in clinical trials. However, it is crucial to conduct research with NHPs in an ethical and responsible manner, considering their welfare and adhering to guidelines and regulations. By doing so, we can further our understanding of substance abuse and develop effective interventions to improve human health and well-being[15].

2.4. NHP Models of Cerebrovascular Diseases

Cerebrovascular diseases, which are characterized by their rapid progression, high incidence rates, and significant impact on mortality and disability, pose a serious threat to human health. Among these diseases, ischemic cerebrovascular disease is the most common form. In order to effectively address ischemic brain disorders, it is crucial to develop animal models and evaluation methods for stroke drugs that are more rational and based on scientific principles. Although cell and rodent models are frequently used in the development of stroke medications, many potential drugs identified through these models often yield unsatisfactory results in clinical settings. Therefore, the integration of non-human primate (NHP) models becomes essential for advancing stroke drugs and validating surgical procedures. For instance, a study conducted by Meloni et al. utilized macaque monkeys to establish an ischemic stroke model through transient middle cerebral artery occlusion (MCAO) surgery. The aim of this study was to investigate the therapeutic potential of poly-arginine-18 in enhancing recovery from post-ischemic brain injury.

3. Challenges in NHP Experimental Animal Application

Non-human primates (NHPs), such as primates, play a vital role in animal research experiments. However, ensuring their biosafety is of utmost importance. NHPs differ from commonly used rodent species like mice and rats in terms of size, activity space, food requirements, breeding difficulty, and complexity of experimental techniques and personnel requirements. Additionally, the diseases and viruses they may carry pose a significant threat to humans. Therefore, it is crucial to prioritize biosafety in the breeding, experimentation, and post-experimental processes. Unlike human studies, research involving NHPs allows for comprehensive control of the experimental environment, including living conditions, diet, and social interactions. This is possible due to the close evolutionary relationship between humans and these animal models. The disposal of older animals and the continued use or breeding of animals, especially those involved in genetic and reproductive experiments, require specialized care. The high cost of using these animals and the potential biosafety and ethical concerns associated with genetic and reproductive experiments have generated considerable attention.

3.1. Biosafety in Disease Surveillance

Experimental monkeys such as macaques, crab-eating macaques, and other macaque animals play a crucial role in medical research. Over the past few decades, the breeding and cultivation of these animals in China has experienced significant growth due to changing international supply and demand as well as increased demand in domestic research. However, the breeding and cultivation of experimental monkeys also come with biosafety risks, given the close evolutionary relationship between humans and non-human primates. While proper measures can minimize these risks, it's important to acknowledge the potential hazards. Despite these challenges, the population of bred experimental monkeys in China has reached a scale that will continue to be essential to make breakthroughs in medical research.

To ensure the well-being and safety of experimental monkeys, it is necessary to provide suitable living conditions based on their size and habits. This includes arranging appropriate breeding spaces, providing suitable food, and maintaining the correct temperature and humidity levels. It is also crucial to establish clear distinctions between different categories of experimental monkeys, such as those that have undergone specific experiments (modeling, infection, disease treatment, etc.) and those that have not. When newly acquired animals are brought in, they should undergo an epidemic isolation period. For non-human primates (NHP), this isolation period typically lasts for 40-60 days, during which their status as carriers of zoonotic pathogens can be confirmed. Strict documentation is essential throughout the breeding process to comply with regulatory requirements. These records include animal medical records, breeding records, induced disease records, and research records. Furthermore, personnel involved in the breeding process, such as management staff, veterinarians, and feeders, must receive proper training before assuming their duties. This training is necessary to ensure their competence in handling and caring for the experimental monkeys effectively.

The Experimental Animal Center of Tsinghua University has reported that due to the close evolutionary relationship between non-human primates and humans, there are more zoonotic diseases that can be shared between humans and NHPs compared to commonly used rodent experimental animals like mice and rats.

In order to prevent the transmission of zoonotic diseases, it is crucial to have standardized operating procedures (SOPs) and quarantine processes in place. Previous reports have indicated that monkeys bred in zoos have been found to carry viruses, leading to a few animal caretakers testing positive for monkeypox virus antibodies in their blood. While the breeding practices and management regulations may vary between experimental monkey breeding and zoo monkey breeding, it is still essential to strengthen preventive measures and regularly monitor the health of individuals involved in breeding.

3.2. Experimental Operation Safety

Experimental monkeys are subject to stringent regulations and necessitate comprehensive documentation. Given their substantial stature and propensity for aggression, trained personnel equipped with suitable monkey harnesses must be present to apprehend and immobilize them during drug administration or anesthesia procedures. Any operations involving infectious agents must take place in laboratories that fulfill the appropriate biosafety levels, similar to those employed for studying animal infectious disease models. Laboratories or embryo facilities dedicated to the study of genetically modified or gene-edited monkeys or embryos should be exclusively used for such experiments.

3.3. Post-Experiment Safety after experiments

In most cases, experimental animals are euthanized after they have been studied, but certain disease models require researchers to study how the animals recover after treatment or to monitor the long-term health of animals that have been immunized for vaccine research. Animals that fall under this category are typically looked after until their natural death. In the case of infectious disease models, animals must be verified to have fully recovered or lost infectivity before they can be reintroduced to breeding facilities. Special care must be taken when dealing with genetically modified or gene-edited animals, as allowing them to breed can lead to the transmission of modified genes to their offspring or to other animals in the population. As such, these animals must either be reintroduced to breeding facilities separately or their reproduction must be blocked.

3.4. Ethical Issues in the Use of NHPs

Animal welfare and ethical concerns have always been taken into account in animal experimentation. Before conducting any experiments, they must first be reviewed by an animal welfare and ethics committee. Non-human primates (NHPs), such as monkeys, are particularly relevant to humans and can greatly contribute to scientific research in fields like medicine and biology. However, the use of NHPs has also attracted attention from animal welfare organizations and the public, resulting in ethical and social challenges.

When NHPs are needed for experimentation, more comprehensive scientific evidence must be provided, and ethical considerations must be thoroughly discussed and researched. Approval from the ethics committee is required before testing can begin. Prior to conducting such research, the necessity and significance of using NHPs must be fully considered. Additionally, the principles of the 3Rs (Reduction, Refinement, and Replacement) must be taken into account, the harm-to-benefit ratio of the study must be evaluated, and the proper disposal of experimental animals must be ensured.

Currently, there are no universally standardized regulations regarding the use of NHPs internationally. To promote the rationalization of NHP utilization, ensure animal welfare protection, and encourage rigorous and innovative scientific research, international exchange and cooperation are necessary. Efforts should be made to reduce the unreasonable and illegal use of animal experimentation. Ultimately, internationally recognized standardized regulations should be established in this regard.

4. Conclusion

As we look towards the future, non-human primate (NHP) experimental animals are poised to play a crucial role in advancing scientific research, particularly in the realm of understanding and addressing complex brain-related problems that cannot be adequately studied using smaller animal models. The utilization of NHP experimental animals offers unique opportunities for researchers to delve deeper into intricate neural processes and develop effective interventions. However, it is important to acknowledge that the use of NHP experimental animals also comes with its own set of challenges. Biosafety concerns arise due to the potential transmission of zoonotic diseases between NHPs and humans. Additionally, ethical considerations surrounding the treatment and welfare of these highly intelligent and sentient animals must be carefully addressed.

Fortunately, there is a growing recognition of these challenges, and there have been significant advancements in laws and regulations governing the use of NHPs in research. Strict protocols are now in place to ensure the proper care, ethical treatment, and humane conditions for NHP experimental animals. This includes providing enriched environments, regular veterinary care, and minimizing any potential discomfort or distress. With the continued progress in refining laws and regulations, we can unlock the full potential of NHP experimental animals in the field of neuroscience. Ethical guidelines and oversight help to ensure that the use of NHPs is justified by the potential scientific and medical advancements that can be achieved. By collectively addressing biosafety concerns and ethical considerations, we can pave the way for groundbreaking discoveries and solutions to some of the most significant problems in neuroscience.

Acknowledgements
If any, should be placed before the references section without numbering.

References

[1] Sandstrom, P. A., Phan, K. O., Switzer, W. M., Fredeking, T., Chapman, L., Heneine, W., & Folks, T. M. (2000). Simian foamy virus infection among zoo keepers. The lancet, 355(9203), 551-552.

[2] Bliss-Moreau, E., Amara, R. R., Buffalo, E. A., Colman, R. J., Embers, M. E., Morrison, J. H., ... & National Primate Research Center Consortium Rigor and Reproducibility Working Group. (2021). Improving rigor and reproducibility in nonhuman primate research. American journal of primatology, 83(12), e23331.

[3] Lu, S., Zhao, Y., Yu, W., Yang, Y., Gao, J., Wang, J., ... & Peng, X. (2020). Comparison of nonhuman primates identified the suitable model for COVID-19. Signal transduction and targeted therapy, 5(1), 157.

[4] Leblanc, H., & Ramirez, S. (2020). Linking social cognition to learning and memory. Journal of Neuroscience, 40(46), 8782-8798.

[5] Dewi, F. N., & Cline, J. M. (2021). Nonhuman primate model in mammary gland biology and neoplasia research. Laboratory Animal Research, 37, 1-10.

[6] Trichel, A. M. (2021). Overview of nonhuman primate models of SARS-CoV-2 infection. Comparative Medicine, 71(5), 411-432.

[7] Van Dam, D., & De Deyn, P. P. (2017). Non human primate models for Alzheimer's disease-related research and drug discovery. Expert opinion on drug discovery, 12(2), 187-200.

[8] Hollander, C. F., & Mos, J. (1986). The old animal as a model in research on brain aging and Alzheimer's disease/senile dementia of the Alzheimer type. Progress in brain research, 70, 337-343.

[9] Flint, J., & Kendler, K. S. (2014). The genetics of major depression. Neuron, 81(3), 484-503.

[10] Li, W., Wu, Y. E., Min, F., Li, Z., Huang, J., & Huang, R. (2010). A nonhuman primate model of Alzheimer's disease generated by intracranial injection of amyloid-beta42 and thiorphan. Metabolic brain disease, 25, 277-284.

[11] Philippens, I. H., Ormel, P. R., Baarends, G., Johansson, M., Remarque, E. J., & Doverskog, M. (2017). Acceleration of amyloidosis by inflammation in the amyloid-beta marmoset monkey model of Alzheimer's disease. Journal of Alzheimer's Disease, 55(1), 101-113.

[12] Zhai, R., Rizak, J., Zheng, N. A., He, X., Li, Z., Yin, Y., ... & Hu, X. (2018). Alzheimer's disease-like pathologies and cognitive impairments induced by formaldehyde in non-human primates. Current Alzheimer Research, 15(14), 1304-1321.

[13] Zhang, Z. Y., Mao, Y., Feng, X. L., Zheng, N., Lü, L. B., Ma, Y. Y., ... & Hu, X. T. (2016). Early adversity contributes to chronic stress induced depression-like behavior in adolescent male rhesus monkeys. Behavioural brain research, 306, 154-159.

[14] Ding, H., & Ko, M. C. (2021). Translational value of non-human primates in opioid research. Experimental neurology, 338, 113602.

[15] Jimenez, V. A., & Grant, K. A. (2017). Studies using macaque monkeys to address excessive alcohol drinking and stress interactions. Neuropharmacology, 122, 127-135.