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# Why non-human primates are needed in stroke preclinical research

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## ABSTRACT

Numerous seemingly promising cerebroprotectants previously validated in rodents almost all have failed in stroke clinical trials. The failure of clinical translation strikes an essential need to employ more ideal animal models in stroke research. Compared with the most commonly used rodent models of stroke, non-human primates (NHPs) are far more comparable to humans regarding brain anatomy, functionality and pathological features. The aim of this perspective was to summarise the advantages of NHPs stroke models over rodents, discuss the current limitations of NHPs models, and cast an outlook on the future development of NHPs in stroke preclinical research.

## INTRODUCTION

Until 2006, more than 1000 neuroprotectants with proven safety and efficacy in cells and rodents have been investigated,[1] but almost all have failed in clinical trials. In 2009, The Stroke Therapy Academic Industry Roundtable (STAIR) recommended that neuroprotective therapies need to be further tested in gyrencephalic non-human primates (NHPs).[2] Later on, STAIR XI still voices concerns about NHPs models, suggesting that demonstration of efficacy in NHPs may contribute to predicting clinical efficacy.[3] Of note, several promising cerebral cytoprotectants verified in NHPs have emerged in recent years. In 2012, Cook *et al*[4] successfully validated the cerebroprotective effect of Tat-NR2B9c, a PSD95 inhibitor, on cerebral infarction model of NHPs. Subsequently, Hill *et al*[5] reported the ESCAPE-NA1 phase III clinical trial which revealed the possible beneficial effects of PSD95 inhibitor NA-1 for patients who had a stroke, though the positive effects are still under evaluation to determine the optimal window. Besides, Jia *et al*[6] found DL-3-n-butylphthalide improved cognition and general functions in patients with subcortical cerebral infarction. Recently, we successfully verified the ameliorative effects of DL-3-n-butylphthalide on post-stroke cognitive impairments (PSCI) in cynomolgus monkeys and revealed its possible mechanism.[7] All these studies highlight the extremely high value of NHPs, especially for gyrencephalic ones, in stroke preclinical research.

## HIGH CONSISTENCY IN BRAIN ANATOMY WITH HUMAN

NHPs have highly comparable brain anatomy to those of humans. First, NHPs have complete Willis circle and collateral arteries similar to humans and the distribution and branching of middle cerebral arteries (MCAs) largely resemble those of humans.[8] Occlusion of the M1 segment of MCA in NHPs causes infarcts quite similar to those found in patients who had a stroke. Second, NHPs have multiple convoluted sulci and gyrus in cortex as well as complex lobes and brain subdivisions. The brains of NHPs are much larger and more evolved than rodents, especially for the prefrontal cortex.[9] Moreover, NHPs have similar cerebral grey and white matter proportion and abundant white matter fibres to those of humans,[10] making NHPs an ideal model of ischaemic white matter injury.

Because of the high similarities between NHPs and humans regarding brain anatomy, neuroimaging techniques represented by MRI, positron emission tomography (PET) and PET/MRI can be fully applied in NHPs. Unlike rodents, which have high requirements for field strengths, the 3.0 Tesla MRI commonly used clinically are directly applicable to NHPs. Additionally, scholars have established public data platforms of NHPs neuroimaging for data exchange and better collaboration.[11] As to PET, despite the fact that it is more often applied to disorders like Parkinson's disease and Alzheimer's disease with anomalous accumulations of specific proteins,[12] it also plays key roles in cerebrovascular diseases. [15]O-water PET serves as the gold standard modality to quantify cerebral blood flow (CBF) in absolute units, which is a critical index for stroke.[13] Besides, PET, especially for simultaneous PET/MRI system, not only can help detect the abnormal deposition of metabolites directly such as cerebral β-amyloid (Aβ) deposits after stroke,[14 15] but also contribute to monitoring blood–brain barrier permeability by imaging and quantifying the transfer rate of nanoparticles[16] and evaluating the spatio-temporal evolution of brain inflammation through matched apparent

diffusion coefficient (ADC) decrease in brain regions,[17] thus enhancing our understanding of the neuropathological basis and furnishing indispensable data for evaluating clinical outcomes.

## HIGH COMPATIBILITY IN BRAIN FUNCTIONALITY WITH HUMAN

NHPs are highly similar with humans in terms of advanced cognition, motor behaviour, sleep cycle and neuroplasticity. NHPs have advanced cognition close to humans, which can perform multiple complex cognitive tasks after training, thus allowing for better evaluation of PSCI. Various cognitive assessment methods for NHPs have been established and widely used.[18] Additionally, NHPs have more complex emotions than rodents, and their facial expressions, body movements and social behaviours to express emotions are abundant, which are of great value in decoding post-stroke affective disorders.[19]

For patients who had a stroke, the ability to perform fine movements significantly affects life quality, especially for hands, which cannot be precisely evaluated in rodents. NHPs have extensive corticospinal projections with innervation patterns similar to humans,[20] thus they can precisely control the independent movements of a single finger, realising the 'pincer grasp' movement that specifically belongs to primates. Additionally, NHPs can walk bipedally over short distances which bears a strong resemblance to human.[21] The above advantages make NHPs irreplaceable in exploring post-stroke fine motor deficits.

NHPs share similar sleep traits to humans, such as sleep–wake cycle, sleep length and stability. Various characteristic brain waves of NHPs during sleep can be easily distinguished, which show similar periodic changes to humans.[22] Rodents, on the other hand, are diurnal, polyphasic sleepers with multiple short naps, and both rapid and non-rapid eye movement sleep cycles are significantly shorter. Besides, the sleep monitoring system of NHPs has been established, such as electroencephalogram, electromyogram, electrooculogram, body movement recorder and manual analysis after video recording, making NHPs promising models for studying post-stroke sleep disorders.

Rodents have strong neuroplasticity, some of which show only temporary hemiparesis and spontaneous neurological recovery after stroke, causing false-positive results that ineffective therapies are effective. In contrast, the neuroplasticity and the effects of stroke modelling of NHPs are more similar to humans,[23] which help to judge the true efficacy more accurately.

## HIGH CORRESPONDENCE IN PATHOLOGICAL FEATURES WITH HUMAN

Apart from the aforementioned similarities in physiological states, NHP stroke models also bear high correspondence with patients who had a stroke on pathological features, such as cerebral Aβ deposits. The earliest autopsy study reported cerebral Aβ deposition in patients who had a stroke traces back to 1990s.[24] Nevertheless, a more recent autopsy study with a larger sample size found no significant difference in cerebral Aβ deposition between patients who had a stroke and healthy control group.[25] Moreover, several recent clinical PET imaging studies fail to testify the neurotoxic role of cerebral Aβ deposition after stroke.[14 15] Generally, evidence from clinical studies seemingly drawn negative conclusions on the pathological mechanisms of cerebral Aβ deposition after stroke.

There are also plentiful experimental studies on Aβ pathology. Numerous studies have confirmed Aβ deposition in ischaemia-free brain regions in rodents after stroke, indicating that Aβ pathology may play a role in the damage of remote brain regions and PSCI in rodents.[26] By contrast, in our study using cynomolgus monkeys, there was obvious damage to remote brain regions 12 months after stroke, but neither cerebral Aβ deposition, nor significant changes in Aβ levels of blood and cerebrospinal fluid were found.[27] Similar negative results of Aβ pathology were reported in other study using marmosets 45 days after stroke.[28] The above only two NHPs researches available to date are quite in accord with results from clinical studies. Notably, the inconsistency of cerebral Aβ deposition among humans, NHPs and rodents strongly suggests that NHPs can better modulate pathological conditions after stroke.

Of note, NHPs models share similar critical pathophysiological parameters with human beings. The concept of the penumbra was defined for the first time in NHPs, assessed by the technique of hydrogen clearance in MCA occluded baboons.[29] Furthermore, Jones *et al* first clarified the importance of occlusion duration and local CBF which defined the threshold for paralysis and infarction in awake-primate model of reversible MCA occlusion during physiological monitoring.[30] The conception above laid the critical foundation for the proposal of revolutionary therapies for stroke like intravenous thrombolysis and endovascular thrombectomy and meanwhile emphasised the therapeutic time window.

## CONCLUSIONS AND PROSPECTIVES

In conclusion, NHPs have irreplaceable advantages over rodents in stroke research, and are extremely important tools for promoting basic and translational research to achieve major breakthroughs. However, it is undeniable that current NHP models still bear some limitations. First, NHPs resources are scarce, the purchase and feeding costs are high and the experimental period is long, making it difficult to conduct large-scale experiments. Second, the inter-individual heterogeneity in NHPs is larger than rodents. Besides, there is a lack of detailed information on the physiological, biochemical, immunological and other basic parameters of NHPs. Research tools such as antibodies, targeted drugs, biochemical reagents, behavioural evaluation systems, imaging equipment and gene editing technology are not as mature as those in rodents.

Finally, although NHPs are ideal model animals for neuroscience research, their ethical scrutiny is far more stringent. When using NHPs, the principles of 'Reduce, Replace, Refinement' should always be implemented to strictly guarantee the animal welfare.

Following the first cloned NHPs born alive in 2018, Liao *et al*[31] produced a cloned rhesus monkey that has successfully lived into adulthood for the first time, by employing somatic cell nuclear transplantation technology-trophoblast replacement strategy, which brings hope for the acquisition of numerous genetically uniform NHPs. In 2022, the first whole-body organ cell atlas of NHPs led by Chinese scholars and involving multinational scholars was published.[32] Later, by means of spatiotemporal genomics technology and single-cell nuclear transcriptome sequencing technology, the most comprehensive cell type, spatial distribution and molecular characteristics of primate cerebral cortex to date were defined.[33] These studies provide a solid foundation for in-depth analysis of human-related brain functions and diseases. Additionally, the rapid advancement of transgenic and gene editing technology enables the establishment of more disease models for NHPs and the development of more promising therapeutics.[34]

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