INTRODUCTION

Alzheimer’s disease (AD), also known as Alzheimer’s dementia, is a neurodegenerative disorder that constitutes the most important form of dementia among the elderly.1 Its main clinical manifestations include progressive memory loss and cognitive dysfunction, followed by behavioral disorders and related impairments.2,3 Neurophysiologically, AD is characterized by the extracellular aggregation of β-amyloid (Aβ) in the form of so-called senile plaques (SPs), as well as the intracellular neurofibrillary tangles (NFTs) caused by the hyperphosphorylation and aggregation of the tau protein within neurons. These are presently the neurophysiological indicators used to diagnose AD.4,5 Additional pathological changes that can arise in the brains of AD patients include age-related brain atrophy, synaptic pathologies, leukoaraiosis, granulovacuolar degeneration, loss of neurons, pathologic TDP43 accumulation, and neuroinflammation.4-6 Since it was first reported in 1906 by professor Alois Alzheimer at the German Southwest Congress of Psychiatry, AD already has a history of medical recognition that spans for more than a century. However, related research has yet to deliver a true breakthrough. To date, no specific drug has been developed for the treatment of AD. However, with the increasingly rapid aging of the global population, the incidence of AD is also quickly increasing. It has been estimated that by 2050, the number of AD patients in the...
According to etiology, AD can be divided into broad types of familial and sporadic disease. The familial type accounts for 5% of the total cases of AD, and is mostly correlated to mutations in genes that encode amyloid precursor protein (APP), Presenilin-1 (PSEN1), Presenilin-2 (PSEN2) and apolipoprotein E (APOE). The etiology of sporadic AD is complex, and may be correlated to many factors, such as age, gender, family history, education level, APOE gene variants, diabetes, environmental factors, or microorganisms. There are many hypotheses on the proximal pathophysiological causes of AD, which include the amyloid cascade hypothesis, the cholinergic hypothesis, and insulin signaling pathway disorders. However, the exact mechanism remains insufficiently understood. One important reason for this may be the lack of animal models that are more closely correlated to humans, which can be employed as more powerful research tools. Furthermore, there are many types of animal models for AD, which include fruit flies, mice, rats, rabbits, dogs and non-human primates, and the most widespread are models based on rodents. However, the physiological and neurobiological characteristics have exhibited comparatively large deviations from those of humans, and the pharmaceutical treatments developed in rodents have consistently failed to show a significant effect on humans in clinical trials. Consequently, despite having a very important supporting role, the insights from these models cannot readily be translated to clinical applications. The identification and establishment of better animal models for the elucidation of the pathophysiological causes of AD and the screening of effective pharmaceuticals have always been an important aspect of AD research. Since non-human primates are evolutionarily the closest to humans, which also means that the anatomy and neurobiology are closely correlated, these are presently generally considered to be ideal for the establishment of animal models of AD. Accordingly, the present status of the research of non-human primate models of AD was reviewed, in order to provide a theoretical basis for further research of this increasingly more pressing globally important disease. Furthermore, the theoretical basis of the model construction, the construction method, the phenotype and application of the model, and the advantages and limitations of the model would be summarized.

2 | EXAMPLES OF SPONTANEOUS AD-LIKE MODELS IN NON-HUMAN PRIMATES

2.1 | Theoretical basis

Advanced age is one of the most important risk factors that influence the incidence of AD, and most patients develop the disease relatively late in life (normally after the age of 65). Furthermore, the age of disease onset in familial AD has a relatively high genetic basis. In cases with early-onset disease, mutations in the APP, PS1 and PS2 genes are already known to account for half of the cases of familial AD. However, most genes involved in the age-related increase in disease incidence remain to be determined. At present, the only widely known and strongly risk-related locus is the APOE E4 gene. APOE is a blood serum protein with important roles in fat and lipoprotein metabolism. Humans possess three important APOE subtypes, APOE2, APOE3 and APOE4. Among these three subtypes, APOE3 is the most common, while the APOE4 gene has a relationship with aging, and has the closest relationship with the incidence of AD.

During the aging process, some non-human primates can present with characteristics similar to symptoms determinative of human AD, which include neuropathy and cognitive behavioral changes. However, aging animals are not models of AD, but are excellent models of normal aging and naturally occurring Aβ deposition, and some have cognitive impairment. To date, amyloid deposition in the brain parenchyma has been observed in most non-human primates, which include rhesus monkeys, chimpanzees, crab-eating macaques, stump-tailed macaques, lemurs, vervet monkeys, marmosets and baboons. This study introduces rhesus monkeys, stump-tailed macaques, mouse lemurs, the common marmoset and cynomolgus monkeys as representative examples.

2.2 | Representative models

Rhesus monkeys (Macaca mulatta) have a body length of 45-64 cm and weigh 5-12 kg. The life expectancy of these monkeys can reach 34-40 years. At an age of over 20 years, these monkeys are considered to have entered old age, during which these monkeys can experience cognitive and behavioral decline. In addition, the deposition of amyloid plaques can be observed in the cerebral cortex. Furthermore, the APP of rhesus monkeys is highly similar to that of humans. Among the cerebral plaques of rhesus monkeys, approximately 1/2 are positive for acetylcholine (Ach) esterase, and share similar biochemical characteristics with humans. Furthermore, approximately 1/5 contain APOE. These histochemical and morphological features are similar to those that arise in AD patients. However, the area of plaque deposition in rhesus monkeys differs from the area affected in human AD patients, in whom this is obvious in the hippocampus, amygdala, olfactory cortex, frontal cortex, temporal lobe and parietal lobe. In contrast, the main regions of plaque deposition in rhesus monkeys encompass the marginal cortex and prefrontal lobe, and not the hippocampus, with great differences between individuals. In addition, there have been no definite reports of NFTs similar to those of humans in rhesus monkeys.

Stump-tailed macaques (Macaca arctoides) have a body length of 45-70 cm, weigh between 7-12 kg, and can reach an age of
30 years. These macaques exhibit morphological and functional degradation of the brain similar to that observed in rhesus monkeys, but have some obvious differences. It has been reported that the cognitive and behavioral function of these animals degrades after the age of 24 years. In many regions of the brain, which include the prefrontal cortex, hippocampus, and parahippocampal region, areas that contain high-density plaques were discovered. These regions were interspersed with regions without plaques, and were also scattered in other regions of the brain. Furthermore, the plaque morphology was diverse, which included small areas visible due to uniform immunostaining, or very small extracellular grains that indicated diffuse immunoreactive sediments, as well as some grains inside the neurons. At the same time, the accumulation of proteins reactive to antibodies against the tau protein (phosphorylated and unphosphorylated) was discovered within small neurons. However, it is noteworthy that AD-like neuropathological changes were observed only in animals with obvious cognitive and behavioral dysfunction, while animals without such abnormalities did not show evidence of neuropathological changes. This shows that behavioral dysfunction and neuropathological changes are consistent in stump-tailed macaques, but this differs from postmortem findings in humans, revealing that the pathological change characteristics of AD can be found in normal people after death.

Mouse lemurs (Microcebus murinus) are non-human primates, which in some aspects resemble rodents. The characteristics of these animals particularly include a small size, with a body length of 12-13 cm, and a tail length similar to the length of the body. Accordingly, these animals only weigh 50-100 g. Furthermore, the lifespan of these animals is comparatively short, with the longest reported age being 14 years. Hence, those over 5 years can already be considered to have entered the aging process. These animals can develop features similar to those found in AD patients, such as amyloid protein aggregations that form plaques and basophilic neurons, in which animals with such neurodegenerative changes lose the ability to complete tasks and socialize. This is similar to the behavioral changes of AD patients. Furthermore, mouse lemurs may exhibit patterns of amyloid plaque deposition similar to those of humans. There are three main types of deposits: diffuse plaques 100 μm in diameter, amyloid plaques with a dense core that are positive for thioflavin, and circular structures surrounding the blood vessels. The first two types were also discovered in AD patients. The Aβ40/Aβ42 ratio of mouse lemurs was higher than that of humans, indicating that the synthesis mechanism of amyloid proteins differs between these two species. The plaque distribution of mouse lemurs can be classified from high to low density, in the order of upper temporal lobe, amygdala, prefrontal lobe, parietal lobe and occipital lobe, which is similar to that of humans. At the same time, in mouse lemurs, it was discovered that tau protein accumulation gradually increases with the progression of aging, which has the most severe effect on the cerebral cortex, even in young monkeys. Merely animals over 8 years (which represents an extraordinarily advanced age for this species) exhibited changes in the hippocampal substratum and internal olfactory cortex, which are very different from human AD.

The common marmoset (Callithrix jaccus) has become an increasingly important primate model in the research filed due to its small size, multiple births, ease of raising in cages, and convenience for laboratory handling. Its body length is 10-12 cm, and its weight is only 80-100 g. Furthermore, its lifespan is 7-17 years, which has been reported only in a cage breeding environment. However, there are still no established criteria to define a marmoset as aged. Furthermore, the reproductive rate significantly declines from the age of 7, and Aβ deposits can be found in the brain at an age of at least 7 years. It is also possible that Aβ exists in the brains of young marmosets, but only in small amounts. In addition, it is remarkable that the distribution of Aβ in the brains of common marmosets resembles the distribution and chemical composition of the human brain. However, these marmosets do not present an immune response to abnormally phosphorylated tau protein. At the same time, the analysis of survival on a group of marmosets revealed that merely 6% of animals live past the age of 7. These data suggest that common marmosets are a low-level primate model for human AD, and can be used to study the pathological effects of Aβ. However, the relatively small number of animals expected to live to old age severely limits the use of this species as a model of Aβ deposition.

Cynomolgus monkeys, also known as crab-eating macaques (Macaca fascicularis), have a body length of 40-65 cm and a weight of up to 9 kg. The lifespan of these monkeys can reach 35 years or more. These animals are considered to be old upon reaching the age of 20. A study revealed that 20-year-old cynomolgus monkeys presented with mature plaques, which are more common than scattered plaques, in the temporal cortex of the superior and inferior gyrus and amygdala. However, there is no clear trend in the age and distribution of diffuse plaques, when compared to mature plaques. Furthermore, the presence of tau accumulation associated with NFTs remains uncertain, and some could be spontaneously observed in the brains of monkeys with advancing age. Individuals with age spots were found to be somewhat closer to human AD in terms of histological and cognitive behavioral functions, when compared to those with less age spots.

### 2.3 Advantages and limitations of the models

Naturally aged non-human primate models with cognitive impairment can be regarded as natural AD-like models, with obvious advantages that include a natural disease incidence, making it possible to identify biological or environmental factors correlated to AD through careful observation and research. In addition, SPs and tau protein aggregations can also be present at the same time, providing a basis for investigating their role in pathogenesis. One important aspect that can be investigated is the epidemiology of
neuropathy in non-human primates and the possible AD-like cases, which can be useful for testing diagnostic and therapeutic agents that target aggregated forms of Aβ. Furthermore, it is important to understand the similarities and differences of AD-related neuropathology between human patients and non-human primates. Finally, the relationship between normal physiological aging of the brain and pathological age-related neurodegeneration should be studied in non-human primates.

However, spontaneous AD-like models have two main limitations, which are difficult to ignore. First, although AD is somewhat similar to age-related dementia, there is a pathological basis to age-related dementia, which is essentially different from normal aging. Second, there are few sources of aged non-human primates, which when combined with their long lifespan, makes these very expensive. Mouse lemurs have the shortest life expectancy, but these also must be at least 8 years old to have any probability to display the two main pathological features of AD. Therefore, the paucity of positive samples is the main constraint that limits the broader use of spontaneous AD models (Table 1).

| Animal species | Body length | Weight | Lifespan | Age considered as old | Similarity to human AD | Cognitive behaviors | Differences from human AD | Advantages of models | Disadvantages of models |
|----------------|-------------|--------|----------|-----------------------|------------------------|---------------------|--------------------------|-----------------------|------------------------|
| Rhesus macaques | 45-64 cm | 5-12 kg | 34-40 years | 20 years old | Aβ deposits can be seen | Cognitive deficits | Aβ is distributed in the hippocampus, amygdala, olfactory cortex, frontal cortex, temporal lobe and parietal lobe | Include natural pathogenesis | Normal aging is not the same process as AD |
| Stump-tailed macaques | 45-70 cm | 7-12 kg | >30 years | 24 years old | Aβ deposits and accumulation of tau protein can be seen | Partial cognitive deficits | Neurpathological changes with or without cognitive behavioral degeneration | Exhibit senile plaques and tau protein aggregations | Significant individual differences, long breeding cycle, small sample size |

3 | ARTIFICIALLY INDUCED MODELS OF AD IN NON-HUMAN PRIMATES

3.1 | Cholinergic nervous system injury models in non-human primates

3.1.1 | Theoretical basis

The central cholinergic nervous system encompasses cholinergic neurons in the central nervous system and their projecting nerve fibers. The main areas that contain cholinergic neurons are the hippocampus, striatum and cortex. Acetylcholine (Ach) is an important transmitter of the eponymous cholinergic nervous system, which plays roles in learning, memory and similar high-level cognitive functions. In the 1970s and 1980s, the cholinergic hypothesis of AD was proposed. According to this idea, the basis for the cognitive impairment observed in AD patients was the degeneration of cholinergic neurons in the basal forebrain, and loss of cholinergic transmitters in the cerebral cortex and other regions of the brain. The basal forebrain mainly encompasses the gray matter areas near the surface...
of the brain in the anteromedial and basal hemispheres, that is, the ventral basal ganglia, extensions of the amygdala, Meynert’s basal ganglia, the septal nucleus, and Broca oblique zone nucleus. The main changes of neurotransmitters observed in the brains of patients with old-age dementia are correlated to Ach. In normal brains of the elderly, this is especially true for the hippocampus, AchE activity, and the decrease of the muscarinic cholinoreceptor. Scopolamine can reduce the short-term memory ability of normal young probands, who subsequently exhibit symptoms that resemble the early stages of senile dementia. Furthermore, neostigmine, arecoline and other agents that strengthen the postsynaptic activity of cholinergic neurons can also improve short-term memory ability. These observations further support the cholinergic hypothesis of memory impairment in senile dementia. The cholinergic system injury models were established based on this theory, and a number of studies have used central cholinergic nervous system injury to establish AD models.

3.1.2 | Methods for establishing the models and their special features

The cholinergic M-receptor inhibitor scopolamine hydrobromide (SCP) was dissolved in phosphate buffer and subcutaneously injected into marmosets at 0.03 mg/kg, with an injection volume of 1 mL/kg. The control group was administered with the same volume of phosphate buffer. Both groups were independently subjected to spontaneous object-location (SOL) and fear-motivated behavior (FMB) tests. The results revealed that the exploration time of moving objects by animals from the control group was significantly longer, when compared to that of the same stationary object. In contrast, the SCP-treated marmosets exhibited a similar exploration time for both objects. In the FMB experiment, the control group spent significantly less time in the location of the fear stimulus and spent more time on vigilance of the surrounding environment than before exposure. Again, the change in the length of behaviors before and after exposure to the fear-inducing stimulus in the SCP-treated group was not significant. These results show that SCP reduced the short-term cognitive and memory ability of marmosets, which masked any behavioral changes in spatial location (SOL task) and related stimuli (FMB task) before and after exposure. However, SCP can only reversibly block postsynaptic Ach M receptors, and only partially mimic some of the characteristics of AD, such as memory decline, which is again reversible. Another study skillfully utilized the reversibility of the effect of SCP on the cholinergic system to establish an animal model of AD, and investigate the pharmacodynamics of SCP-reversing drugs. The researchers used an SCP dose of 20 µg/kg, and dissolved and subcutaneously injected this into rhesus macaques, in order to establish a cognitive- and memory-impairment model of AD. Subsequently, the model animals were administered with anti-AD drug donepezil at different concentrations and subjected to a delayed matching-to-sample task test to analyze and evaluate the drug’s reversing effect on cognitive impairment. The results were in agreement with the clinical reports, confirming that non-human primate models of AD established using this methodology offer an excellent transitional research subject for the in vivo evaluation of new cognitive enhancement drugs before clinical trials. Other neurotoxic pharmaceutical agents that are often used to destroy the cholinergic system include anisodine, hemicholine hydrobromic acid, kainic acid and immunotoxins (eg ME20.4 IgG-saporin), which are usually injected into the basal Meynert nucleus and ventricle of great apes.

3.1.3 | Advantages and limitations of the models

The advantages of this type of model include the suitability to evaluate the cognitive impacts of drug-induced damage to cholinergic function, and the usefulness as good AD animal models for the preclinical evaluation of cognitive enhancement drugs. However, the side effects of the cholinergic drugs themselves may affect animal behavior, which means that supporting evidence pertaining to appropriate concentrations, dosage and mode of action should be provided as much as possible, in order to ensure the credibility of the experimental data. In addition, from the point of view of behavioral science, it is necessary to observe and record the reactions of animals in various ways, in order to facilitate the follow-up analysis.

The establishment of cholinergic nervous system injury models is relatively simple, but such models also have certain limitations. First, simply destroying only the cholinergic system cannot reflect the complex pathophysiology of AD in its entirety. Second, such models cannot or can only partially mimic the pathological features of AD, with mostly only changes in behavioral aspects. Third, the effects of a number of neurotoxic substances, such as SCP and anisodine, are reversible, which is inconsistent with the irreversible neural damage observed in AD. Finally, the necessary surgical procedures bring harm to these animals.

3.2 | Non-human primate models of AD induced by Aβ injection

3.2.1 | Theoretical basis

Aβ is a fragment of its precursor, the integral membrane protein APP. The processing of APP mainly follows two routes, among which one route is under the influence of α secretase. In this pathway, hydrolysis produces two fragments that do not form deposits, but form the nonamyloid peptide-derived pathway or structural secretion pathway, which is the main pathway of APP processing. The second route functions via the β and γ secretases, in which β secretase hydrolyses the peptide bond between positions 596 and 597 in APP695, while γ secretase cuts the peptide bonds between Aβ39-44, forming the complete Aβ. This is the amyloid peptide-generating pathway. Thus, the formed peptides are mostly Aβ40 and Aβ42, and the latter has the highest propensity to aggregate, and is the major component of SPs. This leaves open the question how Aβ exerts its neurotoxic effect in AD. Another possible explanation was proposed in the form of the Aβ cascade hypothesis. First, the synthesis of Aβ is...
increased, and degradation and clearance are decreased, while the ratio of Aβ42/Aβ40 is increased. Following aggregation, Aβ influences the function of synapses and forms SPs, after which the injury becomes more severe, further disrupting the molecular homeostasis of neurons, leading to damage due to reactive oxygen species. Subsequently, the hyperphosphorylation of tau occurs, which finally leads to large-scale neuronal dysfunction and impairment of signal transmission. Some studies have also reported that the deposition of Aβ can lead to changes in presynaptic structure and function, such as the slowing down of the bidirectional transport of mitochondria in axons, the decrease in the number of presynaptic vesicles, the increase in vacuoles, and worsened synaptic transmission fatigue.

3.2.2 | Methods for establishing models and their special features

One method uses a brain-stereotactic apparatus to microinject insoluble and fibrous Aβ at a concentration consistent with the plaque formation (200 pg) into the cerebral cortex of old (25-28 years old) rhesus monkeys. The control group was injected with phosphate buffered saline. At and surrounding the injection site, a large-scale loss of neurons was visible, the abundance of phosphorylated tau protein increased, and microglia proliferated. Regardless of whether the injection contained Aβ40 or Aβ42, the results were comparable in all cases. In contrast, when the same concentration of Aβ was injected into the brains of young (5 years old) rhesus monkeys, there was no obvious neurotoxicity. Researchers have also applied the same methodology to old marmosets and old rats, and it was revealed that the toxicity of Aβ is greater for old rhesus monkeys than for marmosets, but there was almost no obvious neurotoxicity in the rodent model for old rats.

An injection of soluble Aβ (1-40) was also used to investigate the neurotoxicity. Under the guidance of a stereotactic instrument, Aβ (1-40) was injected into the prefrontal cortex of old rhesus monkeys, while the control groups were injected with nontoxic peptides, peptide fragments (CA4) and antisense Aβ (1-40). The results revealed that the Aβ (1-40) group developed cerebral cortex injury, which was dose-dependent and obviously larger than that in the control group. Furthermore, young monkeys did not develop visible changes of neuronal bodies or axons. These experiments corroborate the neurotoxic effect of Aβ in the brain tissues of non-human primates, and reveal that the cytoskeletal response to Aβ is specific and age-related.

3.2.3 | Advantages and limitations of the models

Alzheimer’s disease models established via the direct injection of Aβ into the brain have certain advantages, since these can be used to directly produce amyloid deposits, which are a classical neuropathological change observed in AD. Furthermore, research has shown that the toxicity of insoluble Aβ is age-related, with brains from older individuals showing a stronger reaction. This indicates that this approach can be used to investigate factors that sensitize the brain to Aβ toxicity. Research has also revealed that Aβ has highly species-specific features in vivo, and that its toxicity is greater in rhesus monkeys than in other experimental species. Consequently, it may be pertinent to concentrate on rhesus monkeys in future research.

Nevertheless, these types of models also have certain disadvantages. First, the successful establishment of the model requires that Aβ reaches a certain concentration, and consequently produces damage at the injection site and the surrounding tissue. Second, the precision required for the injection site is quite high, which requires experienced surgical personnel. Finally, the success or failure of the model establishment is influenced by many factors, such as the Aβ form, its purity, and the vehicle form and components, and the requirement for aged animals also somewhat constrains related research.

3.3 | Non-human primate models based on intrinsic formaldehyde

3.3.1 | Theoretical basis

Formaldehyde is the simplest aldehyde molecule. It is colorless, and has a pungent odor. Furthermore, it has a comparatively high toxicity, and has been registered as a confirmed carcinogen and teratogen by the World Health Organization. In addition, it is a widely known allergen, and one of the strongest potential mutagens. In recent years, it has received increasing attention both in China and internationally. Research has shown that in addition to environmental sources, it can also be produced by biological factors, including the metabolism of bacteria, animals and plants. This type of formaldehyde, which can be detected within the body of living organisms, is termed intrinsic formaldehyde.

In the human body, many pathways can also produce intrinsic formaldehyde, with enzyme-catalyzed reactions being the most important. For example, semicarbazide-sensitive amine oxidase produces formaldehyde by converting histamine, polyanine and methylamine in vivo. DNA demethylases produce formaldehyde by demethylating DNA as part of their role in controlling transcription activity in the nucleus. Mitochondrial cytochrome P450 enzymes can also produce formaldehyde by oxidizing extrinsic chemicals. Under physiological conditions, intrinsic formaldehyde is a methyl donor that takes part in the folic acid cycle, DNA methylation, genetic modification, control of gene expression and other diverse cell biological processes, and its concentration is maintained in a state of equilibrium. In order to maintain the balance of formaldehyde concentration, the body also has a set of highly efficient endogenous formaldehyde removal systems. Formaldehyde can be enzymatically converted to formic acid, which is excreted via urine, or converted to CO₂ and removed via respiration. Furthermore, it can be converted through nonenzymatic reactions, and can alkylate proteins, DNA and RNA, which can influence the normal gene expression, or the structure and function of proteins. The accumulation of endogenous formaldehyde can also lead to chronic damage to the nervous system, which can lead to learning...
and memory impairment.\textsuperscript{67,68} Formaldehyde is therefore considered a possible factor that can induce AD, which can mainly occur through the following pathways. First, formaldehyde can damage the balance of neurotransmitters. Research has shown that the amount of monoamine neurotransmitters in hippocampus tissues of mice increases under the influence of formaldehyde, while the content of glycine significantly decreases.\textsuperscript{58,65} Second, in vitro experiments have shown that formaldehyde can induce tau protein misfolding, which leads to the formation of spherical amyloid aggregates that damage the cell membrane, leading to cellular abnormalities and cell death.\textsuperscript{70} Third, intrinsic formaldehyde can influence long-term potentiation (LTP) in the hippocampus, since pathological concentrations of formaldehyde can inhibit the N-methyl-D-aspartic acid receptor, and in turn inhibit hippocampal LTP and cause a decrease in learning and memory ability.\textsuperscript{71} Finally, intrinsic formaldehyde influences DNA methylation, and at increased concentrations, it can destroy the coding of long-term memory-related proteins and DNA methylation patterns in the cortex, induce the apoptosis of hippocampal and cortical neurons, and lead to memory decline. Wang et al\textsuperscript{72} discovered that the formaldehyde concentrations in the urine of elderly (>65 years old) patients with postoperative cognitive dysfunction (POCD) were significantly higher than in patients without POCD. Tong et al and He et al\textsuperscript{73,74} analyzed the levels of intrinsic formaldehyde in the urine of elderly and young probands. It was revealed that the concentration of formaldehyde increases with age, and that urine formaldehyde levels are positively correlated with the severity of AD symptoms. The hypothesis of urinary formaldehyde stress was put forward to explain that endogenous formaldehyde is one of the risk factors for the occurrence and development of Alzheimer's disease.

### 3.3.2 Methods for establishing the models and their special features

In one study, young rhesus monkeys (3-5 years old) were chronically fed with diluted methanol (3%), and the variable spatial delayed response task was used as a cognitive behavioral testing method to determine the learning and memory capacity of experimental animals in regular intervals.\textsuperscript{17} It was discovered that rhesus monkeys that were fed with methanol for 3 months displayed a marked decline in cognitive capacity. At the same time, the immunohistochemical investigations of various brain regions revealed a substance reactive to antibodies against A\textsubscript{β}, as well as the accumulation of abnormally phosphorylated tau protein within neurons. Methanol is considered to be easily converted to formaldehyde under the action of alcohol dehydrogenase in vivo, which in turn exerts a toxic effect.\textsuperscript{75}

Researchers\textsuperscript{76} have also obtained the location coordinates of lateral ventricles based on the brain maps and/or magnetic resonance imaging data of experimental animals, after which catheters of drug delivery devices were implanted into the left and right ventricles of the animals, and were uniformly injected with a specially prepared formaldehyde-containing solution at a daily dose of 100 µL. After its continued administration for 12 months, a non-human primate model of AD was obtained.

At present, there are already many publications on the establishment of cognitive impairment models in rodents using formaldehyde.\textsuperscript{77,78} However, corresponding research on non-human primates is rare. A study conducted by Li et al\textsuperscript{79} revealed that placing mice in an atmosphere that contains gaseous formaldehyde vapor leads to the development of behaviors indicative of depression and anxiety, as well as cognitive decline. Tong et al\textsuperscript{71} continuously injected formaldehyde into the abdominal cavity of rats, or injected high doses of formaldehyde into the rat hippocampus,\textsuperscript{73} which both induced cognitive disability and memory decline, and the latter was correlated to aging.

### 3.3.3 Advantages and limitations of the models

Alzheimer’s disease models in non-human primates established through chronic methanol feeding represent a comparatively simple method, while the chronic injection of methanol into the lateral ventricle also overcomes the inability of rodents to simulate the deposition of hyperphosphorylated tau protein. The experimental scheme of rodent modeling can be further improved by greatly increasing the utilization of the great genetic similarity between non-human primates and humans, as well as their highly similar neuroanatomy and neurophysiology, thereby simulating the toxic injury due to the imbalance of formaldehyde metabolism in vivo. This can further be used in studies on cognitive impairment.

Nevertheless, this modeling methodology still has some shortcomings. First, the liquid intake differs between individual animals. Hence, the time needed for model generation and level of toxicity for each individual varies and uniformity of model generation cannot be easily meet. Second, methanol itself has certain toxic side effects, which may influence the experimental results. Finally, drug administration into the lateral ventricle can lead to brain injury. Furthermore, since the administration period is comparatively long, the extent of injury is difficult to assess.

### 3.4 Using streptozotocin to induce non-human primate models of AD

#### 3.4.1 Theoretical basis

Streptozotocin (STZ) is a methyl nitrosourea sugar compound with a molecular weight of 265 Da, and antimicrobial and antitumor effects. However, this can also induce diabetes as a side-effect. Peripherally, STZ can selectively destroy islet β cells, leading to type II diabetes. Diabetic encephalopathy (DE) is a severe complication of diabetes, and affects the central nervous system. It is characterized by cognitive impairment, a decline in learning and memory ability, pathological changes in brain structure, and neurophysiology and neuropsychiatric symptoms. Epidemiological studies have shown that cognitive impairment and neurodegenerative diseases (such as AD) occur with higher probability in patients with advanced diabetes mellitus.\textsuperscript{80}
Among other factors, insulin resistance (IR) plays an important role in the incidence and development of diabetes mellitus. Insulin is a small protein secreted by pancreatic β cells. It interacts with the insulin receptor to activate the eponymous signaling pathway, and exerts its biological function. The impairment of the insulin signaling pathway can lead to the development of IR. The insulin receptor is a transmembrane glycoprotein that is abundant in both the neurons of the central nervous system and glial cells. IR refers to the need for excessive amounts of insulin to maintain its normal physiological function, which is mainly caused by the decrease in sensitivity of the insulin signaling pathway.

The intracerebroventricular injection of STZ (ICV-STZ) can disturb the phosphorylation of the insulin receptor in the central nervous system, which blocks the insulin signaling pathway. Furthermore, this leads to reduction in function of the cholinergic nervous system, which in turn presents as a reduction in learning and memory ability. At the same time, the dysfunctional expression of insulin degrading enzyme prevents the timely and efficient clearance of Aβ, leading to its accumulation. A blockage in the insulin signaling pathway leads to increase in the activity of its downstream target GSK-3β, and induces tau hyperphosphorylation, which is a classical feature of AD.

3.4.2 Methods for establishing the models and their special features

Yeo et al. used an ICV-STZ to establish an AD model in crab-eating macaques. The method relies on the injection of a small dose (2 mg/kg) of STZ dissolved in artificial cerebrospinal fluid. The results revealed that ICV-STZ induced severe ventricular enlargement and substantial brain atrophy, accompanied by Aβ deposition, loss of neurons in the hippocampus, tau hyperphosphorylation, loss of ependymal cells and astrocytes, and the activation of microglia. These changes were all also observed in the brains of elderly or AD patients, suggesting that ICV-STZ monkey models may be a valuable resource for studying the mechanism and treatment of brain lesions, such as AD or age-related diseases.

Yeo et al. used the ICV-STZ method to successfully establish an AD model, and used fluorodeoxyglucose positron emission tomography (FDG-PET) together with magnetic resonance imaging (MRI) to investigate the brain glucose metabolism. They discovered that the effect of STZ is region-specific, in which the glucose metabolism in the cortical region was similar to that of early-stage AD patients. This further supports the feasibility of using this type of model to investigate the pathological mechanisms of AD incidence. Park et al. used an ICV-STZ AD model in crab-eating macaques to quantitate the expression of genes related to APP and tau phosphorylation. The results revealed similar gene expression levels in the ICV-STZ and control groups, but there were significant differences in gene expression patterns between these two groups.

Although STZ has been used to establish AD models in rodents in increasing numbers of studies, corresponding research in non-human primates remains limited. One study used intraperitoneal STZ injections in APPswe/PS1 mice to establish an animal model of comorbid AD with type II diabetes. This is of special significance for investigating the mechanistic interplay between the development of these two diseases. It has also been reported that ICV-STZ was used to induce a mouse model of AD, in order to investigate the selenium-containing ATZ derivative, selenothymidine (S1073). The results demonstrated that it has a neuroprotective effect, which may be correlated to the antioxidant activity of S1073 and/or its inhibitory effect against cerebral pain. This study confirms that the ICV-STZ methodology is a good model for pharmaceutical screening.

3.4.3 Advantages and limitations of the models

The advantage of non-human primate models of AD induced using ICV-STZ is that these are nontransgenic models based on the novel theory of the insulin-resistant state of the brain. Furthermore, it is very important to study sporadic AD with higher incidence. In addition, it is also possible to adopt the advances made in rodent models, and use the intravenous injection of STZ for the establishment of a non-human primate model of AD, in order to study the mechanistic relationships between age-related dementia, AD and the specific mechanism of IR. These models can also be used to screen and confirm neuroprotective drugs.

The limitations of the above-described models include the need for surgical procedures that cause penetration injury, which has a certain effect on the brain, and the continuing controversy regarding the validity of the theoretical basis of this model. Thus, more evidence from studies on experimental animals is needed to provide a solid basis for this theory.

3.5 AD models in non-human primates based on genetic engineering

3.5.1 Theoretical basis

The use of genetic engineering to establish animal models is mainly based on genetic modification and gene targeting. Genetic modification entails the artificial insertion of heterologous genetic constructs into the animal’s genome, allowing it to be stably inherited. The main manipulation techniques include microinjection, embryonic stem cell technology and lentiviral transfection. Gene targeting technology uses endogenous site-directed DNA recombination to change a specific locus in the genome, and investigate the function of the gene in question in vivo. These techniques can be used either for gene knockout, or gene knock-in. Traditional gene targeting technology relies on embryonic stem cell lines, with a first step encompassing gene targeting in cultured cells, followed by the introduction into a foster mother, and the delivery of model animals with targeted genetic modifications. However, the lack of appropriate embryonic stem cell lines in non-human primates implies that this methodology cannot be readily used. Nevertheless, the emergence of artificial nuclease-mediated genome editing technology...
represented by TALENs and CRISPR/Cas9 technology has made it possible to target genes directly in embryonic cells, making it possible to achieve accurate genetic modification in primates.\textsuperscript{88} CRISPR/Cas9 technology was derived from a bacterial immune mechanism, in which an RNA guides the Cas protein to edit a specific sequence. This technology is practical, simple, and can produce highly efficient gene knockouts, knock-ins, or targeted expression interference.\textsuperscript{89}

### 3.5.2 Methods for establishing models and their special features

In January 2001, American scientists published a manuscript in Science on the world’s first genetically modified rhesus monkeys. They used lentiviral transfection to successfully introduce a green fluorescence protein (GFP) into early rhesus monkey embryos, and used embryo transplantation to obtain genetically modified monkeys with stable integration and expression of GFP.\textsuperscript{90} This was the first successful attempt of using genetic engineering in non-human primates. In 2008, Yang \textit{et al}.\textsuperscript{91} successfully constructed a genetically modified rhesus monkey model of Huntington’s disease. The authors linked 84 CAG repeats to the first exon of the human HTT gene, and packaged the resulting construct into high-titer lentiviral vectors (10\textsuperscript{9} PFU/mL), followed by oocyte transfection, single-sperm fertilization and embryo transfer. This was the first example of a successfully established, genetically modified non-human disease model. Two Chinese research groups collaborated closely to adapt CRISPR/Cas9 technology for use in primates, and successfully produced the first line of crab-eating macaques with a targeted gene knockout, proving that CRISPR/Cas9 is indeed well-suited for primates, and produces viable animals.\textsuperscript{92} At the same time, a Chinese research team produced the first genetically modified model of Parkinson’s disease in monkeys in 2015.\textsuperscript{93} Genetic modification models of non-human primates are rarely used in AD model construction. However, genes closely correlated to AD pathogenesis (such as APP, PSEN1, PSEN2, APOE, etc) can be used as potential target genes for gene modification for modeling. Meanwhile, tau protein-related genes can also be regarded as an important target for the modeling of gene modification methods.

### 3.5.3 Advantages and limitations of the models

The development of the non-human primate disease models described above has laid a solid basis for further research. Attempts can be made to learn from the advantages of the genetically modified models of other diseases, especially regarding the use of the novel CRISPR/Cas9 gene editing technology. Developing more non-human primate models of AD would provide indispensable tools for studying the role and mechanism of genes involved in the pathogenesis and development of diseases.

The development of genetically modified models in non-human primates has certain limitations. First, ethical considerations restrict the number of available animals. Second, the efficiency of available

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**Table 2: Artificially induced models of AD in non-human primates**

| Model type | Method | Features | Advantages | Disadvantages | Applications |
|------------|--------|----------|------------|---------------|--------------|
| Cholinergic nervous system injury models | Cholinergic neurotoxic drug injection, operation | Memory impairment, partially simulates the pathological features of AD | Simple | Partially simulates AD, the effect is reversible | Evaluation and screening of drugs related to cognitive function |
| Aβ injection-based models | Microinjection of soluble Aβ into the cerebral cortex | Aβ production, neuron destruction, microglial hyperplasia, cognitive impairment | Simple | Aβ is produced directly | Research on Aβ pathogenesis |
| Models induced by endogenous formaldehyde | Oral methanol, intraventricular injection of formaldehyde | Cognitive deficits, Aβ, tau | Simple and accurate | Surgical injuries, Aβ tau, cognitive deficits | Applicable to research on SAD |
| Models induced by streptozocin | ICV-STZ, intraperitoneal injection | Aβ, tau, cognitive deficits | Simple and accurate | Surgical injuries, Aβ tau, cognitive deficits | Applicable to research on SAD |
| Genetic modification models | Transgenic, gene targeting | Precise gene modification, showed typical clinicopathological features | Simple and accurate | Ethical issues, high price, few AD-related models | Gene-related pathogenesis |
gene-transfer technologies remains low. Hence, it is still inconceivable to develop accurate technical routes for routinely obtaining large numbers of genetically modified animals, similar to the case in mice and rats. Finally, when using lentiviral vectors to introduce exogenous genes, the unpredictable nature of genomic integration and the variable suitability of different loci for gene expression lead to a certain degree of expression instability in the corresponding genetically modified animals.

4 | CONCLUSION

Although non-human primates share a great level of similarity with humans in terms of the neuroanatomy and neurophysiology, and are thereby considered ideal model animals for AD, all present modeling methods still retain some limitations. These naturally aged models are very helpful for studying the bio-environmental factors that influence the pathogenesis of AD, but not all aging animals form spontaneous models. Consequently, the establishment of such models requires great amounts of time and capital, and the resulting paucity of positive samples is the main limitation. Artificially induced models (Table 2), either cholinergic system injury, Aβ injection, or ICV-STZ, each offer only a partial simulation based on a specific hypothesis of AD onset. Furthermore, all require surgery, and thereby induce brain injury, which can influence the results. In comparison, the oral administration of pharmaceutical agents for the model induction mediated by intrinsic formaldehyde is simple, and does not suffer from the side effects of surgery. Therefore, this deserves deeper research and further method development. The use of genetic engineering to establish animal models of AD modifies the animals at the genetic level, and is presently the most reliable animal modeling methodology. With the constant improvement of gene editing technology in recent years, genetic modification has become possible both in embryos and adult animals. Nevertheless, there is still a lack of corresponding genetically modified models of AD. Consequently, constructing such models would be one of the main focuses of the investigators in going forward. It is also noteworthy that non-human primates possess complex cognitive abilities and social behaviors that are similar to those of humans, and these can be used to assess learning, memory and complex interactions in a way that is highly important for human patients.24 Therefore, further efforts should be made to establish non-human primate models of AD for the convenience of studying this devastating disease for which a truly efficacious treatment regimen would be hopefully found in the future. Despite many advances, there is a long way to go in translating the knowledge gleaned from animal models into clinical praxis.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

HWL conceived and wrote the original draft of the manuscript. LZ and CQ revised the manuscript. All authors critically read and contributed to the manuscript, and approved the final version.

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