Synthetic amyloid-β oligomers drive early pathological progression of Alzheimer’s disease in nonhuman primates

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Highlights
- The Aβ oligomers (AβOs) drive to develop massive Aβ plaque in the monkey brain.
- Neurofibrillary tangles form in multiple brain regions of AβO-monkeys.
- The co-occurrence of amyloid and tau pathology in AβO-monkeys as in patients with AD.
- The neuroinflammation and neurodegeneration are triggered in AβO-monkeys.

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Synthetic amyloid-β oligomers drive early pathological progression of Alzheimer’s disease in nonhuman primates

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SUMMARY
As an insidious and slowly progressive neurodegenerative disorder, Alzheimer’s disease (AD) uniquely develops in humans but fails in other species. Therefore, it has been challenged to rebuild human AD in animals, including in non-human primates. Here, we bilaterally delivered synthetic Aβ oligomers (AβOs) into the cerebral parenchyma of cynomolgus monkeys, which rapidly drove the formation of massive Aβ plaques and concomitant neurofibrillary tangles in the cynomolgus brain. The amyloid and tau pathology as well as their co-occurrence in AβO-monkeys were reminiscent of those in patients with AD. In addition, the activated astrocytes and microglia surrounding Aβ plaques indicated the triggered neuroinflammation. The degenerative neurons and synapses around Aβ plaques also emerged in cynomolgus brain. Together, soluble AβOs caused the cascade of pathologic events associated with AD in monkeys as occurred in patients at the early phase, which could facilitate the development of a promising animal model for human AD in non-human primates.

INTRODUCTION
Being an aging-related neurodegenerative disorder, Alzheimer’s disease (AD) is characterized by β-amyloid (Aβ) plaques and neurofibrillary tangles at early stages and neuronal loss at late stages. Owing to the biological changes and environmental influences, such as genetics, epigenetics, lifespan, and metabolism, humans are extremely susceptible to AD (Rosen et al., 2016; Walker and Jucker, 2017). In general, other species, such as rodents, are naturally resistant to develop AD even as they grow old. Currently, AD is considered a multifactorial syndrome that is unique to humans and hard to replicate in other species. The various lines of AD mice that have been widely used fail to fully reproduce AD-associated pathological features, such as neurofibrillary tangles (Drummond and Wisniewski, 2017; LaFerla and Green, 2012). The generation of an appropriate mouse model to authentically recapitulate pathological hallmarks of human AD has still proven challenging. Consequently, the understanding of the neuropharmacology, biochemistry, and molecular biology of AD that relied heavily on animal models has been hampered.

It has been well documented that non-human primates share the closest similarity with humans in terms of genetics, brain structure and function, and lifespan (Sereno and Tootell, 2005). The cynomolgus monkey (Macaca fascicularis) shares 100% sequence homology of amyloid precursor protein (APP) 695, and of course Aβ 40/42 peptides, with humans (Podlisny et al., 1991). In particular, some aged primate species naturally display age-related changes that are reminiscent of AD, such as Aβ plaques (Cramer et al., 2018; Edler et al., 2017; Oikawa et al., 2010; Selkoe et al., 1987). Therefore, non-human primates possess distinct suitability in reproducing AD pathological changes that no other animal species have provided. Re-building the AD-like conditions in primate species might yield more informative clues that triggered this human unique brain disorder.

Previous studies showed that the injection of brain homogenates or Aβ fibrillar from patients with AD caused scattered Aβ deposits near injection sites in the cortex of aged rhesus or marmosets (Baker et al., 1993; Geula et al., 1998). However, tau tangle was not detected in these monkeys with AD brain homogenates or insoluble Aβ fibrillar. Later on, Aβ oligomers (AβOs), small soluble and diffusible ligands derived from Aβ peptides, were identified as the most potent neurotoxins in AD brain and played a pivotal...
role in AD pathogenesis (Haass and Selkoe, 2007; Shankar et al., 2008; Viola and Klein, 2015; Walsh et al., 2000). Subsequently, extensive evidence confirmed that soluble AβOs rapidly stimulated tau phosphorylation in vitro and induced neuron degeneration as well as synapse loss in the brain of mouse and rat (Bruijlette et al., 2012; De Felice et al., 2008; Lambert et al., 1998; Lesné et al., 2006; Malm et al., 2006). However, the attempts to test the effects of soluble AβOs in non-human primates in vivo have been sparse. Several years ago, a report has showed that delivering soluble AβOs into the lateral ventricles of macaques caused tau hyperphosphorylation but failed to induce the formation of Aβ plaques in the monkey brain (Forny-Germano et al., 2014). Other than that, the neurofibrillary-like structures detected in these macaques were morphologically different from those in patients (Forny-Germano et al., 2014). Recently, the same procedures performed in rhesus monkeys still failed to induce the amyloid plaques and tau pathology, which only caused the loss of dendritic spines similar to that observed in normal aged monkeys (Beckman et al., 2019). Therefore, it remains largely uncertain whether AD-associated features can be reproduced in non-human primates upon brain administration of synthetic AβOs.

Here, we show that the repeated AβO injections into the cerebral parenchyma rapidly caused massive Aβ plaques, evident neurofibrillary tangles, profound neuroinflammation, as well as selective neurodegeneration in adult cynomolgus monkeys, indicating that the progression of AD, at least the classical neuropathological features of patient at early phase, were reproduced in non-human primates. Moreover, our evidence implicated the correlation between the AβO-stimulated Aβ plaque development and subsequent tau tangle formation in cynomolgus brain. These results suggested that AβO-monkeys could serve as a promising research model for uncovering the pathogenetic events of AD.

RESULTS
Massive Aβ plaques developed in brain of AβO-induced cynomolgus monkeys as in patients with AD

To determine whether the synthetic Aβ oligomers (AβOs) could induce pathological features associated with AD in non-human primates, we recruited 14 adult cynomolgus monkeys (7 in experimental group and 7 in control group) that were around 20 years old from the same colony (Table S1). Instead of lateral ventricle as previously reported (Forny-Germano et al., 2014), we developed a new injection assay and bilaterally delivered soluble AβOs to cerebral parenchyma, the white matter region adjacent to the dorsal and lateral hippocampus of cynomolgus monkeys. A total of 800 µg AβOs were delivered into each cynomolgus brain via four injections over 5 months (Figure 1A). The oligomerization of commercially synthetic human Aβ1-42 peptides was performed before each injection, and the freshly prepared AβOs were analyzed by western blotting before and after each injection. The blotting data revealed that Aβ1-42 peptides consistently polymerized into soluble, low-molecular-weight oligomeric forms, mainly including dimers (~8 kDa), trimers (~12 kDa), and tetramers (~16 kDa) (Figure 1B), which are among the most neurotoxic AβO species detected in the cerebrospinal fluid from patients with AD (McLean et al., 1999; Shankar et al., 2008). The dot blot using both Aβ oligomeric and fibrillary antibodies revealed that these low-molecular-weight assemblies of Aβ1-42 were soluble fibrillar oligomers (Figure 1C). Magnetic resonance imaging (MRI) scanning showed that the synthetic AβOs were successfully delivered to the targeted parenchymal sites (Figure S1A).

As soon as 8 months after the fourth AβOs injection, we started to examine the neuropathological features of AD in AβO-induced cynomolgus brains. Immunohistochemistry with 6E10 was performed to detect possible amyloid deposits on brain sections from all 14 cynomolgus monkeys. To cover as many brain regions as AD affected, at least four serial sections from the anterior to posterior of each cynomolgus cerebrum were examined (Figure S1B). We screened the whole brain sections and found that Aβ plaques intensely and specifically labeled by 6E10 spread to fill much of the striatum and prefrontal cortex (PFC) (Figures 1D and S1C); the frontal cortex (IFC), striatum, temporal cortex (TC), and parietal cortex (PC) (Figures 1E and S1D); the PC, hippocampus (HPC), entorhinal cortex (EC), and TC (Figures 1F and S1E); and the PC, HPC, and TC (Figures 1G and S1F). In general, the Aβ plaques were mainly distributed in gray matter regions of limbic structures and association cortex of cynomolgus monkeys as in patients. Most plaques were compact with a dense core or were small and partially compact, similar to the typical senile plaques in the cortex of patients with AD (Figures 1D–1G and S1C–S1F). The diffuse plaques and finely granular deposits were also observed in numerous brain regions (Figures 1D and 1E). The density of Aβ plaques was distinct from brain region to region and also from monkey to monkey, but all seven AβO-monkeys exhibited similar spatial patterns of amyloid deposition throughout the brain (Figures 1D–1G and S1C–S1F). In contrast, the brain sections from seven control cynomolgus monkeys remained relatively free of Aβ plaques.
Figure 1. The development of Aβ plaques in AβO-monkeys

(A) Schematic diagram of delivering AβOs into the parenchyma of cynomolgus monkeys (n = 7).

(B) Western blot analysis of synthetic AβOs with the monoclonal antibody 6E10 immediately prior to each injection.
or occasionally harbored a few distributing in some limited brain regions, such as TC (Figure 1H). We counted the Aβ plaques and found that three of the seven AβO-monkeys displayed massive and highly clustered Aβ plaques (Figures 1D–1G), two contained moderate numbers of Aβ plaques (Figures S1C–S1F), and the rest two had a few plaques. The number and burden of Aβ plaques in seven AβO-monkeys were apparently higher than those in seven control monkeys, indicating that synthetic AβOs can efficiently drive Aβ deposition in the brain of cynomolgus monkeys (Figures 1I and 1J).

To confirm the formation of AD-like Aβ plaques in AβO-monkeys, the Aβ plaques in monkey brain were carefully characterized by different measurements. The immunostaining with the most frequently used Aβ plaque-specific antibodies, 6E10 and 4G8, revealed multiple plaque subtypes in AβO-monkeys as in patients with AD, such as compact plaques mostly having clear-cut outlines (Figures 2A–2D, left panels), cotton-wool plaques (Figure 2A, right panel), cored plaques with miliary focus (Figure 2C, right panel), as well as diffuse plaques usually displaying ill-defined surfaces and flake-like deposits (Figures 2B and 2D, right panels), indicating that Aβ plaques in the cortex of AβO-monkeys morphologically and immunoreactively resembled those in patients with AD. In addition, the Aβ plaques in AβO-monkey brain were recognizable by other standard measurements that are usually performed to detect Aβ plaques in the patient brain, including silver (Figure 2E), Thioflavin S (Figure 2F), and Congo Red staining (Figure 2G). Aligned with the immunostaining results, the western blot with 6E10 detected Aβ-positive bands in FC and TC of AβO-monkeys, which was similar to the band from 5XFAD mouse brain with Aβ plaques (Figure 2H). There was no detectable Aβ-band from brain tissues of control monkeys (Figure 2H), which was consistent with the previous findings (Oikawa et al., 2010). The cynomolgus monkeys were proven to display age-related increases in Aβ plaques. Yet in brains of cynomolgus monkeys around 20 years old, the Aβ plaques were absent by western blot analysis or unfrequently detected in limited brain regions by immunohistochemistry (Oikawa et al., 2010). Together, Aβ plaques quickly developed in the cortex of cynomolgus monkeys upon AβO administration, which was similar to the Aβ deposits abundantly present in patients with AD and indicative of the amyloid pathology in the AβO-monkeys.

**Overt neurofibrillary tangles formed in multiple brain regions of AβO-induced cynomolgus monkeys**

Microtubule-associated protein tau is abnormally phosphorylated in AD brain and aggregates as paired helical filaments (PHFs) in neurofibrillary tangles that is another hallmark of AD (Lee et al., 2001). Then, we detected whether AβO-monkeys captured neurofibrillary tangles or dystrophic neurites in the brain. Similar to what was performed for the measurements of Aβ plaques, serial sections from anterior to posterior cynomolgus cerebral cortex were performed and examined by different hyperphosphorylated tau antibodies against PHFs. Since the various transgenic mice that have been widely used failed to develop tauopathy, it was exciting to observe neurofibrillary tangles reactive with AT8 (Figure 3) and AT100 (Figure S2) in multiple brain regions of AβO-monkeys, such as PFC, PC, TC, EC, striatum, HPC, thalamus, and medium septum. The developed neurofibrillary tangles in AβO-monkeys were confirmed by a silver-based histological method that was routinely used to visualize tau tangles in patients with AD (Figure S3). The filamentous aggregations with densely immunoreactive signals were generally detected in the cell soma (arrows) or the long axons (arrowheads) and outstretched dendrites, which were morphologically reminiscent of those in the brain of patients with AD (Figures 3 and S2). Apparently, the neurofibrillary tangles consisting of PHF in AβO-monkeys were mostly intracellular. The accumulation of AT8+ PHF was detected in the cytoplasm of either NF-H+ neurons (Figures 3A–3C) or GFAP+ astrocytes (Figures 3D and 3E).
The general shape of tangle-bearing neurons or astrocytes appeared normal (Figures 3 and S2), exhibiting detectable nucleus (Figures 3C and 3E). The more detailed characterization revealed that the neurofibrillary tangles in AβO-monkeys developed typical structures as those in patients with AD, such as bundles of convoluted filaments in the cell soma (Figure 4A, arrows) or droplet-like inclusions appearing like a string of pearls in long axons (Figures 4A and 4B, solid arrowheads). Some neurons displayed nonfibrillar punctate regions in the cytoplasm with diffuse staining, the typical features of pre-tangle state in the brain of patients with AD (Figures 4A and 4B, stars). Neuropil threads with breakdown of dendritic and axonal structures were found in multiple brain regions, such as hippocampus and PC (Figures 4A and 4B, empty arrowheads). Therefore, the neurofibrillary tangles in AβO-monkeys acquired the features of typical tangles described in patients with AD (Augustinack et al., 2002). The classic neuritic plaques are defined as combined deposits consisting of insoluble Aβ with dystrophic neurites that contain aggregated tau, which develop only in the late phase of the disease process (Braak and Del Tredici, 2015). Some Aβ plaques in the brain of AβO-monkeys occurred with AT8⁺ dystrophic neurites (Figure 4C). These plaques displayed amyloid cores and surrounded neurites, resembling the neuritic plaques in patients with AD.

It is noteworthy that the naturally occurring human-like neurofibrillary tangles with PHF have never been definitively identified in aged primate species, even in those with advanced Aβ deposition (Härtig et al., 2000;
Figure 3. The formation of neurofibrillary tangles (NFTs) in AβO-monkeys

(A and B) Immunostaining analysis for detecting NFTs from anterior to posterior brain sections of AβO-monkeys and representative brain regions containing AT8-positive tau tangles in neurons (immunohistochemical images in A and immunofluorescent images in B). The arrows indicate NFTs in soma bodies, and arrowheads indicate long axons with droplet-like staining.

(C) The double immunostaining with AT8 and neuronal antibody NF-H.

(D) Immunohistochemical analysis for detecting NFTs from anterior to posterior brain sections of AβO-monkeys and representative brain regions containing AT8-positive tau tangles in astrocytes.

(E) The double immunostaining with AT8 and astrocyte antibody GFAP. Scale bars: 100 μm (B), 50 μm (A, C, and D), and 25 μm (E). Abbreviations: PFC, prefrontal cortex; PC, parietal cortex; TC, temporal cortex; EC, entorhinal cortex; HPC, hippocampus; MS, medium septum. See also Figures S2 and S3.

Oikawa et al., 2010). The only exception is a 41-year-old chimpanzee that had a left hemisphere stroke, which displayed human-like tau tangles but without the typical AD distribution (Rosen et al., 2008). Consistent with the paucity of neurofibrillary tangles in aged cynomolgus macaque, we did not detect any tau tangles or abnormally phosphorylated tau in the seven control monkeys (Table 1). In general, AβO-induced neurofibrillary tangles in cynomolgus brain were scattered and not as massive as Aβ plaques. The neurofibrillary tangles were observed in six of the seven AβO-monkeys, showing an apparent correlation between the formation of tau lesions and Aβ plaques (Table 1). The soluble AβOs caused two classical AD signatures in the brain of monkeys, arguing in favor of the amyloid-cascade hypothesis for human AD. Together, we detected the formation of intracellular neurofibrillary tangles that are strikingly reminiscent of tauopathy in patients with AD and, therefore, identified a co-occurrence of amyloid and tau pathology in AβO-monkeys.

Pronounced neuroinflammation was triggered in the brain of AβO-induced cynomolgus monkeys

In addition to Aβ plaques and tau pathology, neuroinflammation is another key neuropathological feature of AD and is characterized by the presence of activated astrocytes and microglia surrounding Aβ plaques (Wyss-Coray and Mucke, 2002). Immunostaining analysis revealed that the GFAP+ astrocytes preferred to accumulate in the white matter region of cortex from both control and AβO-monkeys (Figure 5A). The activated astrocytes exhibiting much larger soma and a more complex morphology were often found in the cortex of AβO-cynomolgus brains (Figures 5A and 5B). The total number of astrocytes in brain regions measured was much higher in AβO-monkeys than in controls (Figure 5C). Some activated astrocytes were detected to surround Aβ plaques in some brain regions of AβO-monkeys, such as PFC and PC (Figure 5D). In general, the spatial distribution of Iba1+ microglia spread over the entire cortex of monkey and an abundant of microglia were observed in the gray matter regions of the AβO-monkeys (Figure 5E), which was distinct from that of the astrocytes (Figures 5A and 5B). The activated microglia with larger soma and more complex morphology showed an increased tendency in some brain regions of AβO-monkeys (Figures 5F and 5G). Quite a number of activated microglia surrounded Aβ plaques and drove the neuroinflammation in multiple brain regions of AβO-monkeys, such as PFC, EC, PC, and HPC (Figure 5H). This was distinct from that observed in the brains of the control monkeys (Figure 5I). Compared with the astrocytes, more microglia were activated by and situated within or near the core of Aβ plaques in AβO-monkeys. The activated microglia and astrocytes intimately surrounding or within Aβ plaques in AβO-monkeys were in line with the observations in the brains of patients with AD. These results indicated that neuroinflammation was triggered in the cynomolgus brain upon AβO treatment.

Since the key pathological features as occurring at the early phase of AD were detected in the cynomolgus brain upon the administration of synthetic AβOs, we next sought to investigate the intervening steps among them. Recent studies have shown that Aβ-activated inflamasome in microglia, the central signaling hubs of neuroinflammatory processes, play fundamental roles in the initiation and progression of AD (Kelley et al., 2019; Lucin and Wyss-Coray, 2009; Meyer-Luehmann et al., 2008; Weiner and Frenkel, 2006). The activation of inflamasomes, which is marked by the formation of ASC specks and the increase of cleaved caspases-1, was detected in the cortex of patients with AD (Franklin et al., 2018; Heneka et al., 2013). In the cortex of AβO-monkeys, we found extracellular ASC specks surrounding Aβ plaques or adjacent to tau tangles (Figures S5A and S5B). Moreover, the formation of ASC specks showed a correlation to the abundance of Aβ plaques and tau tangles (Table 1). In contrast, the ASC specks were seldom observed in control monkeys, except a few around Aβ plaques in two control brains (Figures S5A and S5B; Table 1). Consistently, we detected increased level of cleaved caspase-1 in some brain regions of AβO-monkeys, as
in the cortex of patients with AD (Figures S5C and S5D). These observations indicated that the inflammasome/caspase-1 signal was activated in AβO-monkeys as in patients with AD, suggesting a role of inflammasome in the AβO-driven Aβ plaques and tau tangles formation.

Selective neurodegeneration was detected in multiple brain regions of AβO-induced cynomolgus monkeys

In patients with AD, the early pathological changes, including Aβ plaques, tau tangles, and neuroinflammation, ultimately lead to neural degeneration and neuron loss, which are hallmark events occurring at the later phase of AD. Fluoro-Jade C (FJC) is a fluorochrome that has been widely used to detect neuronal neurodegeneration (Schmued et al., 1997). We found the FJC-positive neurons in different brain regions of AβO-monkeys, such as PFC, PC, and TC (Figure 6A). The FJC- neurons were always in close physical contact with Aβ plaques, indicative of the selective neural degeneration in the AβO-cynomolgus brain (Figure 6A). The nuclear staining of FJC- neurons was less intensive than that of FJC+/− cells around, and some even disappeared in neurons with much stronger FJC signals (Figure 6A, arrows). Consistently, the density of immunoreactive signals in the nucleus of NEUN- neurons bearing AT8+ tangles was much weaker than in neurons without tangles (Figure 6B). The expression of necroptosis cell death marker, pMLKL, was detected around or in the nucleus of some neural cells in AβO-cynomolgus brain (Figure S6), which is consistent with the measurements of pMLKL in patients with AD (Caccamo et al., 2017; Koper et al., 2020). Referring to the degeneration or loss of synapses due to AβO treatment, we found that the decline of density and intensity of Synaptophysin+ dots in the brain of AβO-monkeys was profound compared to those in control brain (Figures 6C, 6D, and S7). We also examined the possible neuron loss in specific brain regions of AβO-monkeys. Compared with control monkeys, the numbers of ChAT+ basal forebrain cholinergic neurons of AβO-monkeys were low but not significantly lower (Figures 6E and 6F). Thus, the AβO-monkeys exhibited selective neurodegeneration that appeared to correlate with Aβ plaques but did not display obvious neuron loss yet.

Table 1. The occurrence of amyloid and tau pathology, as well as inflammasome activation in the brain of AβO-induced cynomolgus monkeys

| Monkey number | Ab plaque number/cm² | Ab plaque area/brain area (%) | Tau tangles | ASC specks/Aβ plaque | ASC specks adjacent to tau tangle |
|---------------|----------------------|------------------------------|-------------|----------------------|-------------------------------|
| AβO-1         | 1,071                | 0.7103                       | +           | ++                   | +                             |
| AβO-2         | 1,042                | 0.6686                       | ++          | ++                   | +                             |
| AβO-3         | 426                  | 0.1616                       | +++         | +++                  | +                             |
| AβO-4         | 250                  | 0.1772                       | +           | +                    | +                             |
| AβO-5         | 149                  | 0.1439                       | +           | +                    | ++                            |
| AβO-6         | 16                   | 0.0193                       | ND          | –                    | –                             |
| AβO-7         | 14                   | 0.0106                       | +           | +                    | –                             |
| Ctrl-1        | 15                   | 0.013                        | ND          | +                    | –                             |
| Ctrl-2        | 8                    | 0.0086                       | ND          | –                    | –                             |
| Ctrl-3        | 17                   | 0.0088                       | ND          | –                    | –                             |
| Ctrl-4        | 4                    | 0.0025                       | ND          | –                    | –                             |
| Ctrl-5        | 4                    | 0.0085                       | ND          | –                    | –                             |
| Ctrl-6        | 42                   | 0.0345                       | ND          | +                    | –                             |
| Ctrl-7        | 8                    | 0.0154                       | ND          | +                    | –                             |

Footnotes: ++++, high; ++, medium; +, low; -, absence; N.D., not detected.
reliably reproduced the pathologic phenotypes of early AD in non-human primates. We advanced the approach by delivering the synthetic Aβ into cerebral parenchyma induces AD-like pathological features

Delivering AβOs into cerebral parenchyma induces AD-like pathological features

Previous studies usually delivered the soluble AβOs into the lateral ventricles of non-human primates, such as marmosets or rhesus monkeys (Baker et al., 1993; Geula et al., 1998), which was different from the intraparenchymal injections of AβOs in the current study. Thus, six more adult cynomolgus monkeys were recruited to test the reliability of the parenchyma-based approach (Table S2). Three monkeys received AβOs through parenchyma same as the previous seven monkeys, the other three through lateral ventricles as previously reported (Figures 7A and 7B). Compared with the first experiment, we increased the dosage and injection times of AβOs for each monkey (Figures 7A and 7B). We detected strong Aβ deposition in global brains of two intraparenchymal monkeys 5 months after the last injection (Figures 7C, 7E, and S8A) and very few Aβ plaques in limited brain regions of one intracerebroventricular monkey (Figures 7D, 7F, and S8B; Table S2). More interestingly, only the two intraparenchymal monkeys with intensive Aβ plaques displayed obvious neurofibrillary tangles (Figures 7G and 7I; Table S2). Therefore, the intraparenchymal delivery stimulated AD-like conditions in these two adult cynomolgus monkeys. Finally, we detected the activated astrocytes and microglia in the monkey brains (Figures S8C and S8D). Together, we advanced the approach by delivering the synthetic AβOs into parenchyma of cynomolgus brain and reliably reproduced the pathologic phenotypes of early AD in non-human primates.

DISCUSSION

In this study, we systematically elucidated that the adult cynomolgus monkeys rapidly captured most of key features of early AD, such as extracellular Aβ plaques, intracellular tau tangles, neuroinflammation, and selective neurodegeneration upon the repeated intraparenchymal delivery of synthetic AβOs. The AβO-monkeys rapidly and fully developed the early neuropathological features of AD.

Aβ deposition and neurofibrillary tangles are the central events during the early pathogenesis of AD. In a Matrigel-based 3D-culture system, expressing amyloid β precursor protein (APP) and presenilin 1 (PS1) with familial AD mutations in human neural progenitor cells induced extracellular Aβ deposition and filamentous tau (Choi et al., 2014; Kwak et al., 2020). However, the established AD animals usually display Aβ plaques in the absence of tau tangles. Neither the traditional AD mouse nor newly reported human-mouse chimeric AD model reproduced tangle pathology (Espuny-Camacho et al., 2017). The critical discrepancy existing in currently available AD model animals has hampered the understanding of molecular biology, biochemistry, and neuropharmacology of AD (LaFerla and Green, 2012). In this study, we found that the repeated parenchymal injections of synthetic AβOs induced massive Aβ plaques in the entire brain of adult cynomolgus monkeys (Figures 1, 2, 7, S1, and S8). In addition, eight monkeys with extensive Aβ plaques from two independent experiments displayed neurofibrillary tangles and neuropil threads in multiple brain regions as well as neuritic plaques with Aβ aggregation and dystrophic neurites. These observations revealed the formation of tau tangles in monkeys and also demonstrated that the formation of tau tangles is well correlated with the development of Aβ plaques in cynomolgus brain (Figures 3, 4, and 7; Table 1). The acute treatment (weeks) by delivering soluble AβOs into the lateral ventricles could not induce the co-occurrence of Aβ plaques and tau tangles in cynomolgus monkeys in previous studies.
Figure 6. The neurodegeneration in AβO-monkeys

(A) The degenerative neurons visualized by Fluoro-Jade C (FJC) were physically associated with 6E10-positive Aβ plaques in different brain regions of AβO-monkeys. Note the nucleus with faint signals in degenerative neurons.
The delivery of Aβ caused strong AD-like conditions in 8 adult cynomolgus monkeys, whereas the intracerebroventricular delivery in separate experiments and among all 10 adult cynomolgus monkeys, the parenchymal delivery of Aβ, but not lateral ventricle drove the early AD-like conditions in cynomolgus monkeys. In two mouse lines with only one or two AD pathologic features, we further confirmed that delivering Aβ plaques and tau tangles were carefully characterized by most frequently used antibodies and standard techniques. It is better to further confirm the conformation of tau tangles by other documented approaches, such as electron microscopy, which will be addressed in our future studies. In addition to Aβ plaques and tau tangles, the AβO-monkeys displayed activated astrocytes, microglia, and inflammasome associated with Aβ plaques, which was indicative of the neuroinflammation in AβO-monkeys (Figures 5, S4, and S5). Together, the key pathologic changes that are in the chain of events leading to AD developed in cynomolgus brain and the pathogenic process of early AD were recapitulated in monkeys upon administration of synthetic AβOs. In this regard, the central questions in AD pathogenesis, such as the nature of the pathologic relationship between Aβ and tau, even the molecular and cellular alterations that precede Aβ and tau lesions, could be interrogated over time in the AβO-monkeys.

Being a unique human brain disorder, AD has not yet been discovered in other aged primates, no matter in captive or wild habitats (Rosen et al., 2016; Walker and Jucker, 2017). These observations suggested that primate species might not be as predisposed to AD as humans, even though some aged primate species display numerous biological commonalities and substantial deposition of Aβ in the brain (Cramer et al., 2018; Edler et al., 2017; Oikawa et al., 2010; Selkoe et al., 1987). In this regard, to develop a genetically modified monkey model that can fully capture the complete spectrum of AD might be more challenging than previous anticipations. In addition, the performance of genetic modifications related to AD was more difficult in monkeys than in rodents, even with the recent advances in gene editing technologies. Furthermore, because of the slow and insidious process of AD and long lifespans of non-human primates, AD pathogenesis in genetically modified monkeys might require a long time to develop, years or even decades. In this study, cynomolgus monkeys with AβO treatment rapidly displayed most neuropathologic changes of early AD within a year, which was even much faster than the generation of a transgenic AD mouse line with only one or two AD pathologic features. We further confirmed that delivering AβOs into parenchyma but not lateral ventricle drove the early AD-like conditions in cynomolgus monkeys. In two separate experiments and among all 10 adult cynomolgus monkeys, the parenchymal delivery of AβOs caused strong AD-like conditions in 8 adult cynomolgus monkeys, whereas the intracerebroventricular delivery of AβO did not trigger dramatic neuropathological changes in the brain of rodents (Malm et al., 2006) and monkeys (Forny-Germano et al., 2014), which are in line with the observations shown in Figure 7. The mechanism underlying remains unclear, but we deduced that the dosage, injection site, duration time, and injection frequency of AβOs would be key factors driving the pathological changes in monkey brain. Therefore, the repeated injections of AβOs into the parenchyma might be a reliable way to reproduce AD-like features in cynomolgus monkeys.

Mechanistically, a bunch of key questions still remain unsettled. The biggest one is how the synthetic AβOs instigated the buildup of Aβ plaques and triggered the AD-like pathogenic cascade in cynomolgus brain. We wonder whether the synthetic AβOs directly aggregate into plaques or the synthetic AβOs stimulate the endogenous production of excessive Aβs to form Aβ plaques or both contribute to the Aβ plaque development in cynomolgus brain. Also, it is intriguing to know whether the synthetic AβOs directly initiated tau tangles, neuroinflammation, as well as neurodegeneration or the synthetic AβOs initiated amyloidopathy and thereby drove the subsequent pathogenic process in the cynomolgus monkeys. Evidences recently collected from different experimental systems show that Aβ can activate microglial inflammasomes (Halle et al., 2008), activated inflammasomes are involved in the Aβ deposition (Heneka et al., 2013), and inflammasome activation drives tau tangles (Ising et al., 2019), implicating a link among Aβ, inflammasome activation, and development of amyloidopathy or tauopathy. It is interesting to note that the activated inflammasomes were detected in the cortex of AβO-monkeys as in...
the cortex of patients with AD (Figure S5), suggesting that inflammasome might initiate AβO-induced Aβ deposition and subsequent tau tangles and contribute to rebuilding AD in cynomolgus monkey. Further exploration of these critical questions in the AβO-monkeys would help to understand the crucial link between Aβ deposition and tauopathy, the key juncture in the AD cascade, and eventually contribute to deciphering the pathogenesis of AD.

In this study, we observed some degenerative neurons in different brain regions and the tendency of neuronal loss in basal forebrain of AβO-monkeys (Figure 6). Neurons in cortex and hippocampus with high Aβ plaque burden in AβO-induced cynomolgus brains appeared relatively intact. Although the

Figure 7. The detection of amyloid and tau pathology in adult cynomolgus monkeys receiving intraparenchymal or intracerebroventricular delivery of AβOs
(A) Schematic diagram of administration of AβOs in adult cynomolgus monkeys via intraparenchymal delivery (n = 3). Two monkeys had been treated for 5 months after the last AβO injection, and one for 9 months.
(B) Schematic diagram of administration of AβOs in adult cynomolgus monkeys via intracerebroventricular delivery (n = 3). Two monkeys had been treated for 5 months after the last AβO injection, and one for 9 months.
(C–F) Immunohistochemical (C and D) and immunofluorescent (E and F) analysis with 6E10 in whole brain sections from anterior to posterior cerebrum of intraparenchymal monkeys and representative brain regions with Aβ plaques (C and E), as well as intracerebroventricular monkeys and representative brain regions without Aβ plaques (D and F).
(G–J) Immunohistochemical (G and H) and immunofluorescent (I and J) analysis with AT8 in brain sections from anterior to posterior cerebrum of intraparenchymal monkeys and representative brain regions with tau tangles (G and I), as well as intracerebroventricular monkeys and representative brain regions without tau tangles (H and J). Abbreviations: PFC, prefrontal cortex; PC, parietal cortex; TC, temporal cortex. Scale bars: 200 μm (C and D) and 50 μm (E–J). See also Figure S8 and Table S2.
neuron loss is minor, the AβO-monkeys displayed declined density of synapse associated with Aβ plaques in some brain regions (Figure 6). Mounting evidences confirmed that the decreased synaptic density is the major neuropathological correlate of the degree of dementia in AD (Terry et al., 1991). The Aβ-induced synaptic dysfunction and loss occurred at an early stage of AD, which led to failures of synaptic networks in AD brain directly causing cognitive deficits in patients (Davies et al., 1987; Palop and Mucke, 2010; Selkoe, 2002). The soluble AβOs were reported to induce impairments in synaptic integrity in the brain of rhesus monkeys (Beckman et al., 2019). The intranasal delivery of synthetic human AβOs impaired the long-term spatial memory of rat (Sipos et al., 2010). Although the behavioral effects of administering AβOs to monkeys remains unmeasured, these data imply the possibility of disrupting cognitive functions of cynomolgus monkeys by AβOs. Therefore, one of the next important steps will be to develop behavioral approaches suitable for cynomolgus monkeys, which allows one to measure the pattern of cognitive changes due to AβO treatment. Then, we might interrogate the possibility of generating AβO-monkeys with both typical pathologic features and the behavioral phenotypes that defines AD in humans. This will be evaluated in more detail in future studies.

In conclusion, we provided comprehensive characterization of cynomolgus monkeys receiving intraparenchymal delivery of synthetic AβOs and found that the progression of early AD was rapidly reproduced in the AβO-monkeys. These results suggest that the AβO-induced cynomolgus monkey might be a promising research model of human AD and would help advance our understanding of AD pathogenesis, which will eventually contribute to the development of promising research model for human AD.

Limitations of the study
As already noted, the detected tau tangles in the brain of AβO-monkeys were carefully characterized by most frequently used antibodies and standard techniques as shown in Figures 3, 4, and S2. Independent from the methods applied in our study, it is better to further confirm the conformation of tau tangles by other documented approaches, such as electron microscopy by using well-documented confirmational antibodies. We are planning to perform electron microscopic measurements in future studies.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Animals
- METHOD DETAILS
  - AβO preparation
  - Intraparenchymal or intracerebroventricular delivery of synthetic AβOs
  - Immunostaining
  - Modified Bielschowsky’s silver staining
  - Thioflavin S staining
  - Congo red staining
  - FJC staining
  - Western blot
  - Dot blot
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Counting analysis on brain slices
  - Statistical analysis

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103207.
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AUTHOR CONTRIBUTIONS

F.Y. initiated the study and developed the methodology. S.F., C.L., and T.Z. performed the experiments and collected the data. S.F., T.Z., and C.Y. designed the experiments, analyzed the data, and made figures. C.L. and G.T. performed material preparation and stereotaxic surgeries. F.Y., C.Y., and N.J. conceived the study and interpreted results. C.Y. wrote the manuscript. N.J. supervised the study. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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| REAGENT or RESOURCE                               | SOURCE                     | IDENTIFIER                                      |
|--------------------------------------------------|----------------------------|-------------------------------------------------|
| Antibodies                                       |                            |                                                 |
| Mouse anti-Aβ₁₋₁₆ (6E10)                         | Covance                    | Cat # SIG-39320; RRID: AB_662798                 |
| Mouse anti-Aβ₁₋₁₇₋₂₄ (4G8)                      | Biolegend                  | Cat # SIG-39220; RRID: AB_662812                 |
| Rabbit anti-Amyloid Fibrils QC                   | Sigma-Aldrich              | Cat # AB2286; RRID: AB_1977024                  |
| Mouse anti-PHF-tau (Ser202, Thr205) (AT8)        | Thermo Fisher Scientific   | Cat # MN1020; RRID: AB_223647                   |
| Mouse anti-phospho-Tau (Thr212, Ser214) (AT100)  | Thermo Fisher Scientific   | Cat # MN1060; RRID: AB_223652                   |
| Rabbit anti-NF-H                                 | Proteintech                | Cat # 21471-1-AP; RRID: AB_10734324             |
| Rabbit anti-GFAP                                 | Abcam                      | Cat # ab16997; RRID: AB_443592                  |
| Rabbit anti-Iba1                                 | Wako                       | Cat # 019-19741; RRID: AB_839504                 |
| Goat anti-Iba1                                   | Abcam                      | Cat # ab5076; RRID: AB_2224402                  |
| Rabbit anti-NEUN                                 | Millipore                  | Cat # ABN78; RRID: AB_10807945                  |
| Goat anti-ChAT                                   | Millipore                  | Cat # AB144P; RRID: AB_2079751                  |
| Rabbit anti-ASC                                   | AdipoGen                   | Cat # AG-25B-0006; RRID: AB_2490440             |
| Mouse anti-Caspase1                              | AdipoGen                   | Cat # AG-20B-0048; RRID: AB_2490257             |
| Rabbit anti-Synaptophysin                        | Abcam                      | Cat # ab32127; RRID: AB_2286949                  |
| Rabbit anti-pMLKL (S358)                         | Abcam                      | Cat # ab187091; RRID: AB_2619685                |
| Alexa Fluor® 594 AffiniPure Fab Fragment Donkey Anti-Mouse IgG (H+L) | Jackson ImmunoResearch     | Cat # 715-587-003; RRID: AB_2340859             |
| Normal mouse serum                               | Jackson ImmunoResearch     | Cat # 015-000-120; RRID: AB_2337194             |
| Normal donkey serum                              | Jackson ImmunoResearch     | Cat # 017-000-121; RRID: AB_2337258             |
| Chemicals, peptides, and recombinant proteins    |                            |                                                 |
| Human Aβ₁₋₄₂ peptide                            | Chinese Peptide            | Cat # AMYD-003                                   |
| HFIP                                             | Sigma-Aldrich              | Cat # 105228                                    |
| DMSO                                             | Sigma-Aldrich              | Cat # D8418                                     |
| HEPES                                           | Sigma-Aldrich              | Cat # H4034                                     |
| Fluoro-Jade C                                   | Millipore                  | Cat # AG325                                     |
| Thioflavin S                                    | Sigma-Aldrich              | Cat # T1892                                     |
| Congo Red                                       | Sigma-Aldrich              | Cat # C6277                                     |
| Critical commercial assays                       |                            |                                                 |
| BCA assay kit                                    | Thermo Fisher Scientific   | Cat # 23225                                     |
| ECL western blotting substrate                   | Thermo Fisher Scientific   | Cat # 32209                                     |
| VECTASTAIN ABC Reagent kit                       | Vector Labs                | Cat # PK-4000                                   |
| VECTOR DAB Substrate Kit                         | Vector Labs                | Cat # SK-4100                                   |
| Modified Bielschowsky’s Stain Kit                | American MasterTech        | Cat # KTBIE                                     |
| Experimental models: Organisms/strains           |                            |                                                 |
| Macaca fascicularis animals                      | Wincon Theracells Biotechnologies Co, LTD. | N/A                                            |
| Software and algorithms                          |                            |                                                 |
| ImageJ                                           | ImageJ                     | https://imagej.nih.gov/ij/download.html         |
| Graphpad Prism 6                                 | GraphPad Software          | https://www.graphpad.com/scientific-software/prism/ |
| StrataQuest software version 6.0.1.14S          | TissueGnostics, Vienna, Austria | N/A                                            |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Naihe Jing (njing@sibcb.ac.cn).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
The care of non-human primates and procedures involved in this study were thoroughly reviewed and approved by the Animal Care and Use Committee, in accordance with the Association for Reassessment and Accreditation of Laboratory Animal Care (AAALAC) guideline.

Fourteen cynomolgus monkeys (Macaca fascicularis) aged 18–22 years were recruited in the first experiment of this study and randomly assigned to two groups, AβO-induced group (n = 7, 5 females and 2 males) and noninjected control group (n = 7, 6 females and 1 male) as shown in Table S1. Another six cynomolgus monkeys aged 20–24 years were recruited in the second experiment of this study and randomly assigned to two groups, AβO-intracerebroventricular delivery group (n = 3, 2 females and 1 male) and AβO-intraparenchymal delivery group (n = 3, 2 males and 1 female) as shown in Table S2. All these 20 monkeys are wild type animals from the same colony and had never been involved in other pharmacological trials and studies. During the study, animals were individually housed in stainless steel cages at the primate facility of Wincon Theracells Biotechnologies Co, LTD. in Nanning, Guangxi China, which is fully accredited by the AAALAC International. Animals are fed twice daily and supplemented with fresh fruits and the miscellaneous enrichments once a day. All animals are maintained on a 12-h light and/or 12-h dark cycle under room temperature at 22–28°C with a relative humidity of 30%–75% and water supply ad libitum.

METHOD DETAILS

AβO preparation
Human Aβ1-42 peptide was commercially synthesized by Chinese Peptide. We adopted and modified the procedures as previously described for the oligomerization of Aβ peptides (Stine et al., 2003). Briefly, 1 mg lyophilized peptide stored at −80°C was allowed to equilibrate to room temperature for 30 min. Then, the peptide was thoroughly dissolved to 1 mM in 100% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, Sigma). The solution containing the dissolved peptide was then aliquoted in microcentrifuge tubes. The solution was subjected to a gentle stream of nitrogen to evaporate HFIP and a thin clear film at the bottom of the tube was produced. During evaporation, approximately 30–50% of Aβ was lost. The evaporated peptide was subsequently resuspended in 40 μl dimethyl sulfoxide (DMSO) and further diluted in 10 mM HEPES...
to a final concentration of 110 μM. The oligomers were freshly prepared before each injection and were characterized by western blot. The AβOs can be kept on ice for at most 5 h before injection. Vehicle controls were prepared in an identical manner using the same tubes and solutions in the absence of Aβ1-42 peptide.

Intraparenchymal or intracerebroventricular delivery of synthetic AβOs

For the brain administration of AβOs, four MRI-guided stereotaxic surgeries combined with convection enhanced delivery system (Fiandaca et al., 2009; Sanftner et al., 2005) were performed on each cynomolgus monkey as shown in Figure 1A. Each monkey was anesthetized with intramuscular atropine (20 mg/kg), ketamine (10 mg/kg), and sodium pentobarbital (20 mg/kg). The head of the monkey was fixed in a stereotaxic instrument and the skull over the parietal lobe was exposed under aseptic conditions by a longitudinal skin incision followed by removal of the connective tissue. MRI scanning was performed on each monkey prior to surgery to identify stereotaxic coordinates. A small hole (<2 mm in diameter) on the skull of each side of brain was made using an electric drill guided by parameters from MRI measurements. The AβO solution (0.5 μg/μl) was bilaterally injected into the brain parenchyma between the lateral basal ganglia and medial temporal lobe directly above the hippocampus or lateral ventricles using Hamilton syringes (gauge 22s). The needle was pushed into the target location at a rate of 1 mm/min through the small hole and held in place for 10 min. Then, 200 μl volume of AβO was injected into each side of brain at a rate of 2 μl/min. The needles were held in place for 20 min following AβO delivery and then drawn back at a rate of 1 mm/min. For each animal, four AβO injections (800 μl per side) were performed at 6–8-week intervals as shown in Figure 1.

To verify the injection accuracy and predict AβO diffusion in cynomolgus cerebrum, two animals received a bilateral intra-brain injection of soluble AβO containing contrast agent, gadopentetate dimeglumine (Gd-DTPA), following the same surgery protocol as the AβO injection (Su et al., 2010). An MRI scanning was performed 2 h after surgery to detect the Gd-DTPA signal.

Immunostaining

Animals were perfused with saline under deep anesthesia with sodium pentobarbital (30 mg kg⁻¹ intravenously). Brains were coronally cut into 4–6 mm thick sections before fixation with 4% paraformaldehyde for 3 days at 4°C. These thick sections were then washed with PBS three times and sequentially transferred to 15% and 30% sucrose solution at 4°C. The thick sections were further cryosectioned at 40-μm thickness using a microtome (Leica SM2000R) and stored in ethylene glycol solutions at −20°C. In some cases, before fixation of the thick sections, small samples of various regions were obtained and flash frozen at −80°C for subsequent immunoblot analysis.

For immunohistochemistry (IHC) assays, the brain sections of 40-μm thickness were washed with PBS three times and quenched of endogenous peroxidase activity in 0.3% H2O2 in PBS for 30 min. After permeabilization and blocking in the 10X blocking buffer containing 5% donkey normal serum, 1% BSA, and 0.4% Triton X-100 for 2 h, the sections were incubated in primary antibody diluted with 1X blocking buffer for 16 h at 4°C. The primary antibodies used were as follows: anti-Aβ1-16 (6E10) (BioLegend, 1:1000), anti-Aβ17-24 (4G8) (BioLegend, 1:1000), anti-PHF-tau (Ser202, Thr205) (AT8) (Thermo Fisher Scientific, 1:1000), anti-phospho-Tau (Thr212, Ser214) (AT100) (Thermo Fisher Scientific, 1:1000), anti-ibα1 (Wako, 1:1000), anti-GFAP (Abcam, 1:1000) and anti-ChAT (Millipore, 1:1000). After washing in PBS between steps, brain sections were incubated in species-appropriate biotinylated secondary antibody (e.g., donkey anti-mouse IgG (H + L) biotin secondary antibody or donkey anti-rabbit IgG (H + L) biotin secondary antibody) for 2 h at room temperature. After 3 washes with PBS, the sections were then transferred to the avidin-biotin-peroxidase complex (prepared from VECTASTAIN ABC Reagent kit) for 30 min, and then reacted with DAB-hydrogen peroxide solution (prepared from VECTOR DAB Substrate Kit). In some circumstances, NiSO₄ was added to amplify the signal. The sections were then washed with PBS, mounted on slides, dehydrated in increasing concentrations of ethanol, cleared in xylenes, and coverslipped using neutral resins.

For immunofluorescent staining, the primary antibodies used were as follows: anti-Aβ1-16 (6E10) (BioLegend, 1:500), anti-Aβ17-24 (4G8) (BioLegend, 1:200), anti-PHF-tau (Ser202, Thr205) (AT8) (Thermo Fisher Scientific, 1:400), anti-NF-H (Proteintech, 1:800), anti-ibα1 (Wako, 1:500), anti-GFAP (Abcam, 1:200), anti-MLKL (Abcam, 1:100), anti-NEUN (Millipore, 1:500), anti-ASC (AdipoGen, 1:200) and anti-Synaptophysin (Abcam, 1:1000). Fluorescent staining with species-appropriate Alexa secondary antibodies (Jackson Immunoresearch Laboratories) was used. To reduce the autofluorescence in
fluorescently labeled brain tissue, the brain sections were dipped briefly in distilled water, and treated with 5 mM CuSO4 in 50 mM ammonium acetate buffer (pH 5.0) for 30 min. The brain sections were then counterstained with DAPI (Sigma), mounted on slides, and coverslipped in Fluoromount-G (Thermo Fisher Scientific).

For co-staining of 6E10 and AT8 on the brain sections, the multiple mouse-on-mouse staining protocol were performed as previously described (Koper et al., 2020). Since the two antibodies are originated from the same host species, coupling method was used to avoid cross-reactivity of secondary antibodies. Briefly, AT8 was stained as described above, followed by the Alexa Fluor® 488 donkey anti-mouse secondary antibody, while 6E10 was coupled to a donkey anti-mouse Fab fragment conjugated to Alexa 594 (Jackson ImmunoResearch). For coupling of 6E10, the antibody was incubated with the Fab fragment for 40 min at RT (2 μg Fab fragment per 1 μg 6E10). Normal mouse serum was then added to capture the unbound Fab fragment (10 μl of serum per 1 μg Fab fragment) for another 20 min. The coupled 6E10 antibody was subsequently used to stain the brain sections, following the standard protocol described above.

For collecting images, a fully automated and high-resolution scanning system, VS120 Virtual Slide Microscope (Olympus), was used to scan the whole brain slides.

**Modified Bielschowsky’s silver staining**
To furtherly identify the intracellular neurofibrillary tangles, modified bielschowsky’s silver staining was performed using a Modified Bielschowsky’s Stain Kit (American MasterTech). Ammoniacal silver solution, developer solution and ammonia water were prepared according to the kit procedure. 40-μm free-floating brain sections were mounted on slides and then rinsed in distilled water. Place the slides in preheated silver nitrate solution for 15 min at 40°C. After 3 washes with distilled water, treat the slides with preheated ammoniacal silver solution for 10 min at 40°C. Slides were immersed in developer solution for 3–30 sec until the tissue became golden brown and removed quickly into ammonia water for 30 sec. Slides were rinsed in running distilled water and then treated with 5% sodium thiosulfate for 2 min. After wash in running distilled water for 2 min, the slides were dehydrated in absolute alcohol, cleared in xylenes, and coverslipped using neutral resins.

**Thioflavin S staining**
Free-floating brain sections were washed with PBS for 3 x 5 min, then mounted on the slides and dried. The slides were then incubated with 0.025% Thioflavin S (dissolved in 50% ethanol) at room temperature for 8 min. The slides were then decolorized in 50% ethanol for 2 x 1 min, washed in PBS, and subsequently co-stained with DAPI and coverslipped using Fluoromount-G. Images were captured with Leica TCS SP8 confocal laser scanning microscope.

**Congo red staining**
Congo red staining was performed as previously described (Zheng et al., 2020). Briefly, free-floating brain sections were washed with PBS for 3 x 5 min, then mounted on the slides and dried. The slides were incubated in a sodium chloride solution (3% NaCl, 80% ethanol and 0.01% NaOH) at room temperature for 20 min, and then 0.2% Congo red containing 3% NaCl, 80% ethanol and 0.01% NaOH for 40 min. The sections were subsequently decolorized in ethanol, cleared in xylenes, and coverslipped using neutral resins.

**FJC staining**
Fluoro-Jade C staining is used to visualize degenerated neurons on 40-μm Macaque brain sections as previous described (Schmued et al., 2005). Following standard immunostaining using Aβ1-16 antibody 6E10 and Alexa594-conjugated secondary antibody, the sections were mounted onto premier charged slides and dried at 50° for 30 min. They were rinsed with distilled water and then dipped in 0.06% potassium permanganate solution for 5 min. After a 2-min water rinse, the slides were incubated with 0.0004% FJC in 0.1% acetic acid for 20 min. The slides were then stained with DAPI and coverslipped using Fluoromount-G. Images were captured with Leica TCS SP8 confocal laser scanning microscope.

**Western blot**
To characterize the Aβ1 species in the oligomerized Aβ1-42 peptide, the AβO sample was electrophoresed on 16% acrylamide Tricine-SDS-PAGE gels and transferred onto 0.2 μm PVDF membranes at 30 V for 2 h.
Filters were boiled for 5 min in PBS and blocked at room temperature for 1 h with 5% fat-free milk in PBS. Monoclonal antibody 6E10 (1:2000) was used to probe the blots. Bound antibody was visualized using HRP-conjugated anti-mouse IgG (1:7000) (Abcam) and ECL detection (Thermo Fisher Scientific).

For detection of Aβ in brain tissue samples from cynomolgus monkeys, tissues were homogenized in RIPA buffer containing protease and phosphatase inhibitors (50 mM Tris-HCl (pH7.6), 150 mM NaCl, 0.1% SDS, 1% NP40, 1 mM EDTA, 1 mM EGTA, 1% deoxycholic acid sodium salt, 1 mM DTT, 1 mM PMSF, 1 mM NaF, 1 mM Na3VO4, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin) and then rotated at 4°C for 30 min. The homogenates were then centrifuged for 30 min at 4°C, 12,000 rpm. The protein concentration of supernatant was measured by BCA assay. The resulting pellet was resuspended with 2% SDS in 25 mM Tris-HCl (pH7.5), centrifuged again, and the supernatant was collected as the detergent-insoluble fraction of the brain samples. These samples were then diluted in SDS sample buffer and resolved on a 4% stacking gel and 10% separating gel containing 6 M urea in Tricine-SDS buffer. The gel was transferred onto 0.2 μm PVDF membrane and the western blot was performed following the same protocol as for Aβ samples described above. The membrane was probed with 6E10 and anti-β-actin antibody, the latter serving as a loading control.

For immunoblot detection of Caspase1, the supernatant samples were diluted in SDS sample buffer and separated by 12% SDS-PAGE gels. The proteins were transferred onto 0.45 μm PVDF membranes at 100 V for 2 h. The membranes were then blocked in 3% BSA in TBS for 1 h at room temperature followed by incubation of anti-Caspase1 (AdipoGen, 1:1000) in 3% BSA in TBS-Tween overnight at 4°C. The membrane was also visualized using HRP-conjugated anti-mouse IgG and ECL detection. Signal intensities were quantified by ImageJ software.

Dot blot
The Aβ aliquots (1 μl) were spotted on a nitrocellulose membrane (Pierce). The membrane was blocked for 1 h at room temperature with 10% nonfat milk in Tris-buffered saline containing 0.01% Tween 20 (TBS-T) and probed with 6E10 (1:10000 in 3% BSA), 4G8 (1:10000 in 3% BSA), OC (1:1000 in 5% nonfat milk). HRP-conjugated anti-mouse/rabbit IgG secondary antibodies (Abcam) were used at 1:5000 for 1 h at room temperature. Blots were detected using ECL chemiluminescence detection reagent (Thermo Fisher Scientific).

QUANTIFICATION AND STATISTICAL ANALYSIS
Counting analysis on brain slices
For counting analysis, image processing of whole brain sections (for Aβ plaques) or specific brain regions (for astrocytes, microglia, synaptic dots, and neurons) and analysis was performed using StrataQuest software version 6.0.1.145 (TissueGnostics, Vienna, Austria). The quantification of Aβ plaques were performed on four serial whole brain sections from the anterior to posterior of each cynomolgus cerebrum. For quantifying astrocytes, microglia, and synaptic dots, three fields on each cortical region were randomly selected and at least two cortical regions of each cynomolgus cerebrum were measured.

Statistical analysis
All of the statistical details of experiments can be found in the figure legends. All data are presented as mean ± SEM. Student’s t test (two-tailed) was performed for statistical analysis between two groups. A value of p < 0.05 was considered significant in all analyses. All statistical analyses were conducted using Prism 6 GraphPad Software (San Diego, CA).