Amyloid-β Deposition and Olfactory Dysfunction in an Alzheimer’s Disease Model

Nan Wua, Xiaoping Raoa,c, Yunling Gaoc, Jie Wanga and Fuqiang Xua

State Key Laboratory of Magnetic Resonance, Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, China

State Key Laboratory of Freshwater Ecology and Biotechnology, Center for Water Environment and Human Health, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

University of the Chinese Academy of Sciences, Beijing, China

Handling Associate Editor: Ling-Qiang Zhu

INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by the progressive impairment of cognition. Currently, there is no effective treatment; however, attention has been paid toward the establishment of methods used to diagnose the disease before irreversible deterioration occurs. Olfactory dysfunction has been proposed as a potential marker for AD diagnosis [1–3] because the functions of odor...
Aβ deposition and olfactory defects in AβPP/PS1 mice

All animal procedures were approved by the Chinese Academy of Sciences. AβPP(E9)Tg mice, co-expressing human PS1 encoding the E9 deletion and mouse AβPP containing humanized AβPP and Swedish mutations (K595N, M596L), were adopted. Mice were bred and maintained within the animal facility at the Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences. Age-matched NTg littermates of the AβPP/PS1 Tg mice were used as controls. To examine the possible contributions of accumulating Aβ pathology on olfaction over time, we used four separate age groups, including 1-2 mo, 3-4 mo, 6-7 mo, and 9-10 mo. Mice were genotyped by PCR analysis of tail DNA using standard methods.
Protein sample preparation and Western blot analysis

Different brain areas within the olfactory and cognitive systems, including the OE, OB, AON, PC, EC, and HP, were dissected following the method as previously described [32]. Briefly, the brain is thermal fixed rapidly with a microwave so the brain tissue can be cleanly and accurately dissected. The segmented brain tissues were homogenized in the previously described protein extraction buffer [33]. In addition, the reducing agent (DTT or β-mercaptoethanol) was not included in the sample buffer used for the 6E10 Western blot. Protein samples were analyzed on 10–20% Tris/Tricine gels for 6E10, and on 12% Tris/Glycine gels for anti-OMP (olfactory marker protein). The proteins were transferred to PVDF membrane and incubated in 5% skim milk (Difco™, BD) and then treated with the primary antibody overnight at 4°C. The antibody signal was detected by an HRP-conjugated secondary antibody (1:1000 diluted) and an ECL advance Western blotting detection kit (GE Healthcare). Images were acquired with a Multilmage TM Light Cabinet (Alpha Innotech).

Immunohistochemistry and thioflavine-s staining

Mice were deeply anesthetized with an overdose of chloral hydrate (1 g/kg) and perfused through the aorta with 100 ml of 0.9% NaCl followed by 400 ml of phosphate buffer containing 4% paraformaldehyde. The brains were removed and post-fixed in the fixative solution overnight at 4°C and then sectioned (20 µm) with a vibratome (Leica, Nussloch, Germany; S100, TP1). The sections were collected consecutively in PBS for IHC staining. All sections were permeabilized in 0.2% Triton X-100 (Sigma Aldrich, Stockholm, Sweden) for 30 min. Primary antibody incubation was carried out overnight at 4°C in a humidity controlled chamber with gentle shaking. Next, the sections underwent 1-hour secondary antibody incubation (the Cy3-labeled secondary antibody against mouse IgG [Jackson ImmunoResearch] or the FITC-labeled secondary antibody against rabbit IgG [Invitrogen]) was diluted 1:250) at room temperature. For double immunostaining, primary antibodies were incubated overnight, simultaneously or stepwise at 4°C, followed by incubation with the appropriate secondary antibodies in a darkened chamber at room temperature for 1 hour. After immunostaining, the sections were stained by 1% thioflavine-s at 37°C for 1 hour in a darkened chamber and then dehydrated using graded ethanol. Then sections were observed and images were taken by a laser confocal microscope (Carl Zeiss, NOL-LSM 710). Following, the IHC signal in the images was quantified following the methods previously described [34, 35] with subtle variation, as that we use mean density to measure rather than area, because the 6E10 signals was dispersed in this study.

Olfactory behavior test

To assess odor discrimination, the olfactory habituation/dishabituation test was completed based on Yang’s method [36]. All olfactory behaviors were administered in a neutral cage with metal lid and fresh wood chip bedding (PC, size: 325 × 210 × 185 mm, Fenyshi, Suzhou) by a blind observer. In the pre-testing, the mouse was placed in the testing cage, then a Q-tip, used as odor applicator, was fixed with plasticene onto the cage lid, and the animal was allowed to acclimate for 12 min (equal to the whole experimental time). During the experiment, the cotton end of the Q-tip was impregnated with liquid odor, and the cumulative sniffing time, defined as the animal extruding its nose toward the tip within a distance of 1 cm, was recorded with a stopwatch. Vanillin and ethyl acetate were diluted to 1 × 10⁻³ and 1 × 10⁻⁶ with distilled water. The Tg and NTg mice were tested at 2, 3, 4, 6, and 10 mo. For each age and odor, 10–14 animals per group were used in olfactory behavioral test. The sequence of odor exposure consisted of water for 3 trials, and then vanillin or ethyl acetate for an additional trial. For each odor, 2-min exposure with a 1-min inter-trial interval was used. No animal was exposed to the same odorant solution more than once. The data were analyzed using the paired samples t-test with the level of significance set to p<0.05.

Spatial learning and memory

Spatial learning and memory of the Tg and NTg mice were tested in the Morris water maze (MWM) at 3 and 9–10 mo. The water temperature was kept stable at 20 ± 1°C. Regarding the place navigation training and spatial probe tests, all animals’ performance in the MWM was recorded with an automatic tracking system (ANY-maze, Stoelting). In the place navigation training, an escape platform was located in the center of one quadrant, and several visual cues were distributed on the walls of the pool. Each mouse was trained 3 times per day in 30–60 min intervals for 7 consecutive days. Each training session was started with the mouse facing the inner wall of pool and then ended with the
mouse climbing onto the platform. Each mouse was allowed to find the hidden platform for 60 s and then rest for 20 s on the platform. If the mouse failed to find the platform, it was guided to the platform by the experimenter and allowed to rest for 20 s. In the training, the animal acquires spatial memories to locate the submerged platform. The time to find the submerged platform was defined as the escape latency. On the third day, following place navigation training for 72 h, the spatial memory retention of the trained animals was evaluated by a single 60-s spatial probe test. The animal was placed in the MWM without a platform, and the cumulative time spent in the training platform quadrant was recorded. The escape latency was analyzed using the repeated measures ANOVA, and the time spent in the target quadrant was analyzed by the independent samples t-test.

RESULTS
Deposition of soluble Aβ aggregates in the olfactory system

Recent evidence suggests that soluble Aβ, not plaque-associated Aβ, correlates better with cognitive dysfunction in AD [37]. To investigate the deposition pattern of soluble Aβ aggregates in the olfactory system, Western blotting with the anti-Aβ monoclonal antibody 6E10 was performed. Various sizes of soluble Aβ aggregates, ranging from 25 kDa to 100 kDa (in 25 kDa increments), were detected in the olfactory system. The spatial deposition pattern of the soluble Aβ aggregates was highly age-dependent (Fig. 1). At 1-2 mo, the bands of soluble Aβ aggregates were obvious in the OE. At 3-4 mo, the intensity of the bands increased in the OE and OB. At 6-7 and 9-10 mo, soluble Aβ aggregates continued to increase and spread throughout the olfactory system, including the HP. Meanwhile, the AβPP signals remained very strong in all examined brain regions at all ages. However, no signals were observed in control samples prepared from the wild type mice at corresponding ages (data not shown).

Aβ deposition and atrophy in the primary olfactory pathway

Odor detection ability largely relies on the primary olfactory pathway, with olfactory sensory neurons in the OE receiving odor signals, which are then relayed to the OB. Thus, we focused on the pathology found in the OE and OB in the Tg mice. As shown in Fig. 2A, the OMP signal in the OE was obviously detected at 1-2 mo, faint at 3-4 mo, then undetectable at 6-7 and 9-10 mo. Degeneration of olfactory sensory neurons (OSNs), including cell bodies and axon bundles, accompanying Aβ deposition, was observed (Fig. 2B). Aβ was found primarily as intracellular granules or vesicle-like cytoplasmic aggregates, with occasional extracellular puncta and aggregates of more diffuse staining primarily localized in the apical layer (containing the OSN cell body and its dendrite) and basal layer (containing the axon bundles of OSNs) of the OE. Aβ signal co-localized with OMP signal in OSNs in the OE of the AβPP/PS1 Tg mice. Moreover, the thickness and morphology of the OE changed as Aβ deposition progressing. Both the apical and basal layers were much thinner at 6-7 and 9-10 mo than 1-2 and 3-4 mo, and the basal layer at 9-10 mo showed signs of further degeneration when compared to the same layer at 6-7 mo. Additionally, thioflavine-s staining was not detected in the OE of AβPP/PS1 Tg mice (data not shown), indicating the lack of β-sheet fibrillary Aβ (Aβ form in SPs) deposits. While in the OB (Fig. 2C), 6E10 signal was detected in olfactory nerve layer containing OSNs axon bundles from the OE. We found a gradual increase of amount, aggregating degree, as well as spatial distribution from outer layers to inner layers in Aβ deposits as the Tg mice growing older. At 3-4 mo, the mitral cell layer developed obvious non-SPs Aβ deposits. At 6-7 mo, Aβ burden (also non-SPs) spread to the granule cell layer. At 9-10 mo, an increased Aβ burden was found in all layers and a small number of SPs was detected within the granule cell layer. And notably, at 9-10 mo, the external plexiform layer and glomerular layer were much thinner, whereas the olfactory nerve layer was thicker than before.

Early elevation of Aβ in the olfactory cortices

To assess the localization of Aβ aggregates and the development of SPs within the olfactory system, we characterized Aβ deposition in the Tg mice with two separate methods, including immunostaining of Aβ with the anti-Aβ antibody 6E10 (1:2000 diluted) and thioflavine-s (Sigma, 1:10 diluted) staining SPs. From the OB, the mitral/tufted cell axons fasciculate to form the lateral olfactory tract, which projects to the central olfactory cortices, including the AON, PC, and EC. In layer II of the AON (Fig. 3A), the 6E10 signals were detected as early as 3-4 mo and the SPs were detected at 6-7 and 9-10 mo. Both signals increased with age. In the PC (Fig. 3B), the 6E10 signal was detected in layer II/III as early as 3-4 mo and increased with age, and...
from Signet Laboratories, Deadham, MA, USA, diluted 1:2000 from a 1 mg/ml stock. That at 1-2 mo, soluble Aβ/H9252 entorhinal cortex (EC), and hippocampus (HP). The quantity of every loading protein sample was the same (50 µg). Western blotting shows that at 1-2 mo, soluble Aβ aggregates with a molecular weight ranging from 25 kDa to 100 kDa (largely in multiples of 25 kDa) were greatly deposited in the OE; at 3-4 mo, the signal spread to the OB; at 6-7 mo, the signal could be clearly detected in all examined samples, with the most dense signal found in the OE and OB; then at 9-10 mo, soluble Aβ aggregates were markedly deposited in all examined samples. 6E10, from Signet Laboratories, Dedham, MA, USA, diluted 1:2000 from a 1 mg/ml stock.

Fig. 1. Spatial-temporal pattern of Aβ/H9252 deposition in the olfactory system of AβPP/PS1 Tg mice. For every age group, the same sampling sequence was taken (from left to right): olfactory epithelium (OE), olfactory bulb (OB), anterior olfactory nucleus (AON), piriform cortex (PC), entorhinal cortex (EC), and hippocampus (HP). The quantity of every loading protein sample was the same (50 µg). Western blotting shows that at 1-2 mo, soluble Aβ aggregates with a molecular weight ranging from 25 kDa to 100 kDa (largely in multiples of 25 kDa) were greatly deposited in the OE; at 3-4 mo, the signal spread to the OB; at 6-7 mo, the signal could be clearly detected in all examined samples, with the most dense signal found in the OE and OB; then at 9-10 mo, soluble Aβ aggregates were markedly deposited in all examined samples. 6E10, from Signet Laboratories, Dedham, MA, USA, diluted 1:2000 from a 1 mg/ml stock.

The SPs were detected at 6-7 and 9-10 mo. In general, layer II/III of the PC developed the greatest density of Aβ burden when compared among all three layers. In the EC (Fig. 3C), a large amount of Aβ deposition appeared at 6-7 mo and further increased at 9-10 mo. Thioflavine-s-positive SPs were mainly found in layer III and IV/V. In the HP (Fig. 3D), a dispersed 6E10 signal was clearly detected beginning at 6-7 mo. Minimal SPs were detected at 6-7 mo, but they were markedly present at 9-10 mo. Regional accumulation in the HP occurred in dentate gyrus, CA1, and CA3. Further, the quantification analysis of the IHC signal and SPs was carried out. The 6E10 signal increased with age, and obviously significantly after 4 mo (Fig. 4A). The pattern of 6E10 IHC signal was similar to that of 6E10 Western blot signal but with high resolution. Thus, the 6E10 signal could be detected in the OE and OB at 1-2 mo, then from OE to PC at 3-4 mo, later after 6-7 mo throughout the olfactory system. Meanwhile, the number of SPs in the olfactory system was also counted (Fig. 4B). SPs could not be detected in the OE, and could be detected only at 10 mo in the OB, whereas in other cortices could be detected at both 7 and 10 mo (with significant increase at 10 mo). At 7 mo the AON got the most and the HP the least, while at 10 mo the PC got the most and the OB the least number of SPs.

Early decline of odor detection ability in the Tg mice

Because of early-onset Aβ deposition in the primary olfactory pathway, including the OE and OB, the odor detection ability was evaluated using the habituation/dishabituation test. As shown in Fig. 5A, the NTg mice recognized a $1 \times 10^{-5}$ vanillin solution from 2 to 6 mo (2 mo: $\xi_{10} = 3.54$, $p < 0.01$; 3 mo: $t_{19} = 2.45$, $p = 0.04$; 4 mo: $t_{20} = 2.93$, $p = 0.02$; 6 mo: $t_{20} = 3.45$, $p < 0.01$), but not at 10 mo ($t_{20} = 0.67$, $p = 0.52$). The Tg mice could recognize the same odor only at 2 mo (Paired samples t test, $t_{9} = 3.24$, $p = 0.01$), but not later (3 mo: $t_{9} = 0.97$, $p = 0.36$; 4 mo: $t_{9} = 1.08$, $p = 0.31$; 6 mo: $t_{9} = 0.10$, $p = 0.92$; 10 mo: $t_{9} = 1.49$, $p = 0.17$). Similar results were found using ethyl acetate. As shown in Fig. 5B, the NTg mice recognized a $1 \times 10^{-6}$ ethyl acetate solution from 2 to 6 mo (2 mo: $t_{12} = 2.83$, $p = 0.02$; 3 mo: $t_{9} = 4.56$, $p < 0.01$; 4 mo: $t_{9} = 4.20$, $p < 0.01$; 6 mo: $t_{9} = 3.48$, $p < 0.01$), but not later (10 mo: $t_{9} = 1.93$, $p = 0.08$). The Tg mice recognized the odor at 2 mo ($t_{9} = 2.27$, $p = 0.049$), but not later (3 mo: $t_{9} = 1.58$, $p = 0.15$; 4 mo: $t_{9} = 1.30$, $p = 0.22$; 6 mo: $t_{9} = 1.61$, $p = 0.14$; 10 mo: $t_{9} = 0.80$, $p = 0.45$). Thus, the odor detecting ability of AβPP/PS1 Tg mice declines beginning significantly from 3 mo.

Late-onset visual-spatial learning deficit in AβPP/PS1 Tg mice

To reveal the relationship between Aβ deposition and cognitive deficits in AβPP/PS1 mice, we used the MWM behavioral test. We adopted 3-month-old mice, because at this age Aβ deposition spreads into the olfactory cortices, including AON and PC, as shown by IHC result (Fig. 3A, 3B). We also used 9-month-old mice because they show a large amount of SPs in the HP (Fig. 3D). As shown in Fig. 6 (A and C), the escape latency or time spent in the platform quadrant of the 3 mo Tg mice did not differ from that
of their NTg littermates in the spatial navigation test (Repeated measures ANOVA, \( F_{1,180} = 9.2, p = 0.34 \)) or spatial probe test, respectively (Independent samples t-test, \( t_{160} = 0.13, p = 0.90 \)). As shown in Fig. 6 (B and C), the escape latency of the 9-10 mo Tg mice in the spatial navigation test was significantly different from the NTg littermates (Repeated measures ANOVA, \( F_{1,14} = 13.7, p < 0.001 \)). Although the \( p \) value does not reach the significant level, there was some difference (Independent samples t-test, \( t_{117} = 1.85, p = 0.08 \)) between 9-10 mo Tg and NTg mice in the time spent in the targeted quadrant. Both the escape latency in the spatial navigation test and the time spent in targeted quadrant are related to learning and memory. Thus, the 9-10 mo Tg mice showed a cognitive deficit, while the 3 mo mice did not.

**DISCUSSION**

Aβ deposits in the olfactory system have been suggested to promote AD pathology in rodents [14, 15]. The present study demonstrates the relationship between the olfactory and cognitive systems in an AD mouse model based on the pattern of Aβ accumulation. In AβPP/PS1 Tg mice, we found that the spatial-temporal pattern of Aβ deposits, mainly soluble aggregates in the olfactory system, and also that OSNs degeneration occurs accompanied with deposition of soluble Aβ aggregates in primary olfactory pathway. Based on our results from the olfactory detection test and MWM, AβPP/PS1 Tg mice exhibit an early-onset of olfactory detection deficit (at 3 mo) that precedes cognitive deficit in memory and learning (at 9-10 mo), and these deficits are correlated with Aβ deposition levels in the olfactory system.

**Spatial-temporal pattern of Aβ deposits in the olfactory system**

The OB receives sensory inputs from OSNs in the OE and then relays odor information to the AON and the other olfactory cortices, including the PC and EC [38, 39], then to the trisynaptic circuit in the HP [40, 41]. In AβPP/PS1 Tg mice, the spatial-temporal deposition of Aβ appeared in the olfactory system from the OE and OB at 1-2 mo, to the AON and PC at 3-4 mo, and then to the EC and HP at 6-7 and 9-10 mo. Notably, in the olfactory system, soluble Aβ deposits and SPs significantly increase after 3-4 and 10 mo, respectively. Therefore, the current experiments have revealed the spatial-temporal patterns of Aβ deposits: from the peripheral to the central olfactory system, and then to the cognitive system in this AD mouse model. The development of the patterns corroborate with the fact that the olfactory deficit occurs earlier than cognitive decline in AD patients.

Further, our data have demonstrated the aggregating manner of the soluble Aβ aggregates. The progression of nontoxic Aβ evolves into soluble oligomers and prefibrillar, then to fibrillar aggregates in SPs [22, 23], as confirmed by the current results of Western blot analysis and IHC staining for Aβ in AβPP/PS1 Tg mice. The toxicity of the aggregates is dependent on the size and the location of the aggregate [42]. The larger aggregates (>80 kDa) are less neurotoxic than the smaller ones (<80 kDa). The intracellular toxic effects of soluble Aβ mainly include apoptotic pathways, and the functions of mitochondria and endoplasmic reticulum [43–45], while the extracellular soluble Aβ aggregates induce synaptic dysfunction which may play an important role in the early pathogenesis of AD [46]. Thus all the components of soluble Aβ aggregates are involved in the early development of AD.
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Fig. 4. Quantification analysis of both the 6E10 signal in IHC result and thioflavine-s stained SPs in olfactory system (including the OE, OB, AON, PC, EC, and HP) of AβPP/PS1 Tg mice. A) Bar graphs showing the mean area density of 6E10 signal in the olfactory system. B) Bar graphs showing the number of SPs in the olfactory system. Error bars represent SD. A paired samples t-test was used for statistical analysis, and asterisks indicate a significant difference in the means (*p<0.05 and **p<0.01).

Our Western blot and IHC results show that the spatial-temporal pattern of Aβ deposits, mainly soluble aggregates, in the olfactory system. In non-reducing buffer, multiple bands of Aβ aggregates in 25-kDa increments (Fig. 1) suggests that the Aβ pentamer is the native aggregating unit in vivo, as revealed by in vitro structure [47, 48]. The Western blotting result of almost undetectable Aβ monomer and dimer (Fig. 1) also agrees with the fact that the 6E10 antibody stains the unaggregated much weaker than the aggregated soluble Aβ [42]. In the OE and OB (Fig. 2), the likely intracellular localization of the Aβ signal is consistent with the concept that the intraneuronal Aβ is a trigger for neuronal loss in the olfactory system [49]. In the olfactory cortices, the soluble extracellular Aβ aggregates may be toxic to synapses [46] and activate microglia [50, 51], leading to the formation of SPs. Therefore, our data suggest that the progressive spatial-temporal deposition pattern of soluble Aβ aggregates might play important roles in the development of SPs in the olfactory system.

Aβ-associated OSNs degeneration and olfactory deficits in AβPP/PS1 Tg mice

In the AβPP/PS1 Tg mice, early degeneration of OSNs within the OE is coincident with the olfactory dysfunction (Figs 2 and 5). This is consistent with clinical AD pathology and observations [52]. Aβ deposition in the OE of AD brains was punctate and small patchy aggregates [52]. OSNs can regenerate throughout a lifetime, but is slower in older mice [53]. OMP, a marker for functional mature OSNs [54], is involved in synaptic formation and odor information processing [55, 56]. Its expression level decreases age-dependently in the AβPP/PS1 Tg OSNs and significantly faster. Accompanying this decline are the faster reductions of the OE thickness (Fig. 2) and more...
Fig. 5. Odor detection ability was evaluated by the habituation/dishabituation test in AβPP/PS1 Tg mice. The x-axis represents the trial number. The sequence of odor exposure consisted of water for 3 trials, and then vanillin or ethyl acetate was used for an additional trial. And the investigation time in the trial is shown as y-axis. A) The Tg mice could not recognize a diluted vanillin solution (1:10⁵ diluted) beginning at 3 mo. As a control, the NTg mice recognized diluted vanillin at 2, 3, 4, and 6 mo, but not over 10 mo. B) The Tg mice did not recognize a diluted ethyl acetate solution (1:10⁶ diluted) after 3 mo, while the NTg mice could detect the solution up to 10 mo. For both solutions, the investigating time of NTg and Tg mice in different age groups were found to be significantly different. A paired samples t-test was used for statistical analysis (*p<0.05 and **p<0.01 was used when comparing the investigation time for odor stimuli with water stimuli).

deposition of soluble Aβ aggregates. Therefore, the current data indicates that the anatomical changes in the OE are associated with deposition of soluble Aβ aggregates.

Interestingly, functional deprivation of olfactory sensation in Tg2576 mouse proves that functional loss promotes amyloid plaque pathogenesis in the OB and PC [57]. Our quantification result of 6E10 signal in the
Fig. 6. In the MWM, the spatial learning and memory of AβPP/PS1 Tg mice was intact at 3 mo, but deficits were found at 9-10 mo. A) In the place navigation test, the escape latency of 3 mo Tg mice shows no difference when compared with NTg littermates (ANOVA with repeated measures, $F_{1,16} = 0.92, p = 0.34$). B) In the place navigation test, the escape latency of 9-10 mo Tg mice was significantly different from NTg littermates (ANOVA, $F_{1,18} = 13.7, p < 0.001$). C) In the spatial probe test, the swimming time of Tg mice in the targeted quadrant showed no difference with the NTg littermates at both 3 mo (Independent samples t-test, $t(16) = 0.13, p = 0.90$) and 9-10 mo (Independent samples t-test, $t(17) = 1.85, p = 0.08$).

Olfactory system also demonstrated that Aβ deposition increase significantly as the olfactory detection ability begins to decline. Thus, OSNs degeneration indicated by decreased OMP expression and thinned OE may lead to the elevation of olfactory threshold and formation of amyloid deposition in the OB, AON, and PC, all of which process basic olfactory sensory functions [58]. Therefore, the Aβ-associated OSNs degeneration correlates with olfactory deficits reflected by behavioral test in AβPP/PS1 Tg mice.

Aβ aggregation in the olfactory cortices and development of olfactory deficits

Western blot analysis revealed that the olfactory system is particularly vulnerable to deposition of soluble Aβ aggregates (Fig. 1) and that olfactory detection ability begins to decline at 3 mo in AβPP/PS1 Tg mice. When soluble Aβ aggregates become detectable by IHC in the OB, AON, and PC, similar facts have been found in 5XFAD mice [16]. The axons of mitral cell terminate in the superficial layer I of the PC and synapse onto the apical dendrites of pyramidal cells, whose somas are located in layer II/III [59, 60]. Our finding that Aβ deposits begin in layer II/III of the PC in 3-4 mo AβPP/PS1 Tg mice indicates that such Aβ deposits may affect pyramidal cells and their synaptic functions. Since PC encodes higher-order representations of odor quality, identity, and familiarity [41], and is associated with olfactory learning and memory, disrupting PC activity would impair corresponding olfactory functions [14]. Along with the facts that Aβ-dependent olfactory dysfunction occurs in young Tg2576 mice [61] and normal odor-guided behaviors are preserved by long-term Aβ oligomer antibody treatment [62], our results suggest that soluble Aβ aggregates in olfactory cortices play an important role in early olfactory deficits.

The relationship between olfactory and memory deficits

Pathologically, brain regions preferentially involved in AD are the PC, EC, and HP. All of them receive primary or secondary projections from the OB [63]. Patients with mild cognitive impairment show increased gray matter loss in the olfactory and polysynaptic hippocampal network [64]. Learning and short-term memory involves the EC and HP, while long-term memory mainly involves the prefrontal cortex [65]. In this study, 3 mo AβPP/PS1 Tg mice showed olfactory deficits but no cognitive decline. Indeed, at this age soluble Aβ aggregates were mainly found in the OE and OB, to a lesser extent in the AON and PC, but not in the EC and HP. The 9-10 mo Tg mice show significant Aβ deposition in all examined brain regions.
regions, as well as a marked olfactory deficit and cognitive deficits, including declined learning ability and memory (severe in short-term and slight in long-term memory).

The EC and HP are overlapping cortices of the olfactory and cognitive system. The EC, which has intensive connections with HP regions, preprocesses information entering the HP and contributes to learning and memory [41, 66]. Although visual-spatial memory is HP-dependent, the EC and forebrain cholinergic system are also functionally involved in MWM performance [67, 68]. Neurons in layer III of the EC project to CA1 [69]. Age-related cognitive deficits are associated with functional and molecular alterations along the projection from layer II in the EC to dentate gyri and CA3 [70]. In 9-10 mo AβPP/PS1 Tg mice, more Aβ deposits in layer II/III of the EC may disrupt the HP-EC connectivity as in AD patient [70], and probably associate with regional accumulation of Aβ deposits in the HP (mainly in dentate gyri, CA1, and CA3). Moreover, memory was found intact at 6-7 mo when small amount of SP deposits, yet impaired at 9-10 mo when large amount of SPs deposits in the HP and EC. These results indicate that the HP- and EC-dependent initiation of cognitive deficits is indicated by the appearance of SPs rather than soluble Aβ aggregates.

In conclusion, combining the behavioral results with the spatial-temporal Aβ deposition pattern, we could deduce that the spread of soluble Aβ aggregates from periphery to olfactory cortices at 3-4 mo may relate to the early odor detection deficit, whereas later SPs deposition progressed to the inner cortices (EC and HP) at around 10 mo may disturb learning and memory, gradually leading to cognitive deficit in AβPP/PS1 Tg mice. Based on our findings, we conclude that in this AD model the degree of soluble Aβ aggregates accumulating through the olfactory pathway to cognitive cortices correlates with the degree of olfactory dysfunctions, from odor detection threshold to spatial memory as the animal ages.

ACKNOWLEDGMENTS

We would like to express our gratitude to Laura Castracane (Loma Linda University in Loma Linda, California, USA) for proofreading. This work was supported in part by grants from the Natural National Science Foundation of China (31000494, 30788002, 20921004, and 2110516). Authors’ disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1817).

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