Hippocampal CysLT1R overexpression or activation accelerates memory deficits, synaptic dysfunction, and amyloidogenesis in young APP/PS1 transgenic mice

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Background: Our previous studies demonstrated that cysteinyl leukotrienes receptor 1 ($CysLT_1R$) knockout, pharmacological blockade, or hippocampus knockdown produced beneficial effects against Alzheimer's disease (AD); however, whether $CysLT_1R$ upregulation has deleterious effects on AD remains elusive.

Methods: In this study, we investigated the changes in behaviors, hippocampal amyloidogenesis, and synapse plasticity after $CysLT_1R$ overexpression by microinfusion of the lentiviral vector, containing its coding sequence of mouse (LV-$CysLT_1R$), into the bilateral dentate gyri (DG) of the hippocampus or $CysLT_1R$ activation by repeated systemic administration of its agonist YM-17690 (0.1 mg/kg, once a day, i.p., for 28 d).

Results: The behavior data showed that overexpression of $CysLT_1R$ in hippocampal DG or administration of YM-17690 deteriorated behavioral performance in Morris water maze (MWM), Y-maze tests, and novel object recognition (NOR) in young APP/PS1 mice. The further studies showed that these treatments significantly destroyed synaptic function, as evidenced by impaired hippocampal long-term potentiation (LTP), decreased spine density, low number of synapses, and decreased postsynaptic protein (PSD95), and promoted the generation of amyloid β (Aβ) through increased expression of BACE1 and PS1 in the hippocampus of young APP/PS1 mice.

Conclusions: Together, our results indicate that $CysLT_1R$ upregulation accelerates memory impairment in young APP/PS1 mice, which is associated with promoting synaptic dysfunction and amyloidogenesis in the hippocampus.

Keywords: Cysteinyl leukotrienes receptor 1 ($CysLT_1R$); Alzheimer's disease (AD); memory; synaptic plasticity; amyloidogenesis

Introduction

Alzheimer's disease (AD) is a progressive synaptic failure disease related to age, characterized by progressive cognitive and behavioral impairments such as the inability to make new memories and loss of important past memories (1). Although the pathogenesis and mechanism of AD progression remain unclear, amyloid β (Aβ) plaques and neurofibrillary tangles are the two principal

Page 2 of 11

Fang et al. CysLT1R accelerates memory deficits in APP/PS1 mice

neuropathological hallmarks of AD (2,3). Therapies targeting Aβ have failed to show clinical efficacy, but the evidence of Aβ aggregates in post-mortem analysis (autopsy), in neuroimaging diagnosis, and data from genetic and biochemical studies lead researchers to think that they cannot rule out the role of Aβ in AD (4,5). According to the amyloid cascade hypothesis, the accumulation of Aβ peptide and consequent aggregation and deposition in the form of amyloid plaques is the main cause of the neurodegenerative process of AD (6). Current treatment for AD (donepezil, galantamine, rivastigmine, and memantine) is only symptomatic and has modest benefits. Thus, the development of drugs with the potential to influence disease progression has become a priority (7).

Cysteinyl leukotriene receptor 1 (CysLT$_1$R) is an important G protein-coupled receptor mediating the biological functions of cysteinyl leukotrienes and mostly expressed in lung smooth muscle cells, interstitial lung macrophages, and the spleen, and it has been thoroughly studied elucidating its role in the etiology of airway inflammation and asthma (8). Antagonists of CysLT$_1$R, such as montelukast, zafirlukast, and pranlukast, have been established as important therapeutics for clinical management of asthma for more than a decade (9). Recently, some studies on CysLT$_1$R have focused on its novel pathophysiological role in CNS disorders, such as cerebral ischemia (10), traumatic brain injury (11,12), experimental autoimmune encephalomyelitis (13), and more. We reported that intracerebral infusions of LTD4, a cysteinyl leukotriene, led to memory impairment in normal mice (14), and CysLT$_1$R antagonists montelukas or pranlukast produced significant protection against cognitive impairment induced by Aβ, lipopolysaccharide, and streptozotocin in mice (15-19). Recently, we generated APP/PS1-CysLT$_1$R$^{-/-}$ mice, and performed lentivirus-mediated knockdown of CysLT$_1$R gene in the hippocampus of APP/PS1 mice, which revealed that CysLT$_1$R knockout or knockdown could conserve synaptic structure and plasticity, and improve cognition in APP/PS1 mice (20). But it is little known that whether CysLT$_1$R upregulation accelerate AD progression. To further clarify the role of CysLT$_1$R in AD, we herein investigated effects of hippocampal CysLT$_1$R overexpression or pharmacological activation on memory deficits, synaptic dysfunction, and amyloidogenesis in young APP/PS1 transgenic mice. We present the following article in accordance with the ARRIVE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4518).

Methods

Materials

The following antibodies were purchased: rabbit anti-CysLT$_1$R from Cayman Chemical (Ann Arbor, MI, USA); rabbit anti-APP, rabbit anti-BACE, rabbit anti-PSD-95, and rabbit anti-PS1 from Cell Signaling Technology, Inc. (Danvers, MA, USA); rabbit anti-β-actin from Boster Biotechnology Co., Ltd. (Pleasanton, CA, USA); Alexa Fluor 488 donkey anti-rabbit IgG were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). All other chemicals were of analytical grade and commercially available.

Animals

Male APP/PS1ΔE9 mice (herein referred to as APP/PS1) and their wild-type (WT) littermates were constructed and bred by Model Animal Research Center of Nanjing University (Nanjing, China). The experimental procedures involving animals and their care were conducted in compliance with the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines (21,22). Experiments were performed under a project license granted by China Pharmaceutical University, in compliance with guidelines of China Pharmaceutical University for the care and use of animals.

Stereotaxic injection of lentivirus in mouse brain

At 5 months of age, WT and APP/PS1 mice were anaesthetized with chloral hydrate (350 mg/kg). Bilateral hippocampal dentate gyri (DG) injection of LV-CysLT$_1$R-EGFP (GeneChem Co., Ltd., Shanghai, China) was performed stereotactically at coordinates 2.1 mm to posterior, 1.7 mm to lateral, and 2.1 mm to ventral relative to brain bregma of APP/PS1 mice. Lentiviral vector-functional enhanced green fluorescent protein (LV-EGFP) served as the control vector. A volume of 2.5 µL of viral suspension containing 2.5×10^6 vector genome (virus titration) was injected at a rate of 0.25 µL/min. After 4 weeks, the mice underwent behavioral testing and biochemical examinations.

Western blot

Hippocampus was homogenized in radioimmunoprecipitation assay (RIPA) buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM ethylenediamine tetraacetic acid (EDTA), 1% Triton

X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS)] and centrifuged at 12,000 g for 15 min. Protein concentrations were determined by bicinchoninic acid (BCA) assay kit (Beyotime, Jiangsu, China). Proteins samples were separated on 12% SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. The membranes were blocked for 2 h with 5% fat-free milk and incubated overnight at 4 ℃ with primary rabbit-derived antibodies. After washing with Tris-buffered saline containing 0.1% Tween 20 (TBST) 3 times, the membranes were incubated with an appropriate secondary antibody (1:5,000) for 2 h at room temperature. The antibody-reactive bands were visualized using Tanon gel imaging system (23).

Immunofluorescence

Mice were transcardially perfused with 4% paraformaldehyde, their brains were then post-fixed overnight in 4% paraformaldehyde at 4 ℃, and dehydrated in 30% sucrose until isotonic. Afterwards, 25 μm coronal sections were sliced on a freezing microtome (Leica, Wetzlar, Germany). After blocking in 2% serum for 2 h, sections were incubated in primary antibody overnight. On the second day, the sections were incubated with secondary antibody and then washed in phosphate-buffered saline (PBS, 3×5 min). The sections examined using fluorescence microscopy (Leica) (24).

Enzyme-linked immunosorbent assay

Detection of Aβ was performed as described previously (20). All procedures were performed in accordance with the manufacturer's instructions. Briefly, fractions of the hippocampi (100–120 mg) were homogenized in ice-cold lysis buffer containing proteinase inhibitor cocktail. After 15 min of incubation on ice, homogenates were centrifuged at 14,000 g for 15 min at 4 ℃, leaving the supernatant containing triton-soluble Aβ peptides. The triton-insoluble pellets were then extracted using 5 M guanidine-HCl, and were briefly re-homogenized and shaken for 4 h at room temperature to promote extraction of insoluble Aβ. The homogenates were centrifuged for 5 min at 8,000 g. Triton-soluble and guanidine-HCl-soluble fractions were used as input for enzyme-linked immunosorbent assay (ELISA) detection of $A\beta_{1-40}$ or $A\beta_{1-42}$. Total protein concentrations were determined in each fraction by BCA protein assay. Levels of proteins were measured using ELISA kits ($A\beta_{1-40}$ or $A\beta_{1-42}$ ELISA kit, Cusabio Biotech Co. Ltd.,

Wuhan, China).

Hippocampal slice preparation and electrophysiology

Hippocampal transversal slices from 6-month-old WT and APP/PS1 mice were prepared using a vibratome as described previously. Briefly, mice hippocampal slices were placed in a recording chamber and perfused with artificial cerebrospinal fluid (ACSF) at 24 ℃. Schaffer collaterals were stimulated with a tungsten monopolar electrode. The field excitatory postsynaptic potentials (fEPSPs) were recorded from the CA1 stratum radiatum by a glass microelectrode filled with ACSF with resistance of 3–4 MΩ. Then, fEPSPs were recorded under current-clamp mode. Long term potentiation (LTP) was induced with a high-frequency stimulation protocol consisting of two one-second long 100 Hz trains (16).

Golgi staining

Mouse brains were removed and Golgi staining was performed using an FD Rapid Golgi Stain Kit (FD Neuro Technologies, Ellicot City, MD, USA) as described. The brains were immersed in solution A and B for 2 weeks and then in solution C for 48 h. Brain samples were serially sectioned into 100 μm coronal slices with a freezing microtome. Slides were then stained with solution D: solution E mixture solutions for 5 min. The sections were dehydrated with graded alcohol solutions, cleared in xylene, and cover slipped. The preparations were observed under a microscope. For morphological analysis of hippocampal DG neurons, 5 granule neurons from each mouse (4 mice/group, 20 neurons from each group) were calculated from the hippocampal DG. The number of spines was quantified by ImageJ (https://imagej.nih.gov/ij/). Spine density was calculated per 10 μm of dendritic length (25).

Electron microscopy

After deep anesthesia, mice were perfused transcardially with 2% glutaraldehyde and 3% paraformaldehyde in PBS. Hippocampal slices were then prepared and fixed in cold 1% OsO4 for 1 h. Ultrathin sections (90 nm) were stained with uranyl acetate and lead acetate, and viewed at 100 kV in a JEOL 200CX electron microscope (JEOL USA Inc., Peabody, MA, USA). Synapses were identified by the presence of synaptic vesicles and postsynaptic densities. The number of synapses was independently counted by two

Page 4 of 11

Fang et al. CysLT1R accelerates memory deficits in APP/PS1 mice

investigators, who were blind to age and species (26).

Morris water maze (MWM)

The mice were assessed for spatial learning and memory in the MWM. During day 1–2 of visible platform training, the platform location was indicated by a flag. During day 3–5, we removed the flag and let the mice discover the hidden platform. Each animal was trained at each quadrant with 1 h interval between trials. The animal had to find the platform in 90 s. If it could not reach the platform within 90 s, the animal was placed on the platform by an experimenter for 30 s. At day 6, mice freely explored in the pool for 90 s without the hidden platform (27).

Y-maze avoidance tests

The maze was constructed with 10 cm high walls and a stainless floor. The mice were tested for 2 days. On training day, the mouse firstly moved freely for 5 min. Then during the learning trial, electric shocks (2 Hz, 125 ms, 10 V) were given in two sectors of the maze through the stainless floor and the light was on in the non-electric sector. Each mouse was trained 10 times. After remaining in the non-electric sector for 30 s, the training was stopped, and this was recorded as a correct choice. The experimenter was required to let the mouse stay in the non-electric sector for 30 s when the mouse failed at entering the sector. On testing day, the same procedure was repeated 10 times (17).

Novel object recognition (NOR) test

On the first day, mice were familiarized with the chamber for 10 min. After 24 h, mice were allowed to explore the same two objects for 5 min. The next day, mice were exposed to two different objects, one familiar object and one novel object, for 5 min each object. The discrimination index was used to reflect the exploration time of the novel object, expressed as the ratio of the total time spent exploring both novel and familiar objects (27).

Statistical analysis

Group differences in the MWM escape latencies were analyzed using a repeated measure analysis of variance (ANOVA) with "days" as the within-subject factor and "group" as the between-subject factor. The remaining data were analyzed using either Student's t-test (2-group comparison) or one-way ANOVA (for more than two groups) followed by a Dunnett's post-hoc analysis, if deemed necessary. Descriptive data were presented as means ± standard error of the mean (SEM). All analyses were carried out using SPSS 20.0 (IBM Corp., Armonk, NY, USA). A P value <0.05 was considered statistically significant.

Results

Hippocampal CysLT₁R overexpression or activation accelerates cognitive impairment in young APP/PS1 transgenic mice

Our previous studies showed that hippocampal $CysLT_1R$ expression increased in APP/PS1 mice with age (20). To confirm the effect of $CysLT_1R$ upregulation on AD, we delivered lentiviral vector encoding $CysLT_1R$ into the hippocampal DG of young APP/PS1 mice (*Figure 1A*). Western blot data showed that hippocampal $CysLT_1R$ expression significantly increased at 3 weeks after the lentivirus injection ($F_{[2, 9]} = 18.37$, P<0.01, *Figure 1B,1C*). The MWM data showed that the mice in each group exhibited similar escape latency in the visible-platform test ($F_{[2,237]} = 9.672$, P>0.05, *Figure 1D*), suggesting no influence of the lentiviral vector infusion on vision or basal motivation of mice, and hippocampal $CysLT_1R$ overexpression significantly increased escape latency in the spatial hidden-platform variant ($F_{[2,237]} = 0.5105$, P<0.05, *Figure 1E*), decreased time spent in the target quadrant and the number of platform location crossings on day 6 ($F_{[2,27]} = 11.410$, P<0.05, *Figure 1F*; $F_{[2,27]} = 5.884$, P<0.05, *Figure 1G*) in young APP/PS1 mice. The young APP/PS1 mice with hippocampal $CysLT_1R$ overexpression exhibited a reduced number of correct choices ($F_{[2,27]} = 7.109$, P<0.05, *Figure 1H*) and more latency to enter the shock-free compartment ($F_{[2,27]} = 11.580$, P<0.05, *Figure 1I*) in the Y maze test, and showed lower discrimination index ($F_{[2,27]} = 16.700$, P<0.05, *Figure 1J*) in the NOR test. To further confirm detrimental effects of $CysLT_1R$, we investigated effects of YM-17690, a specific $CysLT_1R$ agonist, on behavior performance in young APP/PS1 mice. The results showed that YM-17690 treatment (0.1 mg/kg, once a day, i.p., for 28 d) produced similar effects on behavior performance in the MWM task (*Figure 1K,1L*), Y-maze, and NOR tests (data not shown) with hippocampal $CysLT_1R$ overexpression in the young APP/PS1 mice. We also used liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis to confirm its presence in the brain after administration
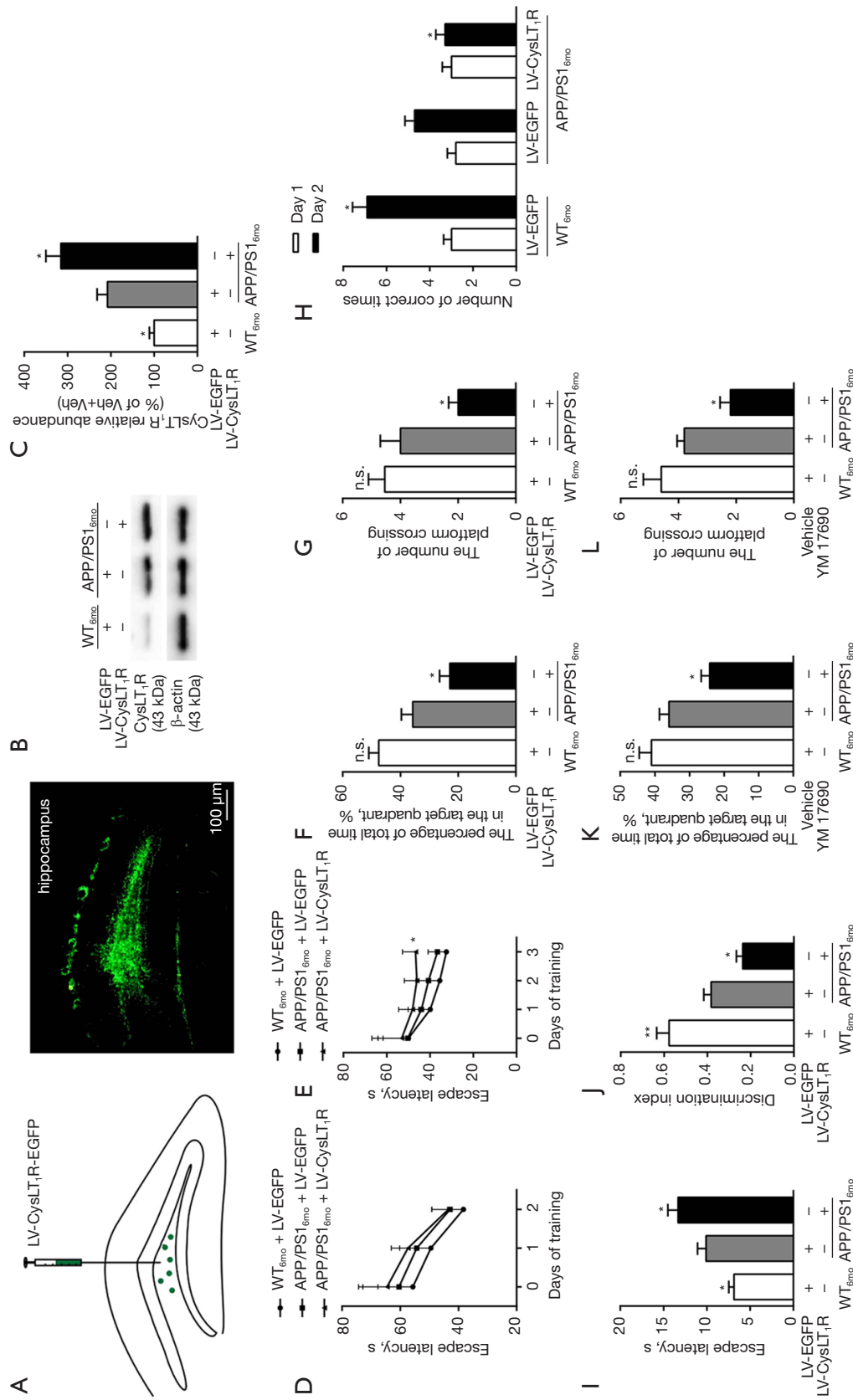
Figure 1 Hippocampal CysLT₁R overexpression or activation accelerates cognitive impairment in young APP/PS1 transgenic mice. (A) Representative hippocampus area with LV-CysLT₁R-EGFP. (B) The hippocampal CysLT₁R protein, and (C) quantified as ration (in%) of WT + EGFP group. (D,E) The mean escape latency to the visible platform and the hidden platform, (F) the percentage of time spent in the target quadrant, and (G) the numbers of platform location crossings during the probe trial in the MWM task, and (H) the number of correct choices, (I) the latency to enter the correct compartment in the Y-maze test, and (J) the discrimination index in the NOR test in young APP/PS1 mice treated with LV-CysLT₁R. (K) The percentage of time spent in the target quadrant, and (L) the numbers of platform location crossings during the probe trial in the MWM task in young APP/PS1 mice treated with YM-17690. Values shown are expressed as mean ± SEM; n=4/group for Western blot, n=9–10/group for behavior tests. *P<0.05, **P<0.01 vs. 6-month-old APP/PS1 + EGFP group or 6-month-old APP/PS1 + Vehicle group. CysLT₁R, cysteinyl leukotriene receptor 1; WT, wild type; EGFP, enhanced green fluorescent protein; NOR, novel object recognition; SEM, standard error of the mean.

Page 6 of 11

Fang et al. CysLT1R accelerates memory deficits in APP/PS1 mice

of YM-17690. Assays using LC-MS/MS for YM-17690 content showed that its content in the hippocampus reached the maximum at 0.5 h after administration, and lasted for 8 h (data not shown), indicating that it can pass through the blood-brain barrier (BBB).

Hippocampal CysLT$_1$R overexpression or activation worsened synaptic plasticity deficiency in young APP/PS1 transgenic mice

Synaptic dysfunction may cause cognitive damage and neurodegeneration (28,29). The present data showed that 6-month-old APP/PS1 mice already had compromised synaptic plasticity, reflected by lower level of fEPSP in the hippocampus, compared to WT mice. When we overexpressed or implemented YM-17690 treatment in young APP/PS1 mice, the level of fEPSP decreased even more significantly (LV-CysLT$_1$R: $F_{[2,9]}$ =7.720, P<0.05, *Figure 2A,2B*; YM-17690: $F_{[2,9]}$ =8.674, P<0.05, *Figure 2C,2D*), suggesting that CysLT$_1$R also has a role in disrupting LTP in the early pathogenesis of AD. The number of synapses and dendritic spine densities were also evaluated by the presence of synaptic vesicles observed using an electron microscope and Golgi staining, respectively. The overexpression or activation of CysLT$_1$R significantly increased synaptic losses (LV-CysLT$_1$R: $F_{[2,9]}$ =53.760, P<0.001, *Figure 2E,2F*; YM-17690: $F_{[2,9]}$ =37.20, P<0.001, *Figure 2E,2G*), and led to significant decreases in spine density (LV-CysLT$_1$R: $F_{[2,9]}$ =68.270, P<0.001, *Figure 2H,2I*; YM-17690: $F_{[2,9]}$ =45.39, P<0.001, *Figure 2J,2K*), in younger APP/PS1 mice. The hippocampal PSD-95, a postsynaptic marker, was remarkably decreased (LV-CysLT$_1$R: PSD-95: $F_{[2,9]}$ =55.950, P<0.001, *Figure 2L,2M*; YM-17690: $F_{[2,9]}$ =45.51, P<0.001, *Figure 2N,2O*) in young APP/PS1 transgenic mice with CysLT$_1$R overexpression or activation.

Hippocampal CysLT$_1$R overexpression or activation promoted Aβ generation in young APP/PS1 transgenic mice

To determine whether hippocampal CysLT$_1$R overexpression or activation increases amyloidogenesis, we measured Aβ, amyloid precursor protein (APP), β-secretase (BACE1), and PS-1, catalytic subunit of the γ-secretase enzyme complex, in hippocampi of young APP/PS1 transgenic mice. The results showed that overexpression or activation of CysLT$_1$R significantly increased hippocampal Aβ$_{1-40}$, a TBS-soluble Aβ (LV-CysLT$_1$R: $F_{[1, 10]}$ =7.234, P<0.05; YM-17690: $F_{[1, 10]}$ =5.227, P<0.05, *Figure 3A,3B*),

but still showed immeasurable hippocampal Aβ$_{1-42}$ in young APP/PS1 transgenic mice. Overexpression or activation of CysLT$_1$R had no effect on hippocampal APP expression (*Figure 3C-3F*; LV-CysLT$_1$R:$F_{[2,9]}$ =12.540, P>0.05; YM-17690: $F_{[2,9]}$ =11.73, P>0.05) but significantly increased hippocampal BACE1 and PS-1 (*Figure 3C,3D,3G-3J*; LV-CysLT$_1$R: BACE: $F_{[2,9]}$ =15.740, P<0.05; PS-1: $F_{[2,9]}$ =16.450, P<0.05; YM-17690: BACE: $F_{[2,9]}$ =21.710, P<0.05; PS-1: $F_{[2,9]}$ =16.35, P<0.05) in young APP/PS1 mice, which suggests that CysLT$_1$R upregulation might increase generation of Aβ via promoting the expression of BACE1 and PS-1.

Discussion

The present studies firstly demonstrated that hippocampal CysLT$_1$R overexpression by microinfusion of the lentiviral vector containing its coding sequence into the bilateral DG or activation by repeated systemic administration of its specific agonist YM-17690 accelerated impairments of long-term learning and memory in the MWM task and short-term memory in the Y-Maze and NOR in young APP/PS1 mice. Simultaneously, these treatments accelerated deficits of synaptic plasticity indicated by decreased synapses and LTP, as well as amyloidogenesis resulting from increased expression of BACE1 and PS-1.

Cognitive function and pathological characteristics are different during different stages of AD (30). In 6-month-old APP/PS1 mice, the short-term learning and memory is impaired, but the long-term cognitive function is not impacted yet. However, 10-month-old APP/PS1 mice display both short-term and long-term cognitive dysfunction (31-33). The 6-month-old (young) APP/PS1 mice in the present study exhibited obvious impairments of short-term rather than long-term learning and memory. Cysteinyl leukotrienes (including LTC4, LTD4 and LTE4), 5-lipoxygenase (5-LO) metabolites of arachidonic acid, are potent inflammatory mediators, which could bind to CysLT1R to mediate pro-inflammatory actions. It has been shown that high levels of neuroinflammation could impair the synaptic structure and function, which induce AD aggravation. Hippocampal CysLT$_1$R overexpression and activation not only caused impairment of long-term learning and memory, but also aggravated short-term learning and memory, which indicates that CysLT$_1$R participates in progressive decline in cognitive function of AD.

Functional synapse formation is critical for the synaptic transmission in the brain, and synaptic plasticity is the
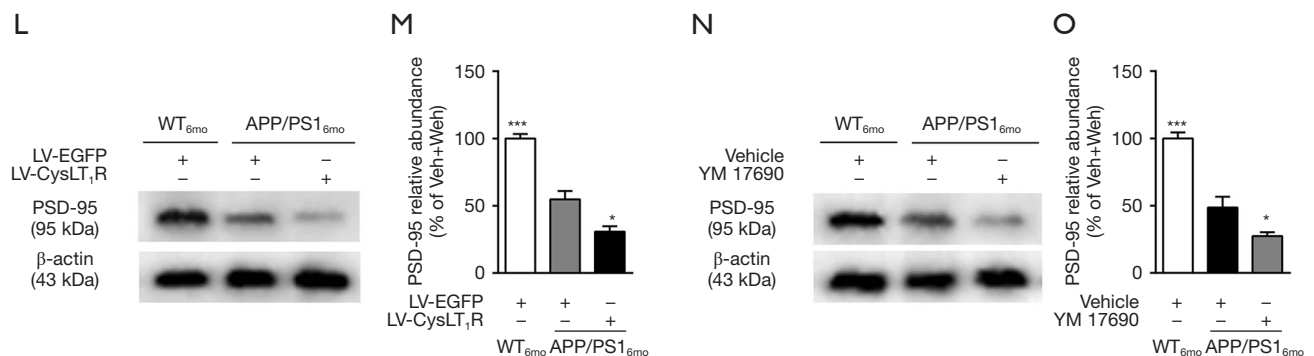
Page 8 of 11

Fang et al. CysLT1R accelerates memory deficits in APP/PS1 mice
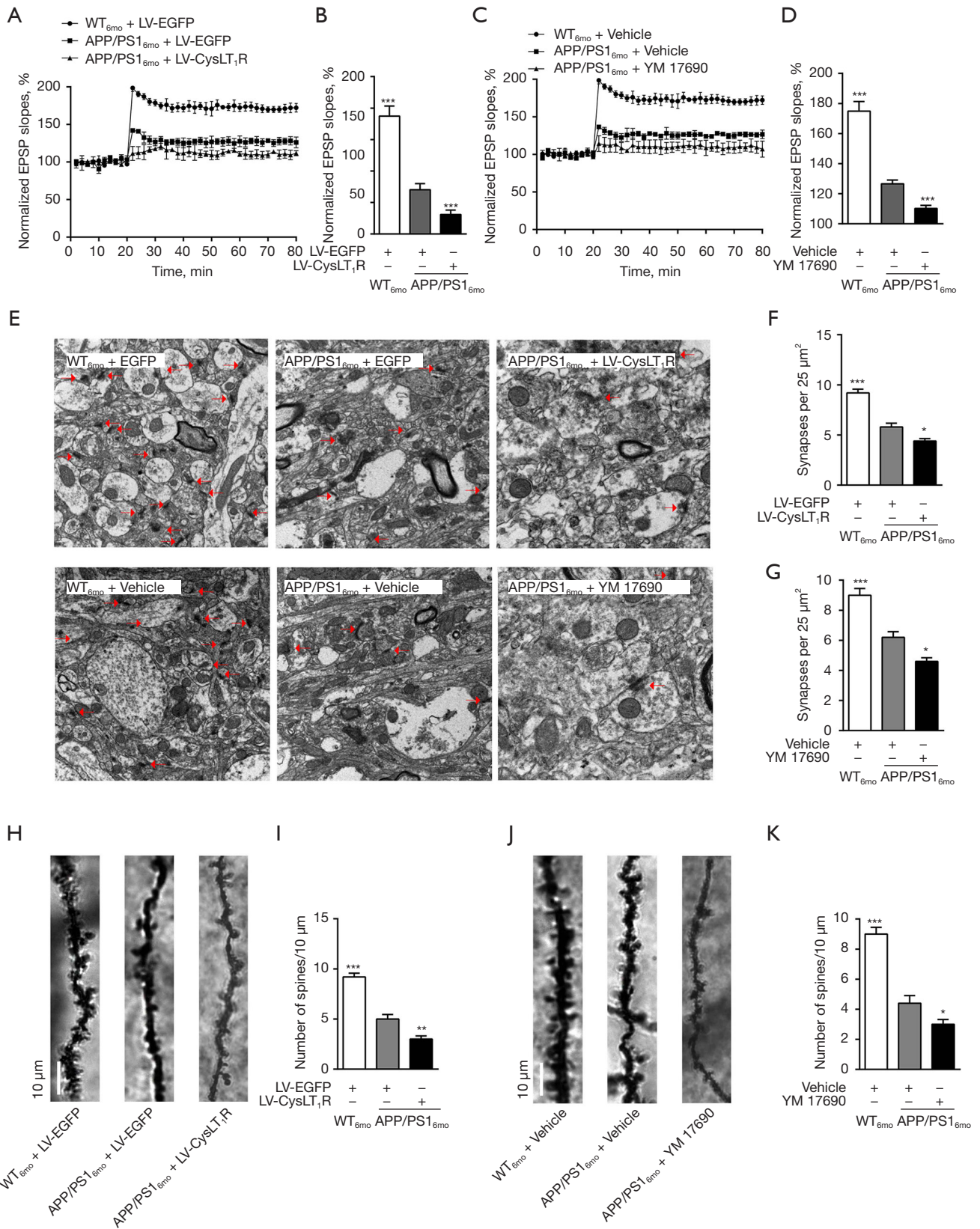
Figure 2 Hippocampal CysLT$_1$R overexpression or activation aggravates damages to synaptic structure and function in APP/PS1 transgenic mice. (A,C) The induction of hippocampal LTP was assessed after high-frequency stimulation and recorded for 60 min post-induction, and (B,D) mean values of fEPSPs were calculated during 55–60 min following the induction of LTP. (E) The synaptic density in the hippocampus was determined by electron microscopy. The red arrows indicate postsynaptic density. Scale bar =1 μm. (F,G) statistical analysis of synaptic density calculated as the number of synapses per 25 μm^2. (H,J) Representative images of Golgi-Cox staining of dendritic spines in the hippocampus. Scale bar =10 μm. (I,K) Statistical analysis of the average number of dendritic spines. (L,N) The protein levels of PSD-95 were detected in hippocampus using western blot and (M,O) quantified as ratio (in%) of WT + EGFP/WT + Vehicle group. Values shown are expressed as mean ± SEM; n=4–6/group; *P<0.05, **P<0.01, ***P<0.001 *vs.* 6-month-old APP/PS1 + EGFP or 6-month-old APP/PS1 + Vehicle group. CysLT$_1$R, cysteinyl leukotriene receptor 1; LTP, long-term potentiation; fEPSPs, field excitatory postsynaptic potentials; WT, wild type; EGFP, enhanced green fluorescent protein; SEM, standard error of the mean.

important neurobiological foundation for learning and memory, and the modification of synaptic strength produced by LTP is widely thought to underlie memory storage (34,35). Marked synapse loss was found in the brain, especially in hippocampus and cerebral cortex, of AD patients in previous findings. At 3 months of age, APP/PS1 mice also showed deficits in synaptic plasticity (36). We found impaired LTP, decreased dendritic spine density, synapse number, and PSD95 proteins in young APP/PS1 mice. More importantly, hippocampal CysLT$_1$R overexpression and activation dramatically accelerate these changes in young APP/PS1 mice. Hippocampal-based LTP is one mechanism for the synaptic plasticity underlying explicit memory storage in mammals; therefore, following overexpression or activation of CysLT$_1$R, lower levels of fEPSP in the hippocampus represent not only impaired synaptic function, but also worsened cognitive function (37,38).

Although the precise cause of AD remains elusive, it has been suggested that synaptic loss in AD is attributed to the accumulation of Aβ (39), which is a cleavage product derived from amyloid precursor protein. On sequential cleavage by aspartyl proteases β- and γ-secretase, APP generates various peptide species, including Aβ$_{1-40}$, Aβ$_{1-42}$, and so on, which are prone to oligomerization, leading to the formation of amyloid plaques (40). Our previous studies showed that

CysLT$_1$R-mediated signaling referred to NF-κB pathway, which led to elevations of APP and β- and γ-secretases and subsequent increase of Aβ production (14,41). In APP/PS1 transgenic mice, CysLT$_1$R overexpression or activation did not influence APP, but increased expression of BACE1 and PS-1, subsequently led to Aβ generation. This difference might be associated with animal status. The vast majority of potential disease-modifying treatments developed in recent years are directed against Aβ, including inhibitors of the synthetic enzyme gamma-secretase and beta-secretase, and Aβ aggregation inhibitors. However, the most elaborated anti-Aβ approach is immunotherapy, including both active vaccines to stimulate the immune system to produce its own antibodies and passive immunization through the administration of exogenous antibodies. Although the development of therapies targeting Aβ for AD has been beset by disappointing results, these failures contain important clues as well as evidence of promise. These therapy trials, especially immunotherapy, may be started too late in disease-when too much Aβ has accumulated and the Aβ cascade is irrevocably initiated. We can expect new trials to be initiated ever earlier in the course of AD.

Taken together, our study indicates that overexpression or activation of CysLT$_1$R accelerates cognitive dysfunction in young APP/PS1 mice, which is involved in Aβ accumulation
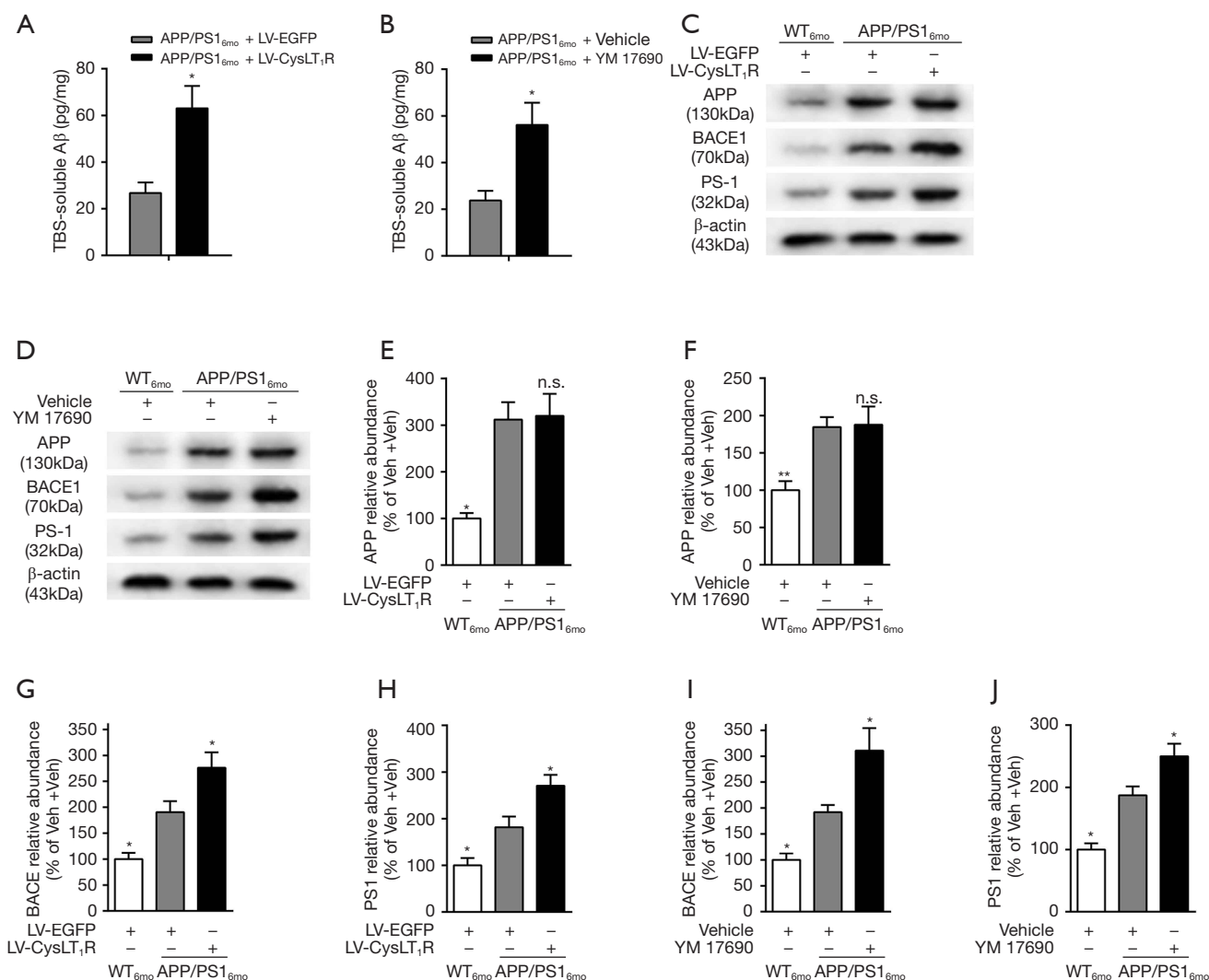
Figure 3 Hippocampal $CysLT_1R$ Overexpression or activation exacerbates Aβ generation in young APP/PS1 transgenic mice. (A,B) The TBS-soluble forms of $Aβ_{1-40}$ in hippocampus were assessed by ELISA. (C,D) Representative western blot of APP, BACE, and PS1 in hippocampus. Quantification of (E,F) APP, (G,I) BACE, (H,J) PS1 levels were expressed as ration (in%) of WT + EGFP/WT + Vehicle group. Values shown are expressed as mean ± SEM; n=4–6/group; *P<0.05 *vs.* 6-month-old APP/PS1 + EGFP or 6-month-old APP/PS1 + Vehicle group. $CysLT_1R$, cysteinyl leukotriene receptor 1; TBS, Tris-buffered saline; Aβ, amyloid β; ELISA, enzyme-linked immunosorbent assay; APP, amyloid precursor protein; BACE, β-secretase; SEM, standard error of the mean.

and synaptic dysfunction. Our findings suggest that $CysLT_1R$ could be a key player in the development of AD and could be targeted for the prevention and/or treatment of AD.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://dx.doi.

Page 10 of 11

Fang et al. CysLT1R accelerates memory deficits in APP/PS1 mice

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license granted by China Pharmaceutical University, in compliance with guidelines of China Pharmaceutical University for the care and use of animals.

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