Sodium Tanshinone IIA Sulfonate Improves Cognitive Impairment via Regulating Aβ Transportation in AD Transgenic Mouse Model

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Research Article

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Abstract

Alzheimer's disease (AD) is a most common neurodegenerative disease. Sodium Tanshinone IIA Sulfonate (STS) has been reported to ameliorate AD pathology. However, the underlying mechanism is still unclear. In this study, APP/PS1 mouse model was used to explore the potential mechanism of STS against AD. Morris water maze and Y-maze tests showed that administration of STS (10 or 20 mg/kg/day) improved learning and memory abilities of APP/PS1 mice. STS reduced the levels of ROS and MDA, while improved the activity of SOD in both hippocampus and cortex in APP/PS1 mice. STS inhibited the activity of AChE, while improved the activity of ChAT in APP/PS1 mice. In addition, STS elevated the protein expressions of neurotrophic factors (BDNF and NGF) and synapse-related proteins (PSD93, PSD95 and SYP) in both the hippocampus and cortex in APP/PS1 mice. At last, STS improved the protein expressions of GLUT1 and LRP1. These results indicated that the potential mechanism of STS on AD might be related to Aβ transportation function via GLUT1/LRP1 pathway.

Highlights

STS improves cognitive impairment of APP/PS1 mice.

STS ameliorates the oxidative stress damage and improves the cholinergic system.

STS protects against neuronal dysfunction and enhances the synaptic plasticity.

STS mediates the Aβ transportation of BMECs.

Declarations

Ethics approval

All the animal experiments were approved by the Guiding Principles for the Care and Use of Laboratory Animals adopted and promulgated by United States National Institutes of Health.

Consent to Participate and Publication

All authors declare consent to participate and consent for publication.

Availability of data and materials

All original data and materials in the manuscript are available from the author.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

Hui-Han Ma, Can Wan and Lu-Di Zhang conducted the experiment. Hui-Han Ma, Can Wan and Shi-Jie Zhang contributed to initial data analysis and interpretation, and drafted the initial manuscript. Rong-Rong Zhang, Dong Peng and Li-Jun Qiao helped revising the manuscript. Shi-Jie Zhang designed the study. Shi-Jie Zhang, Ye-Feng Cai and Hong-Qiang Huang supervised all the aspects of the study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Data Availability

This manuscript has not been submitted to any other journal for simultaneous consideration and has never been published elsewhere in any form or language.

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Introduction

Alzheimer's disease (AD), a common neurodegenerative disorder, is characterized by progressive cognitive impairment and memory loss (Selkoe 2001). As reported by Alzheimer’s Disease International, there was 50 million people suffered from AD in 2018. And the number will reach to 15.2 billion in 2050. However, there is yet no suitable treatment for AD. Almost all phase III clinical trials targeting Aβ have failed (Kryscio et al. 2017; Schott et al. 2019; Sevigny et al. 2016). Therefore, it is urgent to find new drugs for AD treatment.

Numerous studies have confirmed the central role of Aβ and its oligomers in the pathogenesis of AD (Barage et al. 2015). The blood–brain barrier (BBB) protects neurons from neurotoxic factors, BBB breakdown and dysfunction have been shown in early stages during AD pathophysiological progression. The BBB is responsible for 80–85% of the clearance of AD related forms of Aβ from the brain by transvascular transport (Sweeney et al. 2018). Low-density lipoprotein receptor-related protein 1(LRP1) and receptor for advanced glycation end products (RAGE), expressed at BBB, have been proved to play a vital role in the transportation of Aβ, eventually leading to the Aβ clearance (Cai et al. 2016; Horwood et al. 1994; Mooradian et al. 1997). RAGE regulates influx of circulating Aβ into brain, whereas LRP1 mediates the efflux of Aβ into the circulation via BBB. The glucose transporter isoform 1 (GLUT1), expressed at BBB, mediates glucose transport into the brain, which is necessary for the maintenance of BBB integrity. Several studies reported that low GLUT1 level was associated with microvascular impairment and BBB dysfunction in AD patients (Kalaria et al. 1989), and the reductions in BBB transport may be explained by

the fact that the reduced expression of GLUT1 was found in the brain capillaries of AD patients in postmortem studies (Simpson et al. 1994).

Sodium Tanshinone IIA Sulfonate (STS) is a main component of traditional Chinese medicine, *Salvia miltiorrhiza Bge* (Danshen) (Zhou et al. 2018). Danshen has been widely used to treat a variety of diseases with few side effects, especially cardiovascular diseases (Li et al. 2018; Liu et al. 2013). STS has been proved with multiple pharmacological activities, including anti-inflammation, anti-oxidative, anti-apoptosis and interaction with iron channels, and has a broad prospect for the development of neuro-protective drugs (Liu et al. 2020; Shang et al. 2012; Zhang et al. 2020). Nevertheless, whether the STS against cognitive impairment through the transportation of Aβ remained to be unclear.

In the present study, two different doses (10 or 20 mg/kg) of STS were used to treat APP/PS1 mice. The results elucidated that STS may improve learning and memory dysfunction by relieving oxidative stress, regulating cholinergic system, preventing neuron dysfunction, enhancing the synaptic plasticity, and regulating the transportation of Aβ.

Materials And Methods

Materials

STS was purchased from Shanghai NO.1 Biochemical & Pharmaceutical CO.. Kits used for detection of choline acetyltransferase (ChAT), acetylcholinesterase (AChE), reactive oxygen species (ROS), malondialdehyde (MDA), and superoxide dismutase (SOD) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primary antibodies including anti-postsynaptic density 95 (PSD95), anti-postsynaptic density 93 (PSD93), anti-synaptophysin (SYP), anti-nerve growth factor (NGF) and anti-brain-derived neurotrophic factor (BDNF) were purchased from Cell Signaling Technology, Inc. Anti-glucose transporter isoform 1 (GLUT1), anti-low-density lipoprotein receptor-related protein 1(LRP1), anti-receptor for advanced glycation end products (RAGE) and anti-β-actin were purchased from Sigma-Aldrich. All secondary antibodies (horseradish peroxidase conjugated anti-rabbit IgG and anti-mouse IgG) were purchased from Cell Signaling Technology, Inc. Liberase Blendzyme 2 was purchased from Roche, Dulbecco's PBS (D-PBS) was purchased from EuroClone. Penicillin-streptomycin was purchased from Gibco.

Animal and Treatment

APP/PS1 (APPswe/PSEN1dE9) double transgenic mice and wild-type mice (non-transgenic mice) were purchased from the Model Research Centre of Nanjing University with the same background and age. Animals were housed at a standard temperature (22 - 25 °C) with automatic light cycles (12-h light/dark) and a relative humidity of 50 - 60 %. All the animal experiments were approved by the Guiding Principles for the Care and Use of Laboratory Animals adopted and promulgated by United States National Institutes of Health. Twelve-month-old mice were randomly divided into 5 groups (n = 10 each group): WT group (wild-type, 0.9 % saline), WT+STS-H group (wild-type, STS 20 mg/kg/day), APP/PS1 group

(APP/PS1, 0.9 % saline), STS-L group (APP/PS1, STS 10 mg/kg/day), and STS-H group (APP/PS1, STS 20 mg/kg/day). Mice were treated with saline or STS by intraperitoneal injection once a day for 8 weeks.

Morris Water Maze Test

Using Morris water maze test to evaluate the spatial learning and memory ability was mentioned in previous study (Xu et al. 2019). The Morris Water Maze Animal Behavioral Analysis System (Guangzhou Feidi Biology Technology Co., Ltd., Guangzhou, China) consisted of a black circular tank filled with water at 22- 26°C, a hidden platform, and a recording system. The pool was spatially divided into 4 imaginary quadrants (NE, SE, SW, NW) by a computerized tracking/image analyzer system. A circular transparent escape platform (10 cm diameter) was positioned 1- 2 cm below the opaque water surface in the middle of the target quadrant of the pool. The learning and memory abilities of mice were assessed by the Morris water maze test in a dark room. Mice were given orientation navigation tests for 6 consecutive days. Before the measurement, mice were trained once to find the platform. For each daily trial, there were 4 sequential training trials beginning with placement of the animal in the water facing the wall of the pool with the drop location changing for each trial randomly; the recording system then started to record the time. The escape latency and the swim path tracking until the mice landed on the platform were recorded on video tape. If the mouse failed to locate the platform within 60 s, it was guided to the platform and kept there for 10 s. For the probe trials, the mice were allowed to swim freely in the pool for 60 s with platform removal. The time required to cross to the original platform position, the time spent in the target quadrant, and the escape latency were measured.

Y-maze Test

Y-maze tests were used to assess cognitive changes, short-term spatial working memory (by spontaneous alternation), and exploratory activity (by total number of arm choices) of mice. The method was mentioned in previous study (Wang et al. 2018). The Y-maze is a three-arm horizontal maze (30 cm long and 8 cm wide with 15 cm high walls) in which the arms are symmetrically disposed at 120° angles from each other. The maze of floors and walls are made of opaque polyethylene plastic in a black environment. The test consisted of two phases with 1-hour intervals. During the training, the new arm was separated with a baffle and the mice were placed into the starting arm for 10 minutes, during which they had free access to the starting arm and the other arm. The test was performed 1 hour after the training. For this, the baffle in the new arm was removed and the mice were placed into the starting arm. The number of mice access into the three arms within 5 minutes was recorded. An alternation was recorded when mice made consecutive visits to the three different arms. The Y-maze spontaneous alternation was calculated as follows: Alternation behavior (%) = number of alternations / (total arm entries − 2) × 100%. At the end of the experiment, arms were cleaned with alcohol so as to remove the scent of previous mice. All mice were anesthetized and decapitated after behavioral experiments immediately. The hippocampus and cortex were carefully dissected from brains for examination. All the processes were performed on ice-cold plate. Tissues were rapidly stored at - 80°C.

Measurement of ChAT and AChE Activity

The hippocampus and cortex tissues were homogenized with ice-cold saline. The homogenate was centrifuged at 12,000 × *g* for 15 min at 4 °C. The supernatant was collected for the assay of the ChAT and AChE activities according to the manufacturer's instructions.

ROS, MDA, and SOD Assays

The hippocampus and cortex tissues were homogenized with cold saline. The homogenate was centrifuged at 12,000 × *g* for 15 minutes at 4 °C. Supernatant was collected to detect the levels of ROS, which was measured by DCFH-DA as a redox sensitive fluorescent dye. DCFH was oxidized to strong green, fluorescent substance DCF in the presence of ROS, which has a maximum peak at excitation wavelength of 502 nm and emission wavelength of 530 nm and intensity is proportional to intracellular reactive oxygen species. Supernatant was collected to detect the levels of MDA and the activity of SOD using Universal Microplate Spectrophotometer (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Isolation of Brain Microvascular Endothelial Cells (BMECs)

The BMECs were isolated from brain as described before (Navone et al. 2013). Transfer the brain into a sterile glass dish with D-PBS with 0.1% (vol/vol) penicillin-streptomycin. By using sterile scissors and a scalpel, fragment the tissue sample into pieces. Centrifuge at 276g for 10 min. Aspirate the supernatant and resuspend the homogenate pellet with Liberase Blendzyme 2 at a concentration of 0.625 mg/ml. Incubate the suspension at 37 °C on a rotator for 1 h. Resuspend the homogenate with D-PBS. Centrifuge the suspension at 276g for 10 min. Aspirate the supernatant. Coat a 25 cm^2 flask with collagen type I and incubate it for 20 min at 37 °C. Aspirate the collagen solution from the flask, then wash with D-PBS. Resuspend the pellet in culture medium and transfer the suspension to the flask. Maintain the cellular suspension at 37 °C in an atmosphere of 5% CO_2. The BMECs were collected were rapidly stored at -20°C.

Western Blot Analysis

The BMECs, hippocampus and cortex tissues were homogenized and lysed. The lysate was centrifuged at 12,000 × *g* for 10 min at 4 °C and then denatured by boiling at 100 °C with 1 : 4 loading buffer. The protein was fractionated and subsequently transferred onto PVDF membranes. The membranes were blocked in 5 % skim milk for 1 h at room temperature. The membranes containing the protein were incubated with anti-SYP, anti-PSD93, anti-PSD95, anti-BDNF, anti-NGF, anti-GLUT1, anti-LRP1, anti-RAGE and mouse anti-β-actin overnight at 4 °C. Then the membrane was incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse for 1 h at room temperature. Routinely, protein load was monitored by using a super enhanced chemiluminescence reagent (Applygen Technologies Inc., Beijing, China).

Statistical Analysis

Experimental values were given as means ± SEM. SPSS 19.0 statistical software (IBM, Endicott, NY) was evaluated to perform all statistical analysis. Two-way analysis of variance (ANOVA) was applied among the different groups to analyze differences in data for the biochemical parameters, followed by Dunnett's significant post hoc test for pairwise multiple comparisons. Differences were considered as statistically significant at $p < 0.05$.

Results

STS Improves Cognitive Impairment in APP/PS1 Mice

In the Morris water maze test, the time for APP/PS1 group to find central platform was significantly longer than WT group. Compared with the APP/PS1 group, the STS-H group was with shorter escape latency (Fig. 1A). As was shown in Fig. 1B, crossing times of the platform in APP/PS1 group were greatly less than WT group. Both STS-L and STS-H group were with more crossing times than APP/PS1 group. In addition, the APP/PS1 group took less time to stay in the target quadrant than the WT group, while the STS-H group spent more time than APP/PS1 group (Fig. 1C).

Additionally, Y-maze spontaneous alternation test was performed to assess the memory and learning ability of APP/PS1 mice. In contrast to WT group, a prominent decrease of spontaneous alternation index was observed in the APP/PS1 group while (Fig. 1D). However, the spontaneous alternation index was improved after STS treatment. As shown in Fig. 1E, the APP/PS1 group entered the novel arm less frequently than the WT group. STS treatment remarkably increased the frequency.

STS Ameliorates the Oxidative Stress in APP/PS1 Mice

We also detected the oxidative stress state in APP/PS1 mice brain. As shown in Fig. 2, the levels of MDA and ROS in the hippocampus and cortex of the APP/PS1 group were significantly higher, while the SOD activity was significantly lower than WT group. After STS administration, the levels of MDA and ROS showed a remarkable decrease, and the activity of SOD increased significantly.

STS Prevents the Dysfunction of Cholinergic System in APP/PS1 Mice

Cholinergic system plays a vital role in determining the function of cortex and hippocampus. In Fig. 3A and C, the AChE activity of hippocampus and cortex in APP/PS1 group was significantly higher than that in WT group. The activity was significantly reduced after STS treatment. Nevertheless, the ChAT activity of hippocampus and cortex was decreased in APP/PS1 group. STS treatment increased the ChAT activity (Fig. 3B and D).

STS Enhances the Synaptic Plasticity of APP/PS1 Mice

Neurotrophic factors (BDNF and NGF) are propitious to prevent neurodegeneration, further alleviating the learning and memory impairment of APP/PS1 mice. As shown in Fig. 4 and 5, compared with the WT group, the expressions of BDNF and NGF in APP/PS1 group decreased significantly. STS treatment

increased the expressions of BDNF and NGF in APP/PS1 mice. Meanwhile, the expressions of synaptic proteins were also detected. The expressions of PSD95, PSD93 and SYP in APP/PS1 group were decreased. After the administration of STS, there was a prominent increase in the expressions of synaptic proteins. Abovementioned results revealed that STS could improve learning and memory of APP/PS1 mice through increasing the synaptic plasticity.

STS Mediates the Aβ Transportation in APP/PS1 Mice

AD brain endothelium expresses low levels of GLUT1, which leads to diminished glucose transport, and thus the damage to the function of BBB. LRP1 is a major Aβ clearance receptor at the BBB, while RAGE transports Aβ in the opposite direction to LRP1, mediating the reentry of Aβ into the brain. As shown in Fig. 6, compared with the WT group, the expressions of GLUT1 and LRP1 in APP/PS1 group decreased significantly. STS treatment increased the expressions of GLUT1 and LRP1 in APP/PS1 mice. The level of RAGE was barely affected in any group. Abovementioned results revealed that STS could mediate the Aβ transportation of APP/PS1 mice through increasing the expressions of GLUT1 and LRP1.

Discussion

Aβ plaques are considered as the main causes of AD, acting as a pathological trigger for a cascade such as oxidative stress, cholinergic system damage, synaptic dysfunction, and even neuronal damage loss. Sodium Tanshinone IIA Sulfonate (STS) is formed by introducing sodium sulfonate groups into the chemical structure of Tanshinone IIA, resulting in higher water solubility and efficiency (Zhou et al. 2019). According to our previous study, in vivo we found that STS can improve scopolamine-induced cognitive impairment and the damage of cholinergic system in mice (Xu Q.-Q. et al. 2016), then in vitro we found that STS could protect against Aβ - induced damage by modulating Aβ degradation and generation in SH-SY5Y cell (Zhang et al. 2020) and protect Aβ-induced injury by anti-apoptosis, anti-oxidative stress, relieving ER stress, and increasing the expression levels of IDE and NEP to clear Aβ in HT22 cell (Liu et al. 2020). Here we proved that STS effectively improve the learning and memory in APP/PS1 mice.

Oxidative stress, which is defined as the imbalance between pro-oxidants such as MDA and antioxidants such as SOD with associated disruption of redox circuitry and macromolecular damage (Jones 2006), plays an important role in the pathogenesis of AD. The decrease of SOD and the increase of MDA often lead to neurodegenerative diseases. It has been reported that mitochondrial damage caused by oxidative stress could contribute to the accumulation of the Aβ (Uttara et al. 2009). In this study, the levels of MDA and ROS were significantly reduced, and the activity of SOD was increased significantly after STS treatment, which demonstrated that STS could inhibit the formation of reactive oxygen radicals and lipid-free radicals.

The impairment of the cholinergic system often manifests in patients with dementia, including AD (Davies et al. 1976; Whitehouse et al. 1982). Acetylcholine (Ach) is one of the most important neurotransmitters and plays a vital role in the peripheral and central nervous systems, which is synthesized by ChAT (Ferreira et al. 2016). AD can cause a decrease in the levels of ChAT in the basal

forebrain (Babic 1999). AChE is involved in hydrolytic cleavage of Ach to deplete the levels of Ach (Gauthier 2002). It has been reported that Aβ reduced Ach production and discharge, and AChE could induce Aβ aggregation via forming binding sites with it (Carvajal et al. 2011). In the study, we showed that in hippocampus and cortex the activity of ChAT was increased, while the activity of AChE was decreased in the STS groups compared with the APP/PS1 group. Our results indicate that STS may improve cognitive dysfunctions through cholinergic neuron system.

The impaired cognitive ability and memory were associated with synapses dysfunction (Ling et al. 2015). Synapses are structurally specialized regions in neurons that propagate electrical or chemical signals from one neuron to another. Aβ could impair synaptic plasticity and even induce synaptic dysfunction by affecting the activity of N-methyl-D-aspartate (NMDA) receptors or combining with α-amino-3-hydroxy-5-methyl-4-isoxa-zolepropionic acid (AMPA) receptors (Hsia et al. 1999; Hsieh et al. 2006; Newcomer et al. 2000). Loss of synapses or reduced expressions of synaptic markers such as PSD95, PSD93 and SYP, has been reported in the brains of transgenic APP/PS1 mutant mice (Hsia et al. 1999; Mucke et al. 2000). NGF and BDNF are also important for the survival, maintenance and regeneration of specific neuronal populations in the adult brain. NGF, as a secreted growth factor, is produced in the cortex and hippocampus, and the NGF metabolic pathway has been demonstrated to be impaired in AD (Allen et al. 2013). BDNF is produced during the process of synaptic pruning and apoptosis, which regulates neurotransmission through the involvement of synapses (Valente et al. 2012). BDNF administration can prevented cell death and reduced memory deficits. There have been a lot of preclinical studies which suggest that replacement of BDNF may ameliorate both age-related and AD-associated problems (Arancibia et al. 2008; Mamounas et al. 1995). In this study, STS treatment improved the expressions of PSD93, PSD95, and SYP in both hippocampus and cortex of APP/PS1 mice. Significant reductions of NGF and BDNF were found in APP/PS1 mice. After STS treatment, the NGF and BDNF were reinstated. The above results demonstrated that STS improve learning and memory of APP/PS1 mice might through increasing the synaptic plasticity.

Several studies reported that GLUT1 deficiency was found in patients with cognitive impairment (Pearson et al. 2013). AD was characterized by early reductions in glucose transport associated with diminished GLUT1. GLUT1 provides energy for BBB by transporting glucose, the reduction of GLUT1 could result in BBB breakdown. LRP1 and RAGE, two major Aβ transporters, play an important role in Aβ clearance at the BBB. RAGE takes up Aβ from the blood to brain, whereas LRP1 transports Aβ from brain to the blood (Osgood et al. 2017). It has been reported that GLUT1 deficiency in mice might lead to early cerebral microvascular degeneration and BBB breakdown, which causes the decrease of LRP1 and then the reduction of Aβ clearance (Winkler et al. 2015). Here, we found that STS could significantly improve the expression of GLUT1 and LRP1 of brain microvascular endothelial cells (BMECs), while has no effect on the level of RAGE. The above results demonstrated that STS might improve GLUT1 level to improve BBB integrity, and increase the expression of LRP1 to promote the Aβ transportation in APP/PS1 mice.

In conclusion, we demonstrated that STS can effectively improve the learning and memory in APP/PS1 double transgenic mice. Then, we showed that STS effectively ameliorated oxidative stress and

cholinergic system dysfunction. Besides, STS promoted the expressions of neurotrophic factors and synaptic proteins. At last, STS mediated the Aβ transportation by increasing the expression of GLUT1 and LRP1. This study provides a scientific basis for prevention and treatment of STS in AD. However, further mechanisms and clinical trials are needed for a deeper understanding of STS.

Abbreviations

AD, Alzheimer's disease; STS, Sodium Tanshinone IIA Sulfonate; Tan IIA, tanshinone IIA; Danshen, Salvia miltiorrhiza Bge; ChAT, choline acetyltransferase; AChE, acetylcholinesterase; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; PSD95, postsynaptic density 95; PSD93, postsynaptic density 93; SYP, synaptophysin; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; GLUT1, glucose transporter; LRP1, low-density lipoprotein receptor-related protein 1; RAGE, receptor for advanced glycation end products; APP/PS1, APPswe/PSEN1dE9 double transgenic mice; WT, wild-type mice; BBB, blood brain barrier; D-PBS, Dulbecco's PBS.

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Figures

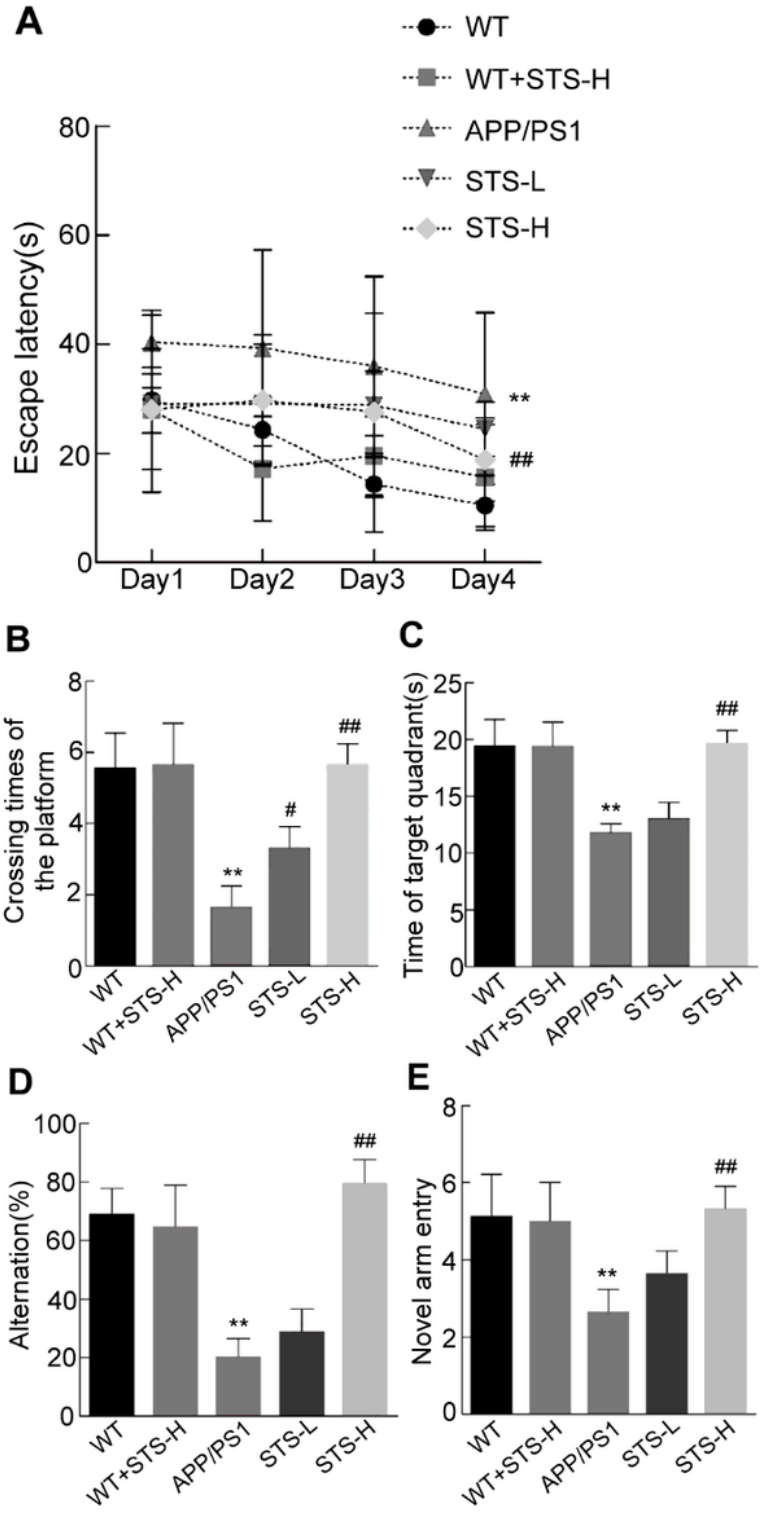

Figure 1

STS improved the learning and memory ability of APP/PS1 mice. Escape latency (A); crossing times of the platform (B); time of target quadrant (C); spontaneous alternation (D); times of novel arm entry (E). Data are mean ± standard error of the mean (S.E.M), n = 10 per group. ∗∗P <0.01 vs. wild-type (WT) mice group; #P <0.05, ##P <0.01 vs. APP/PS1 mice group.

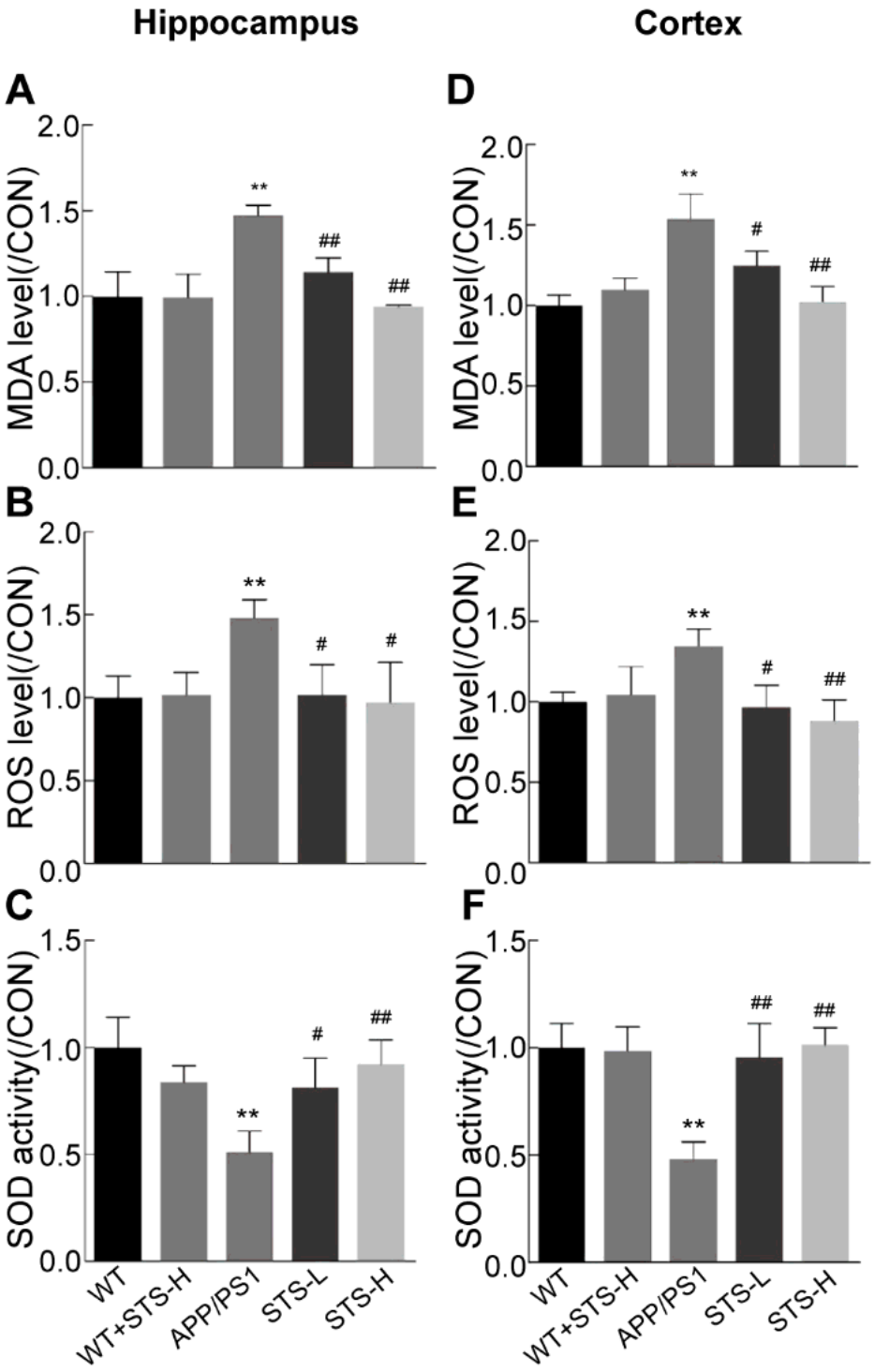

Figure 2

STS alleviates the oxidative stress in APP/PS1 mice. The levels of MDA (A), ROS (B) and the activity of SOD (C) in hippocampus. The levels of MDA (D), ROS (E) and the activity of SOD (F) in cortex. Data are represented as mean ± S.E.M. n = 6 per group. ∗∗P <0.01 vs. wild-type (WT) mice group; #P <0.05, ##P <0.01 vs. APP/PS1 mice group.

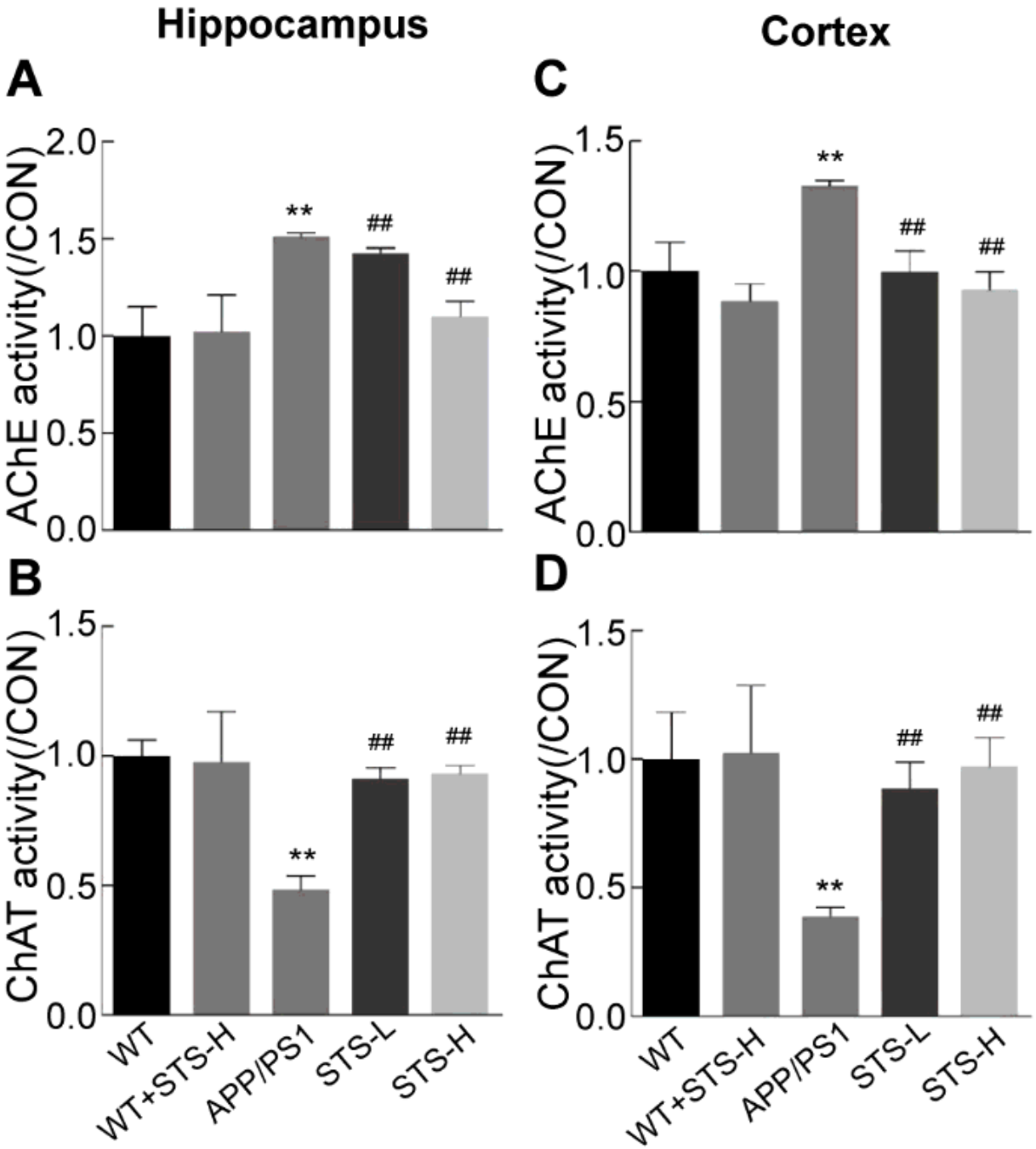

Figure 3

STS protects against cholinergic system dysfunction from APP/PS1 Mice. The activity of AChE and ChAT in the hippocampus (A, B). The activity of AChE and ChAT in the cortex (C, D). Data are represented as mean ± S.E.M. n = 6 per group. ∗∗P <0.01 vs. wild-type (WT) mice group; ##P <0.01 vs. APP/PS1 mice group.

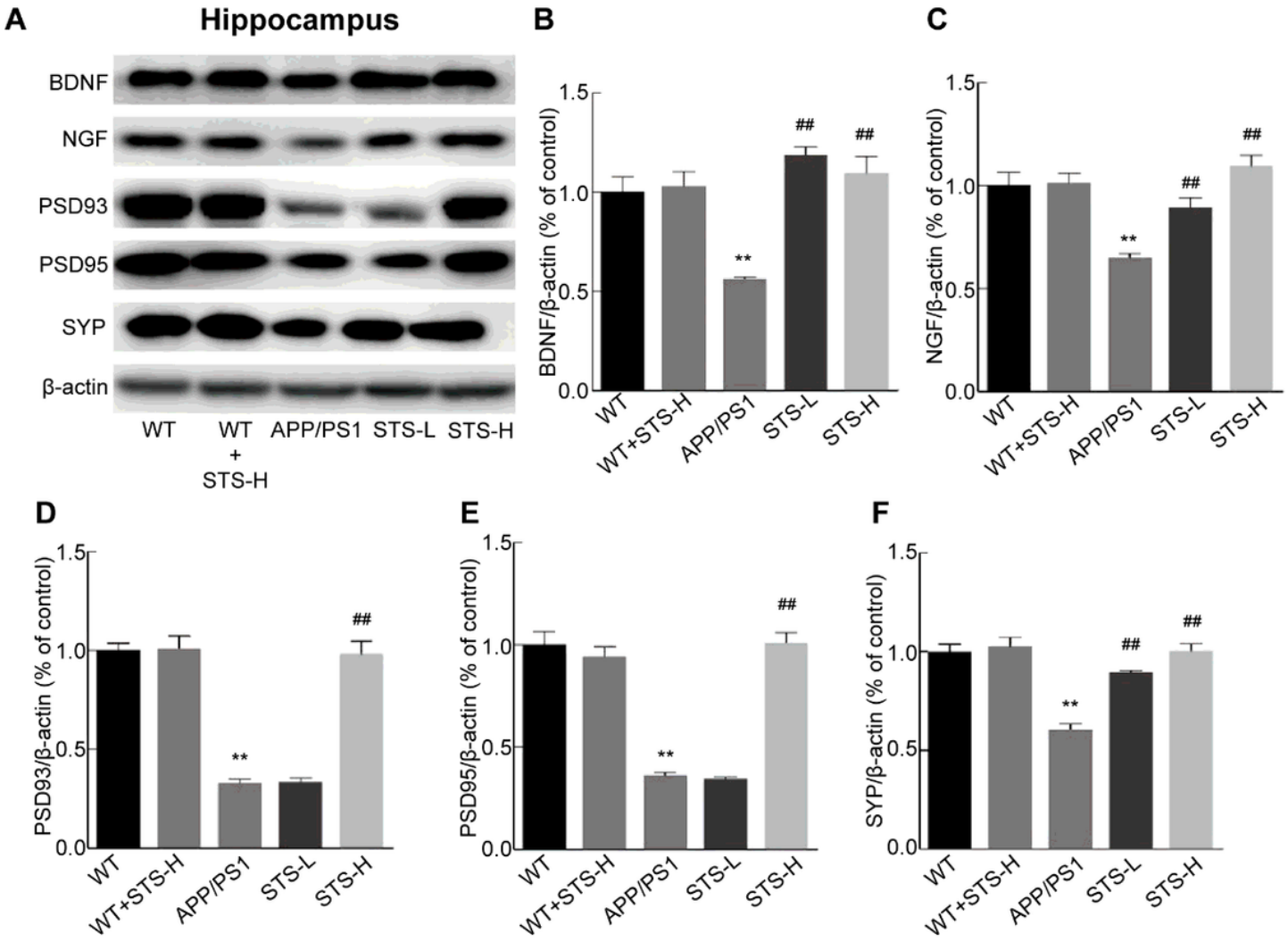

Figure 4

STS prevents neuron dysfunction and increases the synaptic plasticity in the hippocampus of APP/PS1 Mice. Western blotting (A) was used to detect the protein expression of BDNF (B), NGF (C), PSD 93 (D), PSD 95 (E), SYP (F). Data are represented as mean ± S.E.M. ∗∗P <0.01 vs. wild-type (WT) mice group; #P <0.05, ##P <0.01 vs. APP/PS1 mice group.

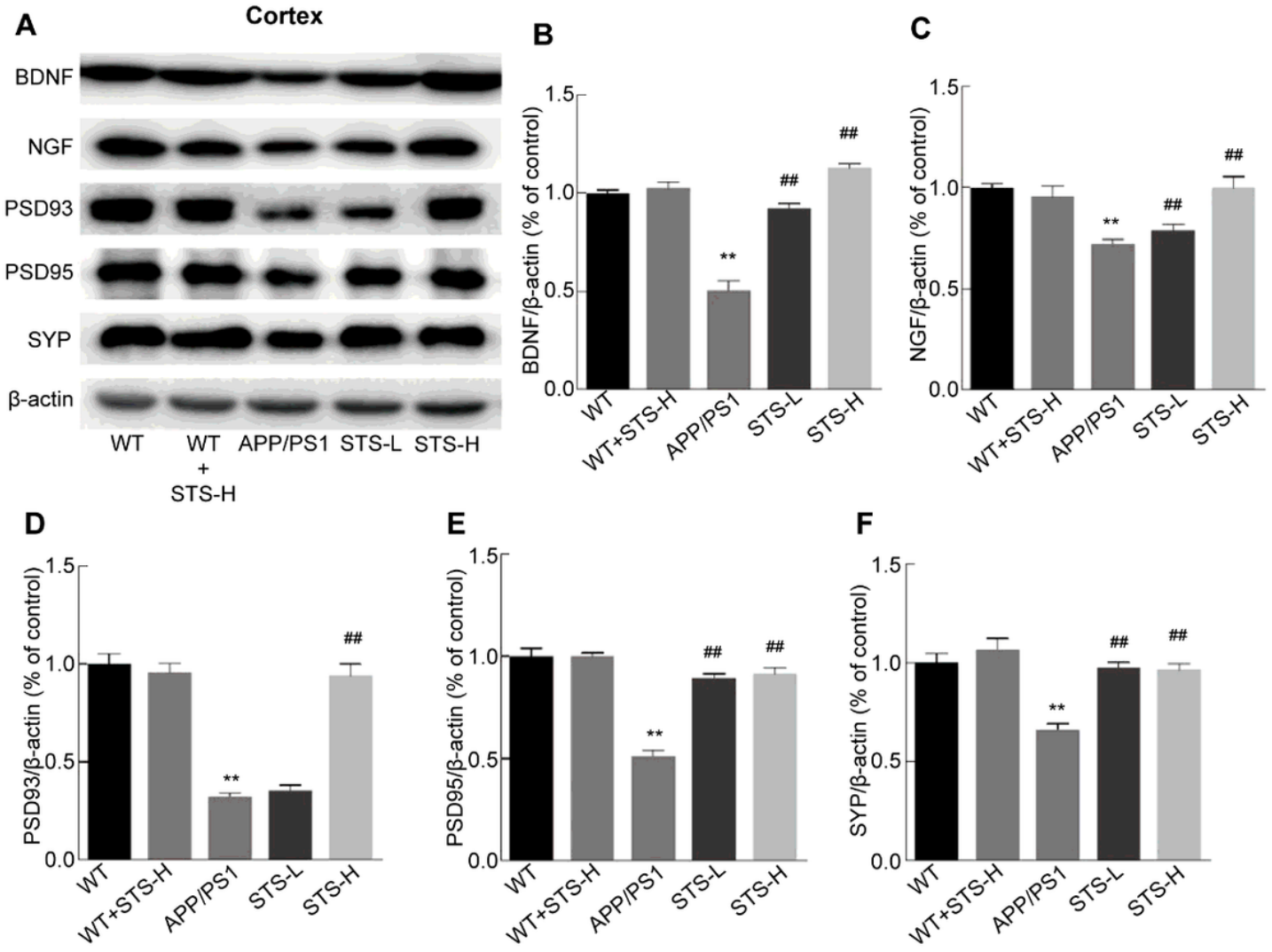

Figure 5

STS prevents neuron dysfunction and increases the synaptic plasticity in the cortex of APP/PS1 Mice. Western blotting (A) was used to detect the protein expression of BDNF (B), NGF (C), PSD 93 (D), PSD 95 (E), SYP (F). Data are represented as mean ± S.E.M. ∗∗P <0.01 vs. wild-type (WT) mice group; #P <0.05, ##P <0.01 vs. APP/PS1 mice group.

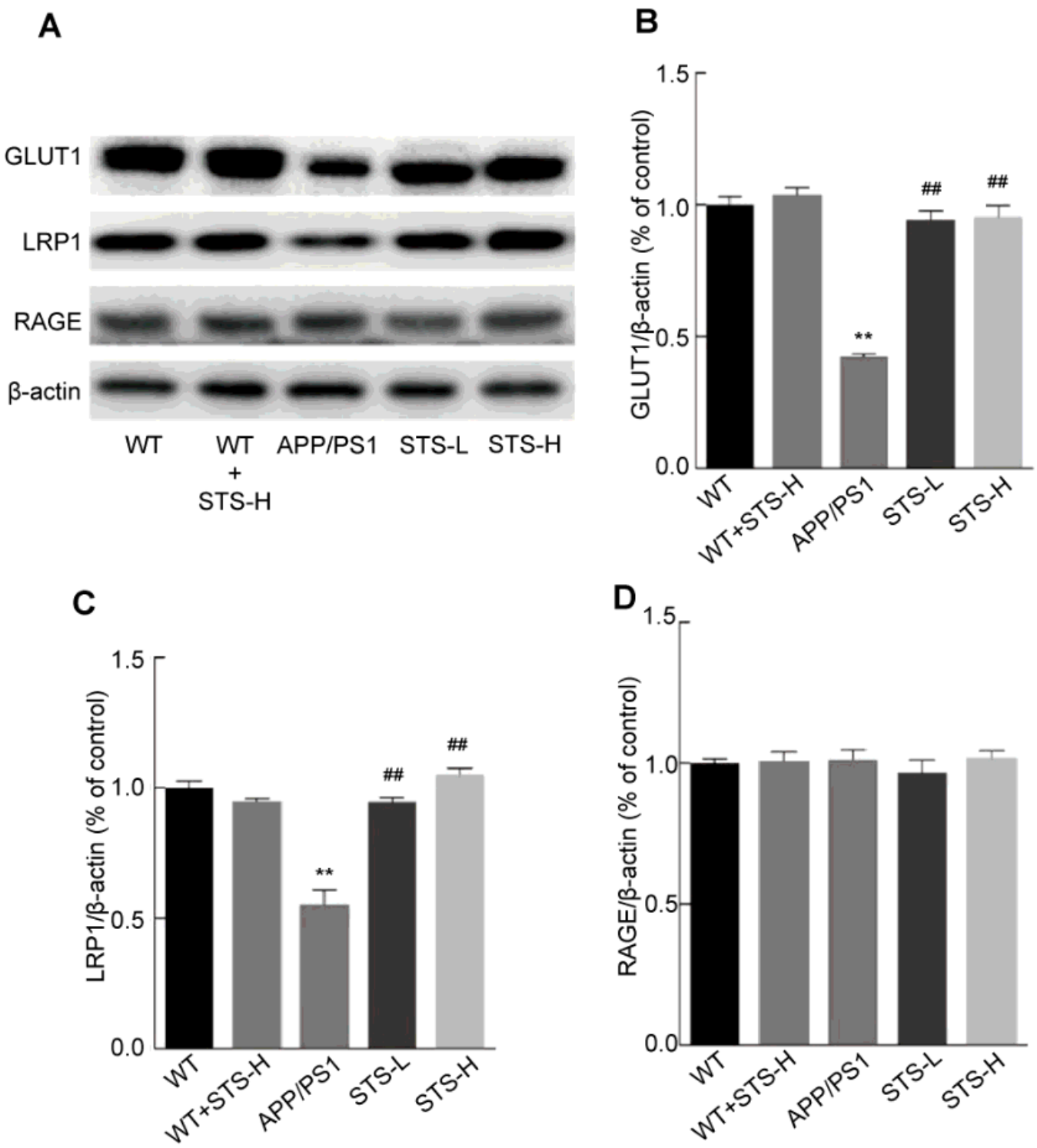

Figure 6

STS Mediates the Aβ transportation in brain microvascular endothelial cells of APP/PS1 Mice. Western blotting (A) was used to detect the protein expression of GLUT1 (B), LRP1 (C), RAGE(D). Data are represented as mean ± S.E.M. ∗∗P <0.01 vs. wild-type (WT) mice group; ##P <0.01 vs. APP/PS1 mice group.