Inhibition of Stat3 Phosphorylation Attenuates Impairments in Learning and Memory in 5XFAD Mice, an Animal Model of Alzheimer's Disease

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Research

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Abstract

The novel functions of astrocytes under normal conditions have been extensively investigated in terms of synaptogenesis and memory formation. Meanwhile, the pathophysiological roles of astrocytes in the reactive state are thought to have important significance in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD). However, the detailed mechanisms underlying the transition of astrocytes from the resting state to the reactive state during neurodegenerative disease largely remain to be defined. Here, we investigated the pathways involved in activating astrocytes from the resting state to the reactive state in primary cultured astrocytes treated with oligomeric Aβ and in the hippocampus of 5XFAD mice, an animal model of AD. Treatment with oligomeric Aβ induced an increase in reactive astrocytes, as assessed by the protein level of glial fibrillary acidic protein (GFAP), a marker of reactive astrocytes and this increase was caused by STAT3 phosphorylation in primary cultured astrocytes. The administration of Stattic, an inhibitor of STAT3, rescued the activation of astrocytes in primary cultured astrocytes and in the hippocampus of 6-month-old 5XFAD mice as well as impairments in learning and memory. Collectively, these results demonstrated that reactive astrocytes in the AD brain are induced via STAT3 phosphorylation and that the increase in reactive astrocytes and the impairments in learning and memory observed in 5XFAD mice are rescued by STAT3 inhibition, suggesting that the inhibition of STAT3 phosphorylation in astrocytes may be a novel therapeutic target for cognitive impairment in AD.

Introduction

Astrocytes are the most abundant cell type in the central nervous system and play a fundamental role in several cerebral functions such as maintenance of the blood-brain barrier, maintenance of ionic homeostasis, metabolic support to neurons and uptake of neurotransmitters of glutamate and GABA by specific transporters [1–3]. They are also involved in synaptogenesis and development of neuronal circuits by facilitating release of gliotransmitters [4]. Meanwhile, astrocytic reactivity is functional and morphological change of astrocytes as a result of variety of brain insults and it is characterized by increased gene expression of a number of astrocyte structural proteins, such as glial fibrillary acid protein (GFAP) and vimentin; morphological changes, such as hypertrophy of the cell soma and processing; and proliferation, which is particularly important in the formation of an astrocyte scar around tissue lesions [5–8]. In addition, reactive astrocytes release cytokines and chemokines such as interleukin 6 (IL-6), interleukin 8 (IL-8), chemokine ligand 1 (CXCL 1), tumor necrosis factor–α (TNF-α) [9]. However, the detailed mechanisms underlying the transition from astrocytes from the resting state to the reactive state during neurodegenerative diseases largely remain to be defined. Additionally, how reactive astrocytes affect memory formation apart from their neurotoxic effects remains to be clarified.

Alzheimer's disease (AD) is the commonest neurodegenerative disorder. The major neuropathological characteristics are neuritic plaques, the main component of which is amyloid beta peptide (Aβ) and neurofibrillary tangles, the main component of which is hyperphosphorylated tau, in the brain [10–12]. AD patients' brains also show neuronal loss and evidence of gliosis including reactive astrocytes [13, 14].

In this study, we investigated the pathways responsible for the activation of astrocytes into reactive astrocytes in the context of AD. It was found that treatment with oligomeric Aβ (oAβ) increased the activation of primary cultured astrocytes into reactive astrocytes via phosphorylated Stat3 (p-STAT3) and that the inhibition of Stat3 rescued this phenomenon. In addition, the administration of Sttatic, an inhibitor of Stat 3, attenuated the impairments in cognitive function, as assessed by the contextual fear conditioning test, in 6-month-old 5XFAD mice, an AD mouse model.

Materials And Methods

Reagents and antibodies

Protein isolation with RIPA lysis buffer was purchased from Elpis biotech (#EBA-1149, Deajeon, Korea) and Pierce™ BCA Protein Assay Kit was purchased from Thermo (#23227, MA, USA). Anti-GFAP, rabbit polyclonal antibody was purchased from DAKO (#Z0334, CA, USA). Anti-NeuN rabbit monoclonal antibody was purchased from Millipore (#3838, MA, USA). Anti-PSD-95, mouse monoclonal antibody, anti-rabbit, goat polyclonal tagged Alexa Fluor 488, anti-mouse, goat polyclonal tagged Alexa Fluor 555 and 4’. 6-diamidino-2-phenylinodole (DAPI) were purchased from ThermoFisher (#MA1-046, #A11034, #A11012, #A21426 and #D3571, MA, USA). Anti-GAPDH, rabbit polyclonal antibody was purchased from AbFrontier (#LF-PA0018, Seoul, Korea). Rabbit polyclonal antibody was purchased from WAKO (#016-20001, Osaka, Japan). Anti-mouse, sheep polyclonal horseradish peroxidase (HRP) tagged antibody was purchased from Abcam (#ab26116, #ab26113 and #ab6808, EA, UK). Anti-p-STAT3 (Tyr 705), STAT3, p-p44/42, p44/42 rabbit polyclonal antibody was purchased from Cell signaling (#9145, #12640, #4377 and #4695 MA, USA). Anti-NF-κB mouse monoclonal were purchased from Santa Cruz (#sc-7151, Taxas, USA)

Experimental animals

All of the experimental procedures were approved by the Animal Care Committee of Seoul National University (Approval number: SNUIBC-171011-2). Transgenic mice with 5XFAD mutations were purchased from Jackson Laboratories (strain: B6SJL-Tg [APP Sw, Fl, Lon, PS1, M146L, L286V] 6799Vas/J) and maintained by crossing hemizygous transgenic mice with B6SJL F1 mice. Six- month-old male mice were used for the experiments. The mice were housed in five per cage with a 12-hour light/dark cycle and ad libitum access to food and water under standard laboratory housing conditions.

Primary astrocyte culture

The primary astrocyte culture was described previously [15]. Briefly, primary mixed glial cultures were prepared from postnatal day 1 (P1) C57B/L6 mice. The cortex was dissociated with 0.25% trypsin and filtered with a cell strainer. The mixed glia was grown in Dulbecco’s modified Eagle’s medium (DMEM),

supplemented with 10% (vol/vol) heat- inactivated FBS and penicillin (10 units/ml) and streptomycin (10 mg/ml) in a humidified cell incubator (Binder, Germany) at 37°C under a 5% CO_2 atmosphere. When the mixed glia filled 80% of the T75 plate, astrocyte and microglia were isolated via a 250 rpm shaking incubator at 37°C for overnight. Microglia were detached from the plate after shaking isolation. The astrocytes were detached with 0.25% trypsin-EDTA and seeded onto poly-L-lysine coated plates for the experiment.

Contextual fear conditioning

Contextual fear conditioning was tested as previously described [8]. Briefly, prior to training, WT and 5XFAD mice were placed into the chamber for 10 min for habituation. Each mouse was placed in the conditioning chamber (13 × 13 × 25 cm) for 3 min and acquired three repetitions of a foot-shock (0.7 mA, 2 sec) at 1 min intertrial intervals during the training day. On the next day, trained mice were placed in the same chamber, and the freezing behavior was measured over periods of 3 min. The freezing behavior was defined as immobility except for respiratory movements. The total freezing time was analyzed as a percentage in the test period. The control group was habituated to the chamber without shock. The 1 hour group received electric shock and was sacrificed after 1 hour. The 24 hour group received electric shock and sacrificed the next day.

T-maze test

T-maze test was performed as previously described [16]. Briefly, prior to training, WT and 5XFAD mice were placed into deem light to habituation for 1 hour. The testing mice was positioned at the starting zone. After the mice choose one way, the way was blocked and the mice stay for 30 sec. During clean the T-maze with 30% EtOH, the mice stayed in the home cage for 1 min and return into the starting zone. If the mice chose the other way, it will get 1 point. If the mice chose the same way, it will get 0 point. This trial performed after 30 min and the next day.

Western blotting

Western blotting analysis was described previously [16]. Briefly, the mice were anesthetized with Zoletil (12.5 mg/kg) and Rompun mix (17.5 mg/kg) and the dissected brain tissues were stored at -80°C before protein lysis. Hippocampi were homogenized with RIPA mix. The primary antibodies were applied in the following concentrations: anti-GFAP (rabbit, 1: 5,000), anti-NeuN (rabbit, 1: 1,000), anti-p-STAT3 (rabbit, 1:1,000), anti-STAT3 (rabbit, 1:1,000), anti-p-p44/42 (rabbit, 1:1,000), anti-p44/42 (rabbit, 1:1,000), anti-p-p65 (rabbit, 1:1,000), anti-p65 (rabbit, 1:1,000), anti-PSD95 (mouse, 1:2,000), anti-GAPDH (rabbit,

1:10,000). Secondary antibodies were conjugated with HRP (1: 2,000). The HRP signals were visualized using an enhanced chemiluminescent substrate.

Immunofluorescence

The protocol was previously described in the paper by Choi et al [8]. The brains were perfused with heparin dissolved in PBS (pH 7.2) for 5 min were fixed with 4 % paraformaldehyde for 24 hours. The fixed brains were transferred into 30 % sucrose solution for 48 hours. The brain tissue was sectioned into 30 μm thick slices with a cryotome with the chamber at -20°C and the bar temperature at -25°C.

For GFAP and NeuN staining, the brain sections were heated in a 95°C water bath for 30 min with 10mM citrate acid (pH 6.0) for antigen retrieval. The sections were blocked with 2 % BSA and 0.3 % Triton X-100 in PBS for 1 hour. Primary antibodies GFAP (1:1,000) and NeuN (1:500) were applied overnight at 4°C. Secondary antibodies, anti-rabbit-488 (1:200) and anti-mouse-555 (1:200) were applied for 2 hours at room temperature. The stained brain tissue samples were imaged with confocal microscopy using LSM 510 (Carl Zeiss, Germany).

Statistical analysis

Data are expressed as the mean ± SEM (means ± standard error of the mean). One-way ANOVA followed by Tuckey post hoc analysis or Student's *t*-test (SPSS, IL, USA) was performed to determine statistical significance. The results were considered to be statistically significant at $p<0.05$.

Results

Six-month-old 5XFAD mice showed altered the activation of astrocytes and hippocampal dependent long-term memory formation

In AD, the evidence of gliosis has been often detected in the patients' brains [17]. In this study, we aimed to investigate the pathological roles of reactive astrocytes and the pathways involved in activating astrocytes from the resting state to the reactive state in primary cultured astrocytes treated with oligomeric Aβ and in the hippocampus of 5XFAD mice, an animal model of AD. in learning and memory in AD.

First, in order to elucidate which cell type is prone to be altered in the hippocampi of 6-month-old WT and 5XFAD mice, we evaluated the protein levels of GFAP, marker for reactive astrocytes, and NeuN, marker for neurons. 5XFAD mice displayed an increase in reactive astrocytes as compared to the WT group as assessed by expression of GFAP.5XFAD mice displayed an increase in reactive astrocytes as compared to the WT group as assessed by expression of GFAP. However, the protein level of NeuN was not different

between WT and 5XFAD mice (Fig. 1a, b). Next, we stained the brain slices of 6-month-old mice with anti-GFAP and anti-NeuN antibodies and DAPI and assessed immnofluorescence. Consistent with the western blot results, GFAP immunoreactivity was higher in 5XFAD mice than in WT mice, whereas NeuN immunoreactivity was not different between the WT and 5XFAD groups (Fig. 1c). Even in the absence of neuronal death in the hippocampus, neuronal function can be altered by the degradation of synaptic proteins. Thus, we evaluated the protein levels of synaptophysin and postsynaptic density 95 (PSD-95), pre- and postsynaptic markers, respectively, to elucidate whether the pre- or postsynaptic components are prone to be altered and damaged in the hippocampus of 6-month-old WT and 5XFAD mice. Altered protein expression was not observed for both synaptic marker proteins such as PSD-95 for postsynaptic and synaptophysin for presynaptic marker proteins in 6-month-old 5XFAD mice (Fig. 1d and de).

Next, we evaluated hippocampus-dependent long-term memory by performing a contextual fear conditioning (CFC) test in WT and 5XFAD mice. There are two types of conditioned stimuli used for CFC: a contextual conditioned stimulus (CS), such as a test chamber, to test hippocampal-dependent spatial memory and a specific cue, such as an additional sound cue, to test amygdala-dependent fear memory [18]. To test hippocampus-dependent memory, we used CS -CFC with WT and 5XFAD mice. The time schedule of CFC is shown in Fig. 1 f. First, we recorded the freezing behavior at intervals between every electric shock on the training day. WT mice showed a significant increase in freezing behavior throughout the training session. The 5XFAD mice group also showed a significant increase in freezing behavior throughout the session (Fig. 1g). These results indicate that neither the WT nor 5XFAD mouse groups showed altered short-term memory formation and both were well trained via the CFC procedure. On the test day, 24 hours after training, the freezing times of the WT and 5XFAD mice were analyzed. 5XFAD mice displayed significantly lower freezing time than WT mice (Fig. 1h). These results showed that 6-month-old 5XFAD mice model displayed an increase in reactive astrocytes as assessed by the protein level of GFAP, and impaired hippocampal- dependent long-term spatial memory; however, significant differences in neuronal and synaptic marker proteins were not observed and that 5XFAD mice showed impaired long-term memory formation.

Astrocytes were activated into a reactive state via the STAT3 pathway

The presence of reactive astrocytes is an well-known neuropathological characteristic in the brains of AD patients and animal models [19]. The 5XFAD mouse which has five types of familial AD mutations, shows increased levels of Aβ beginning at 1.5 months of age [20].

Astrocytes are known to be activated into reactive astrocytes by cytokines, chemokines and aggregated proteins via the JAK/STAT3, NF-κB, MAPK and NFAT pathways. After treating primary cultured astrocytes with 250 nM oAβ for 24 hours, we analyzed the protein levels of GFAP by western blot. The protein level of GFAP was significantly increased in oAβ-treated primary astrocyte cultures (Fig. 2a, b). Next, the protein levels of phosphorylated STAT3 (p-STAT3), total STAT3, phosphorylated p65 (p-p65), total p65, phosphorylated ERK (p-ERK) and total ERK were evaluated via western blot to investigate which pathways are activated by oAβ treatment in astrocytes (Fig. 2c). The expression levels of p-STAT3 and

STAT3 were significantly higher in oAβ-treated primary cultured astrocytes. However, the NF-κB and ERK pathways were not altered in oAβ-treated primary cultured astrocytes (Fig. 2d). The protein levels of p-STAT3 and total STAT3 were significantly higher in the 6- month-old hippocampus of 5XFAD mice than in that of WT mice, while the protein levels of p-p65, p65, p-ERK and ERK was not altered (Fig. 2e, f).

STAT3 is phosphorylated at tyrosine 705 or serine 727 via JAK. This p-STAT3 Y705 is transported from the cytosol to the nucleus [21]. We investigated which cell type expressed activated STAT3 in the hippocampus of 5XFAD mice via immunofluorescence analysis in the hippocampal brain slice using anti-GFAP and p-STAT3 Y705 antibodies. The p-STAT3 signals were increased in the nucleus of astrocytes (Fig. 2g, h). These results indicate that astrocytes are activated into a reactive state via STAT3 phosphorylation in 5XFAD mice.

Oral administration of Stattic, an inhibitor of STAT3, attenuated the cognitive impairments in 5XFAD mice

STAT3 inhibitors such as Stattic, S31-201 and crypotanshinone have been developed for breast and prostate cancer therapy [22, 23]. Primary cultured astrocytes were treated time- dependently with 1 μM Sttatic from 1 hour to 12 hours (Fig. 3a). p-STAT3 expression was significantly lower than in those treated with vehicle in Stattic-treated primary cultured astrocytes from 1 hour to 6 hours (Fig. 3a and 3b). The STAT3 expression was not changed from 1 hour to 12 hours. The p-STAT3 and STAT3 ratio was also significantly lower in Stattic-treated primary cultured astrocytes form 1 hour to 6 hours (Fig. 3c). In addition, GFAP expression was significantly lower in Stattic-treated primary cultured astrocytes from 3 hours to 6 hours (Fig. 3a and 3d).

To test the cognitive and molecular effects of oral administration, Sttatic has been administered orally to WT and 5XFAD mice for 15 days. The time schedule of treatment and behavior tests are shown in Fig. 3e. The hippocampi were removed and the pathway activated in astrocytes was analyzed. The hippocampi of vehicle-treated 5XFAD mice (5XFAD-V) showed significantly higher levels of p-STAT3 than those of vehicle-treated WT mice (WT-V). Stattic significantly reduced the protein level of p-STAT3 in Stattic-treated 5XFAD mice (5XFAD-S), although the p-STAT3 levels were still higher than those in WT-V (Fig. 3f and 3 g). The STAT levels were not altered in WT-S, 5XFAD-V and 5XFAD-S (Fig. 3h). The protein level of GFAP was found to be significantly higher in the hippocampi of 5XFAD-V mice than in those of WT-V mice (Fig. 3f). Stattic administration significantly reduced the protein level of GFAP (Fig. 3i). These results indicate that the systemic administration of Stattic restored the astrocyte activation in the hippocampus via the STAT3 pathway.

We confirmed whether Stattic treatment exerts detrimental effects in experimental animals. Body weight and brain weight were not significantly different between the vehicle- and Stattic-treated groups over 15 days (Additional file. 1a-c). Next, working memory was analyzed with the T-maze test. The percentage of alternation was significantly lower in 5XFAD-V than in the WT-V. However, Stattic treatment significantly rescued the percentage of alternation in 5XFAD-S compared with 5XFAD-V (Fig. 3j). Furthermore, hippocampal-dependent long-term spatial memory was analyzed with CFC. On the habituation day, all groups revealed similar percentages of freezing behavior less than 5% that did not differ significantly

(Additional File. 1d). On the training day, all groups showed a significant increase in the percentage of freezing behavior after each electric shock, but this increase did not differ significantly among the groups (Additional File. 1e). On the test day, the percentage of freezing behavior was significantly lower in the 5XFAD-V group than in the WT-V group. However, 5XFAD-S mice displayed a significantly higher percentage of freezing behavior than the 5XFAD-V mice (Fig. 3k). These results indicate that the systemic administration of Stattic restored the working memory and long-term spatial memory via inhibition of the STAT3 pathway in 5XFAD mice.

Oral administration of Stattic restored the activation of astrocytes in 5XFAD mice

Here, we analyzed the status of astrocytes and p-STAT3 level after the systemic administration of Stattic in mice. We performed immunofluorescence analysis to determine in which cell types p-STAT3 protein expression and astrocyte activation were changed by Stattic treatment in 5XFAD mice (Fig. 4a). In the hippocampus of WT mice, p-STAT3 protein expression was too low to detect. The region of intensity (ROI) of p-STAT3 was significantly lower in 5XFAD-S mice than in 5XFAD-V mice (Fig. 4b). Next, we quantified the colocalization of p-STAT3 and GFAP after the administration of Stattic in 5XFAD mice. The colocalization was significantly lower in 5XFAD-S mice than in 5XFAD-V mice (Fig. 4c). GFAP immunoreactivity was significantly higher in the 5XFAD-V group than in the WT-V group. The ROI of GFAP was significantly lower in 5XFAD-S mice than in 5XFAD-V mice but not fully rescued to the WT-V level (Fig. 4d). These results indicate that the administration of Stattic has the potential to restore the STAT3 phosphorylation observed in astrocytes, the increase in reactive astrocytes and cognitive function in early AD.

Discussion

AD is the commonest form of dementia threatening 35.6 million people worldwide and this phenomenon is expected to double every 20 years [24]. Although the exact mechanism behind AD development and progression is still unclear [25], the disease is characterized by the involvement of different cell types including reactive astrocytes and microglia, characterized by gliosis and neuroinflammation, which in turn contributes to the neuronal dysfunction and death observed in AD [26]. Since AD pathologies are the result of neuronal death, search for mechanisms and therapeutic approaches have been neurocentric until a recent time [27]. However, the importance of nonneuronal cells, such as astrocytes, is now largely accepted and opened new research avenues that aim at better understanding of the pathology of the disease as well as characterizing new cellular and molecular targets for therapeutics development [28, 29].

Astrocytes are thought to reside in three major states, i.e., the resting state, the reactive state and the active state. Astrocytes that are involved in memory formation are defined as active astrocytes, which are different from reactive astrocytes, according to several reports [30]. Astrocytes reveal changes in their morphology and transcription according to their status [31]. Astrocytes release various molecules including glutamate, GABA, D-serine, ATP, cytokines and proBDNF [32, 33]. Based on previous results from

other researchers, it is thought that the transition of reactive astrocytes to resting-state astrocytes prepares them to change into active astrocytes with important functions supporting neuronal function in memory formation, such as synaptogenesis.

Reactive astrocytes were reported to be significantly increased in a hippocampal dentate gyrus region in a hippocampal-dependent cognitive impairment model [34]. It has been recently reported that reactive astrocytes aberrantly produce GABA by monoamine oxidase-B (MAO-B) and abnormally release GABA through the bestrophin 1 channel in the dentate gyrus of APP/PS1 mice, a mouse model of AD [35]. Treatment with a newly developed reversible MAO-B specific inhibitor, KDS2010, significantly attenuated increased astrocytic GABA release and astrogliosis, enhancing synaptic transmission, and rescued learning and memory impairments in APP/PS1 mice [36].

So far, astrocytes have been reported to be activated into a reactive state via several pathways, so far: 1) The JAK / STAT3 pathway, 2) the MAPK pathway, 3) The NF-κB pathway and 4) The NFAT pathway. The detailed mechanisms underlying the transition of astrocytes from the resting state to the reactive state during neurodegenerative disease largely remain to be defined, and the pathways that are responsible for the activation of astrocytes in AD remains to be elucidated.

Our results showed that reactive astrocytes in the hippocampus were significantly increased by STAT3 phosphorylation in the early stage of AD when neuronal loss was not observed. p-STAT3 was increased in primary cultured astrocytes after oAβ treatment. This activation of astrocytes by STAT3 phosphorylation was rescued via Stattic treatment.

Astrocytes have been classified into type A1 and A2 types according to their genetic expression pattern. Type A1 astrocytes have harmful functions and morphology whereas type A2 astrocytes have neuroprotective functions. In neurodegenerative disorders such as AD and Parkinson's disease, more type A1 astrocytes are detected in the brain than type A2 astrocytes [31, 37, 38]. In recent studies, novel AD therapeutic strategies regulating the JAK / STAT3 axis, one of the pathways leading to reactive astrocytes were established. These strategies improved cognitive impairment and neuronal activity in an AD mouse model [39, 40].

In this study, we focused on the pathological roles of reactive astrocytes in impairments in cognitive function and investigated the pathways involved in activating astrocytes from the resting state to the reactive state in primary cultured astrocytes treated with oAβ and in the hippocampus of 6-month-old 5XFAD mice, an animal model of AD. Six-month-old 5XFAD mice, which showed an increase in the protein level of GFAP in the hippocampus and impairments in learning and memory, as assessed by the CFC test, did not display a significant difference in the protein levels of NeuN, PSD-95 or synaptophysin compared with the levels observed in age-matched WT mice. These results suggest that reactive astrocytes could be one of the important causative factors of cognitive dysfunction in 6-month-old 5XFAD mice. In addition, we found that treatment with oAβ increased the protein levels of p-STAT3 and GFAP, a marker for reactive astrocytes, in primary cultured astrocytes. Stattic, a STAT3 inhibitor, attenuated p-STAT3 and GFAP protein levels in primary cultured astrocytes. Furthermore, the administration of Stattic reversed the

impairments in learning and memory of 5XFAD mice compared with wild type mice. These results suggest that transitioning of reactive astrocytes to the resting state via STAT3 phosphorylation may be a novel therapeutic strategy before neuronal alterations in AD.

Taken together, our results suggest that the inhibition of STAT3 phosphorylation rescues the activation astrocytes from reactive into resting state in the hippocampal dentate gyrus. Furthermore, our results may describe the relationship between the regulation of astrocyte activity and cognitive function. A drug that specifically inhibits STAT3 phosphorylation in astrocytes may be a novel therapeutic strategy before neuronal loss is observed.

Declarations

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Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All animal procedures were carried out following the National Institutes of Health Guidelines for the Humane Treatment of Animals, with approval from the Institutional Animal Care and Use Committee of Seoul National University (IACUC No. SNU-180410-8).

Competing interests

The authors declare that they have no competing interests.

Author contributions

M-Choi performed most of the experiments and designed the project. H-Kim and EJ-Yang contributed to the primary cultures. HS-Kim supervised the project, the manuscript and supported funding acquisition.

Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Abbreviations

5XFAD: Five human familial AD; $A\beta_{1-42}$: β-amlyoid$_{1-42}$; AD: Alzheimer's disease; APP: amyloid precursor protein; CFC: contextual fear conditioning test; CNS: central nervous system; CS: a conditioned stimulus; CXCL 1: chemokine ligand 1; EAAT2: excitatory amino acid transporter 2; GABA: serotonin, γ-aminobutyric acid; GFAP: glial fibrillary acidic protein; GPCR: G-protein coupled receptors; IL-6: interleukin 6; IL-8: interleukin 8; IP3R2: inositol 1, 4, 5 – triphosphate receptor type 2; JAK/STAT3: the Janus kinase/signal transducers and activators of transcription: LAA: L-α-aminoadipate; MAPK: the mitogen-activated protein kinase; NeuN: neuronal nuclei; NF-κB: the nuclear factor κ light chain enhancer of activated B cells; NFAT: the nuclear factor of activated T-cells; oAβ: oligomeric Aβ; p-ERK: phosphorylation ERK; p-p65: phosphorylation p65; p-STAT3: phosphorylation STAT3; PS1: presenilin-1; PSD-95: postsynaptic density protein 95; ROI: region of intensity; TNF-α: tumor necrosis factor–α; US: an unconditioned stimulus; WB: western blotting; WT: wild type

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Figures

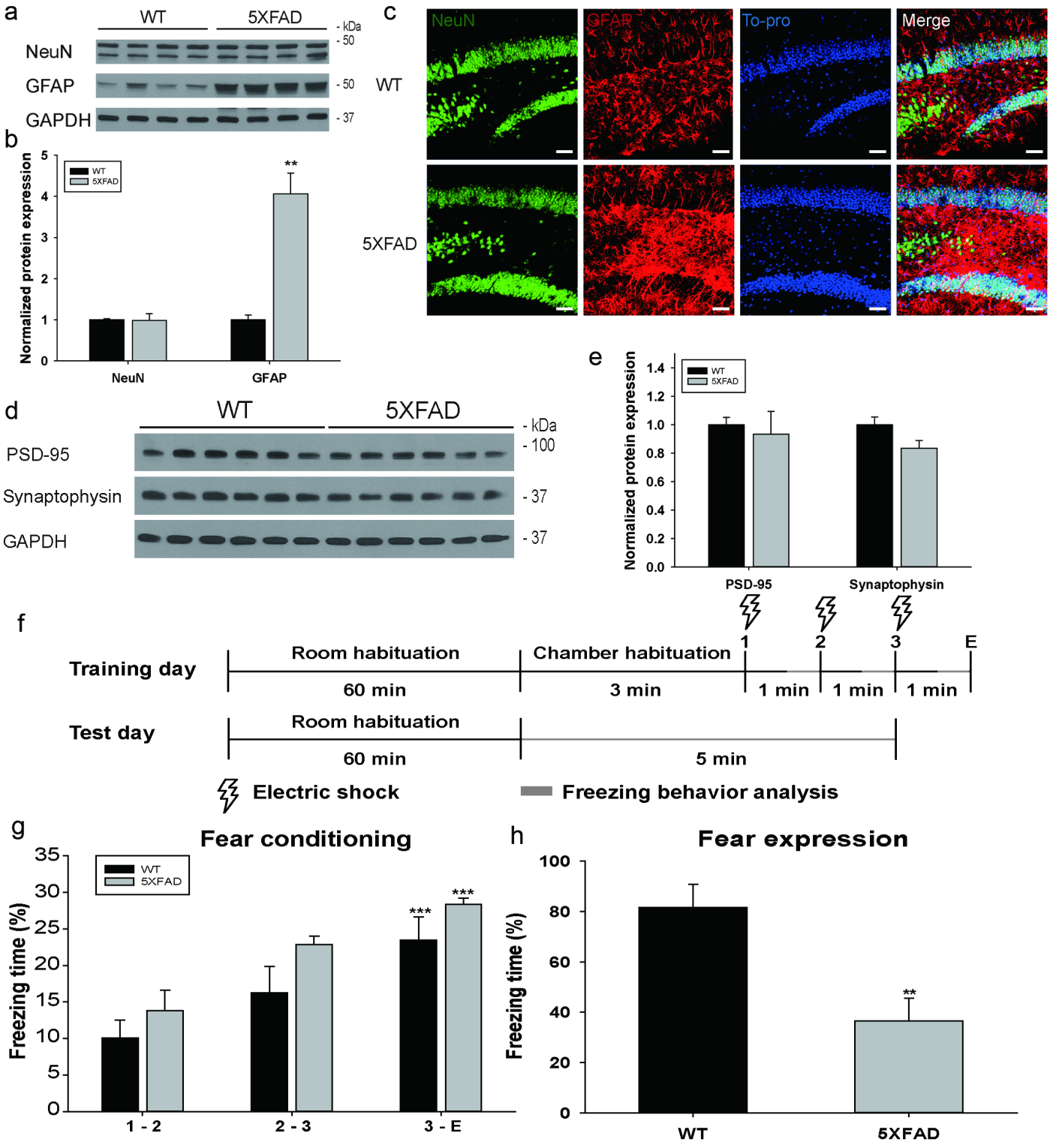

Figure 1

Six-month-old 5XFAD mice exhibited the reactive astrocytes and cognitive impairments (a) Representative immunoblots for neuronal marker NeuN and astrocyte marker GFAP from the WT (N = 7) and 5XFAD (N = 9) hippocampus. (b) Densitometric analysis of immunoblots for NeuN and GFAP. (c)

Immunofluorescence for NeuN and GFAP in the hippocampal dentate gyrus of both WT and 5XFAD mice (scale bar = 50 μm). (d) Representative immunoblots for postsynaptic marker. PSD-95 and presynaptic marker, synaptophysin from the WT and 5XFAD hippocampus. (N = 6) (e) Densitometric analysis of immunoblots for PSD-95 and synaptophysin. (f) A time schedule for the contextual fear conditioning test is shown. (g) Quantification of freezing behavior for fear conditioning in age-matched 6-month-old WT and 5XFAD mice (N = 8) (h) Quantification of freezing behavior for fear expression in age-matched 6-month-old WT and 5XFAD mice (N = 8) *p<0.05; **p<0.01; ***p<0.001

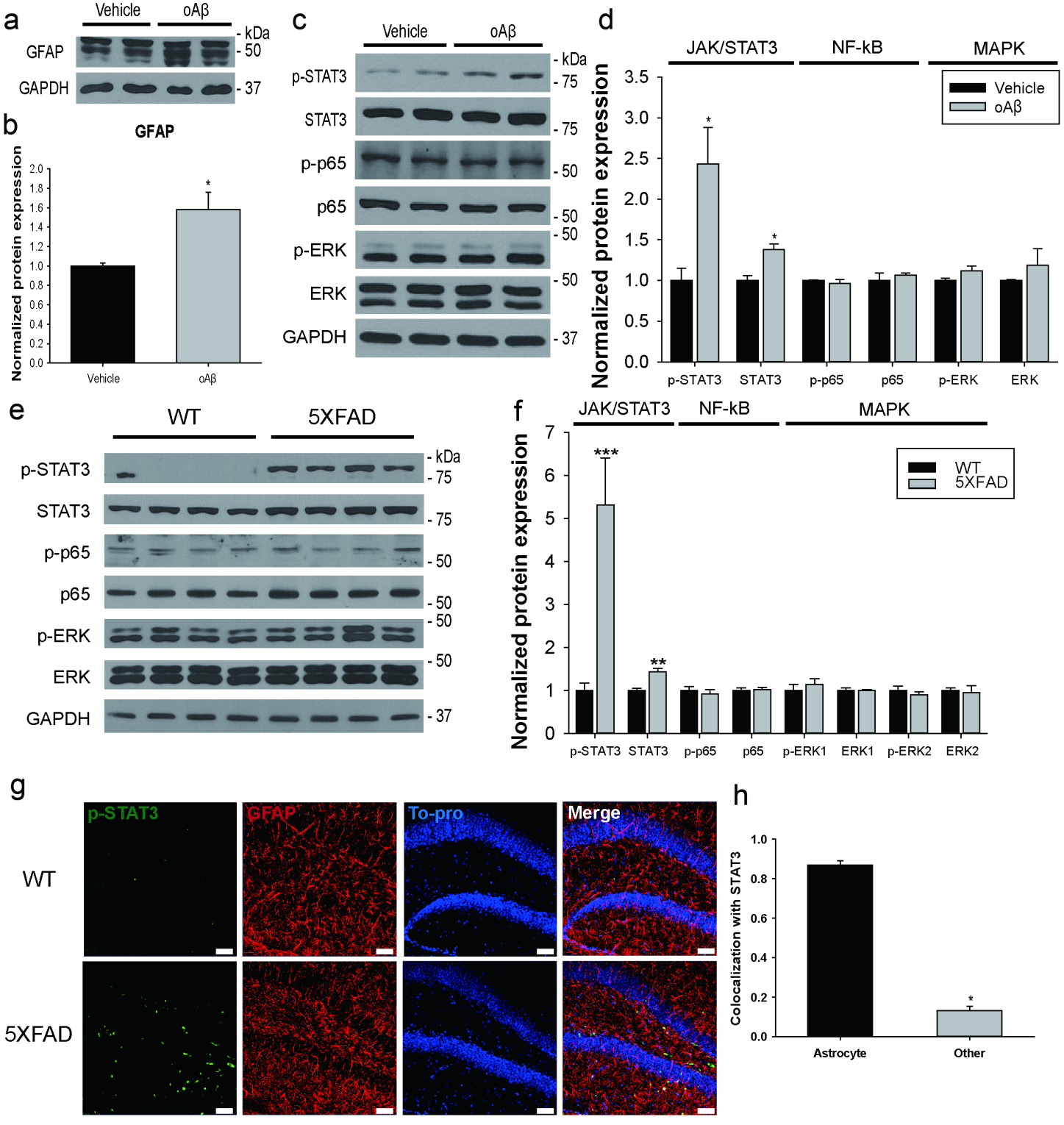

Figure 2

Astrocytes were activated via STAT3 phosphorylation in the 5XFAD hippocampal dentate gyrus (a) Representative immunoblots for p-STAT3, STAT3, p-p65, p65, p-ERK and ERK for vehicle- and oAβ-treated primary cultured astrocytes from C57B/L6 p1 mice. (N = 4) (b) Densitometric analysis of immunoblots for p-STAT3, STAT3, p-p65, p65, p-ERK and ERK. (c) Representative immunoblots for GFAP in vehicle- and oAβ-treated primary cultured astrocytes from C57B/L6 p1 mice. (N = 4) (d) Densitometric analysis of immunoblots for GFAP. (e) Representative immunoblots for p-STAT3, STAT3, p-ERK, ERK, p-p65 and p65 in the WT (N=7) and 5XFAD (N = 9) hippocampus. (f) Densitometric analysis of immunoblots for p-STAT3, STAT3, p-ERK, ERK, p-p65 and p65. (g) Immunofluorescence for p-STAT3 and GFAP in the hippocampal dentate gyrus of both WT and 5XFAD mice (scale bar = 50 μm). (h) Colocalization of p-STAT3 and GFAP in the hippocampal dentate gyrus (N = 5). *$p<0.05$; **$p<0.01$

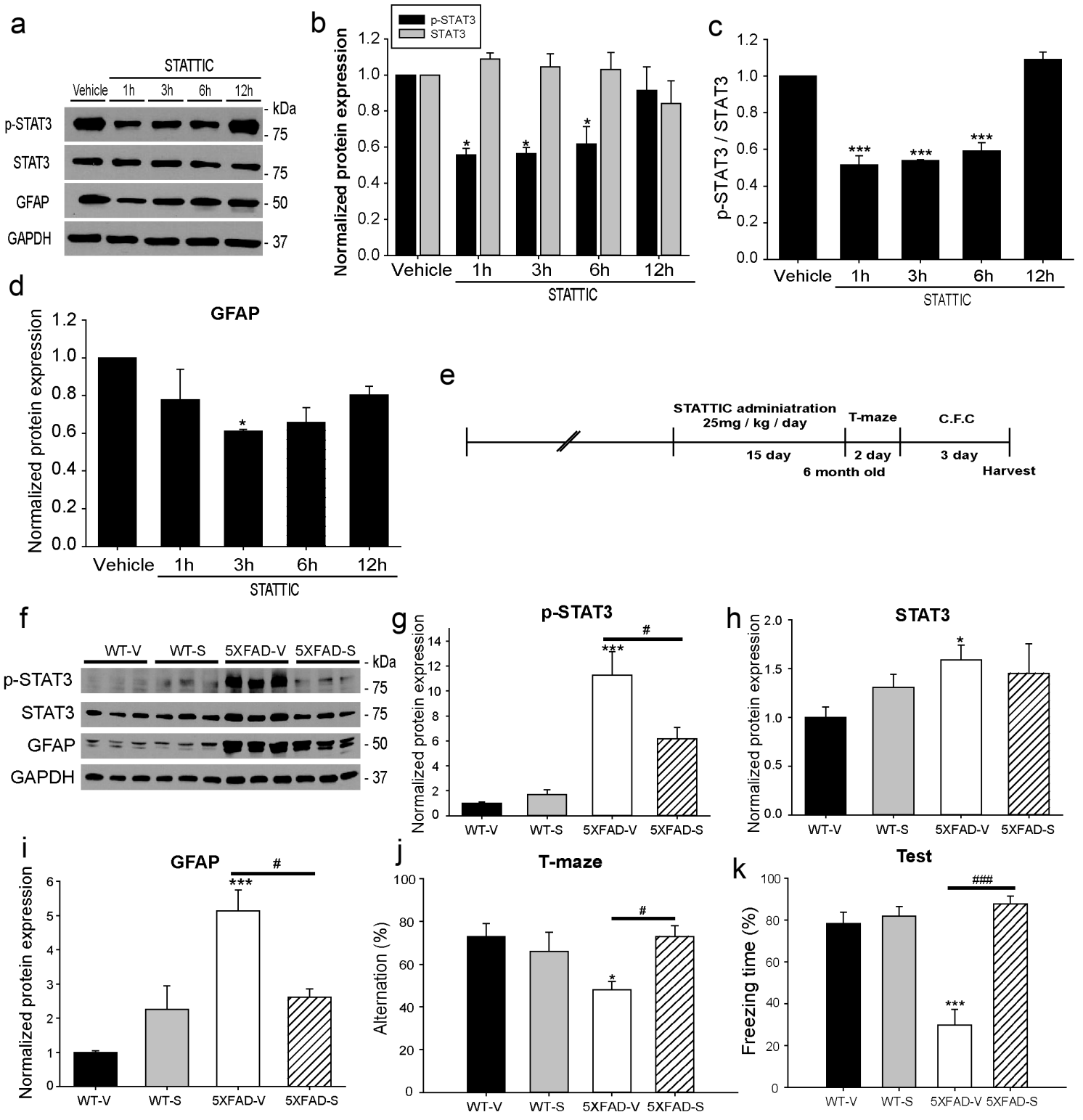

Figure 3

Systemic administration of Stattic restored the impairments in cognitive function in 5XFAD mice (a) Representative immunoblots for p-STAT3, STAT3 and GFAP from vehicle and 1 μM Stattic-treated time dependently in primary cultured astrocyte. (N = 3) (b) Densitometric analysis of immunoblots for p-STAT3 and STAT3 in the vehicle and Stattic treatment groups. (c) Quantification of the ratio of p-STAT3 and STAT3. (d) Densitometric analysis of immunoblots for GFAP. (e) A time schedule for the Stattic

administration and behavior testes with WT and 5XFAD mice is shown. (f) Representative immunoblots for p-STAT3, STAT3 and GFAP from WT and 5XFAD mice that received vehicle or Stattic treatment. (N = 6) (g) Densitometric analysis of immunoblots for p-STAT3. (h) Densitometric analysis of immunoblots for STAT3. (i) Densitometric analysis of immunoblots for GFAP. (j) A graph showing the alternation percentage in the T-maze test after vehicle or Stattic oral administration to 5XFAD mice (N ≥ 10). (k) A graph showing the freezing time percentage in the contextual fear conditioning test on the test day after vehicle or Stattic oral administration to 5XFAD mice. (N ≥ 10) *p<0.05; **p<0.01; ***p<0.001; #p<0.05; ###p<0.001, Data are displayed as the mean ± SEM.

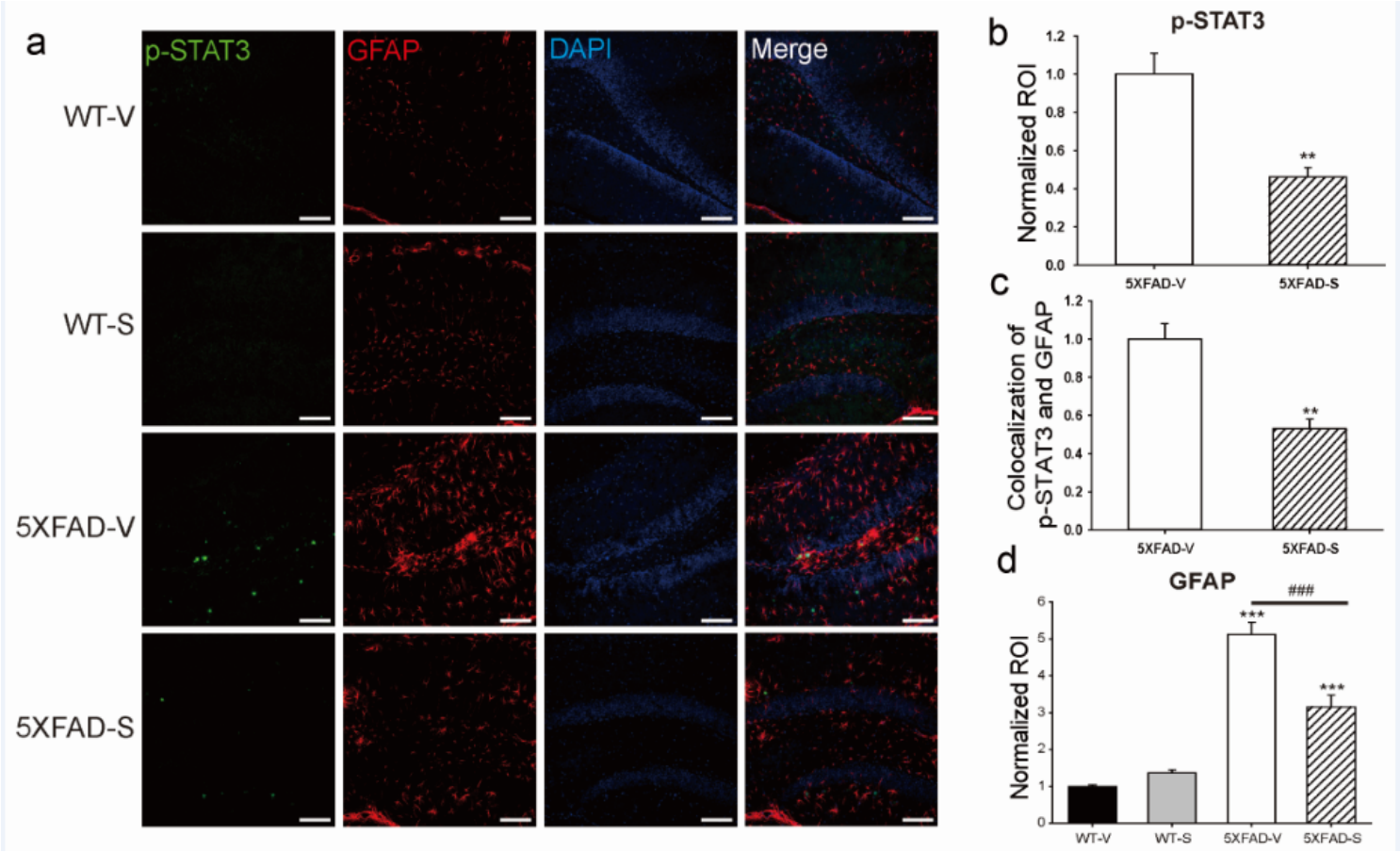

Figure 4

Systemic administration of Stattic restored the activation of astrocytes in the hippocampal dentate gyrus of 5XFAD mice (a) Immunofluorescence for p-STAT3 and GFAP in the hippocampal dentate gyrus of both WT and 5XFAD mice that received vehicle or Stattic treatment (scale bar = 100 μm). (N ≥ 9) (b) ROI analysis of immunoblots for p-STAT3. (c) Bar graph showing the colocalization percentage of p-STAT3 and GFAP-positive astrocytes. (d) ROI analysis of immunoblots for p-STAT3. *p<0.05; ***p<0.001; ###p<0.001

Supplementary Files

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