Dysfunction of the BDNF-TrkB signaling pathway contributes to learning and memory impairments induced by neuroinflammation in mice

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Abstract

Background

Evidence suggests neuroinflammation is the main mechanism in cognitive dysfunction. The brain-derived neurotrophic factor (BDNF) is involved in learning and memory via binding to tyrosine kinase B (TrkB) receptors. Herein, we mainly tested roles of the BDNF-TrkB signaling pathway and its downstream cascades in lipopolysaccharide (LPS) induced cognitive dysfunction in mice.

Methods

Mice were treated with LPS and 7,8-DHF for 7 days, and learning and memory function was evaluated by the novel object recognition test (NORT). Western blot elucidated roles of the BDNF-TrkB signaling pathway and its downstream cascades in LPS mice.

Results

The NORT showed that LPS induced learning and memory deficits in mice. LPS increased the levels of IL-1β, IL-6, and TNF-α in the serum of mice. In the hippocampus and medial prefrontal cortex (mPFC) regions, LPS reduced protein levels of BDNF, p-TrkB, Bcl-2, p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 and increased the expression of Bax. In the entorhinal cortex (EC), levels of BDNF, p-TrkB, Bcl-2, p-CaMK2 and p-CREB proteins were reduced and the protein level of Bax was increased in LPS mice. 7,8-DHF could alleviate these disorders in LPS mice and improved their learning and memory function, however, the TrkB antagonist ANA12 effectively reversed the effects of 7,8-DHF.

Conclusion

These results revealed that the BDNF-TrkB signaling pathway and its downstream cascades disorders contributed to neuroinflammation induced cognitive dysfunction in mice. 7,8-DHF could become a new therapeutic drug for cognitive dysfunction induced by BDNF-TrkB signaling pathway disorders in neurodegenerative diseases.

Introduction

Systemic inflammation often promotes central neuroinflammation by up-regulating pro-inflammatory cytokines, increasing the permeability of the blood-brain barrier and further causing neuroinflammation in the central nervous system[1, 2], which further activates the inflammatory signaling pathway, that triggers synaptic plasticity changes and neuronal apoptosis, ultimately leading to cognitive dysfunction and neurodegenerative diseases[3–5]. For example, systemic infection which occurs with surgery[6, 7], can trigger systemic and hippocampal inflammation resulting in cognitive decline[8].

The brain-derived neurotrophic factor (BDNF) is the main neurotrophin growth factor that participates in mediating synaptic plasticity and neuronal survival, differentiation, as well as neurogenesis[9, 10]. BDNF exerts its biological functions mainly through binding to tyrosine kinase B (TrkB) receptors[11, 12] and the BDNF-TrkB signaling pathway is involved in the formation of dendritic spines. Furthermore, activation of TrkB receptors promotes activations of downstream cascades including the ERK1/2, CaMK2, CREB, GluR1 and the mechanism of apoptosis which are related to regulating learning and memory[13]. The ERK1/2, a member of the mitogen-activated protein kinase superfamily, is involved in regulating cell proliferation, survival, and apoptosis[14]. The CaMK2 is essential for synaptic plasticity and memory formation[15]. The CREB, a transcription factor, is one of the main downstream transcription factors of ERK1/2 and plays significant roles in neuronal plasticity, learning and memory[16]. The GluR1 is also necessary for learning and memory processing and mediates neuronal plasticity[17]. Therefore, the reduction of BDNF expression in the brain is associated with impairment of synaptic plasticity and learning and memory failures.

7,8- Dihydroxyflavone (7,8-DHF), a member of the flavonoid derivative, has been identified as an effective agonist of the TrkB receptor which mimics the properties of BDNF[18–20]. 7,8-DHF can penetrate the blood-brain barrier and plays neuroprotective roles including increasing dendritic spine density and exerting neurotrophic effects, via activating the BDNF-TrkB signaling pathway and its downstream cascades. ANA12 is a small molecule antagonist of TrkB and crosses the blood-brain barrier in order to exert its antagonism effect[21]. In the current research, we mainly investigated the roles of the BDNF-TrkB signaling pathway and its downstream cascades on neuroinflammation-induced learning and memory impairments and whether 7,8-DHF could alleviate these disorders in mice.

Materials And Methods

Animals

Male c57BL/6J mice (8 weeks old) were obtained from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. A total of 106 mice were used in our research. Mice were raised five per cage under 12 hours light/dark cycle and given water and food ad libitum. All animal studies were approved by the Experimental Animal Committee of Tongji Medical college and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental design

Mice were divided into four groups: Control group, LPS group, LPS+7,8-DHF group and LPS+7,8-DHF+ANA12 group. In the LPS group, mice were intraperitoneally injected with LPS at a concentration of 0.25 mg/kg. In the Control group, mice were intraperitoneally injected with the same amount of saline. In the LPS+7,8-DHF group, 7,8-DHF (5 mg/kg) were injected 30 minutes before LPS treatment. And in the LPS+7,8-DHF+ANA12 group, ANA12 (0.5 mg/kg) were injected 30 minutes before 7,8-DHF and LPS. The novel object recognition test was performed to detect cognitive function 7 days after treatment.

Novel object recognition test

7 days after LPS treatment, the novel object recognition test (NORT) was applied to examine cognitive function in mice[22]. Mice were handled 6 days before the test, for 1 minute a day. Then, putting mice into the box for 5 minutes to acclimatization. 24 hours later, mice were allowed to explore two identical rectangular blocks for 5 minutes. After 2 hours, a cylinder was used to replace one of the rectangular blocks, and mice explored for another 5 minutes. Exploratory behaviors were performed as sniff, lick and climb. Discrimination ratio was used to assess learning and memory.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected and centrifuged for 10 minutes at 3000g at 4 °C to obtain supernatants. Expressions of IL-6, IL-1β, and TNF-α were determined using commercially available ELISA kits (MDL Biotech, Beijing, China). Concentrations of inflammation factors were tested according to the manufacturer's instructions and presented as pg/ml of serum protein.

Western bolt

Hippocampal tissues were prepared as previously described[22] and homogenized with radioimmunoprecipitation assay lysis buffer containing protease and phosphatase inhibitors. The commercially available BCA protein assay kit (Boster, Wuhan, China) was used to determine protein concentrations. Then, protein samples were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). Bands were blocked with 5% BSA for 1.5 hours at room temperature. Primary antibodies were incubated overnight at 4 °C, and then, bands were washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 hours. The antibodies used in this research include rabbit anti-BDNF (1:1000; Abclonal), anti-TrkB (1:1000; Abclonal), anti-p-TrkB (1:500; Abclonal), anti-Bax (1:1000; Abclonal), anti-Bcl-2 (1:1000; Abclonal), anti-ERK1/2 (1:1000; Cell Signaling Technology), anti-p-ERK1/2 (1:1000; Cell Signaling Technology), anti-CaMK2 (1:1000; Proteintech), anti-p-CaMK2 (1:500; Abclonal), anti-CREB (1:1000; Abclonal), anti-p-CREB (1:1000; Cell Signaling Technology), anti-GluR1 (1:1000; Abclonal), anti-p-GluR1 (1:500; Abclonal), anti-GAPDH (1:1000; Abclonal), and goat anti-rabbit (1:5000; Promoter). The bands were visualized using chemiluminescence (Pierce ECL Western Blotting Substrate, Abbkine) and measured by a computerized image analysis system (ChemiDoc XRS+, BIO-RAD, CA, USA).

Statistical analysis

All data were analyzed using GraphPad prism version 7.0 and presented as mean ± SEM. For two groups, unpaired Student's *t* test was applied. For multiple groups, One-way ANOVA was used. In addition, two-way ANOVA was used to analyze data of the time spent with the object in the novel object recognition test. $P < 0.05$ was considered statistically significant.

Results

1. Lipopolysaccharide resulted in learning and memory impairment in mice

The NORT is a widely used to evaluate learning and memory function in mice[22, 23]. In this study, no significant difference was found in time spent with objects between the Control and LPS mice (Fig. 1A). In the testing stage, spent more time was spent on the novel object than on the familiar object in the Control mice (Fig. 1B), but there was no statistic difference in LPS mice (Fig. 1B). The result of discrimination ratio was similar to that in Fig. 1B which shows that Control mice had greater discrimination ratio than LPS mice (Fig. 1C). Then, we extracted serum to detect the concentrations of IL-6, IL-1β and TNF-α after LPS treatment. The result indicates that levels of IL-6, IL-1β and TNF-α were increased in the LPS group (Fig. 1D). These findings demonstrate that the administration of LPS resulted in systemic inflammation that induced learning and memory impairment in mice.

2. Lipopolysaccharide disturbed the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus

After the novel object recognition test, we segregated the hippocampus to detect protein levels of BDNF-TrkB signaling using western blot. The results show that, compared with the Control group, the protein level of BDNF in the hippocampus was reduced in LPS mice (Fig. 2A-B). No statistical difference was found in TrkB protein levels between the Control and LPS mice (Fig. 2A, C). However, the protein level of the p-TrkB was reduced in LPS mice (Fig. 2A, D). Then, we detected levels of apoptosis-related proteins. Compared with the Control mice, the level of pro-apoptotic protein Bax was distinctly increased, while the level of anti-apoptotic protein Bcl-2 was decreased in LPS mice (Fig. 2E-G). In addition, phosphorylation protein levels of TrkB downstream cascades were tested. The protein levels of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1were obviously reduced in LPS mice (Fig. 2H, J-K, M-N, P-Q, S). However, no difference was found in levels of ERK1/2, CaMK2, CREB and GluR1 proteins (Fig. 2H-I, K-L, N-O, Q-R). These results illustrate that the BDNF-TrkB signaling pathway and its downstream cascades were regulated by inflammation and may be involved in learning and memory processes.

3. Lipopolysaccharide disturbed the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC

Then, we tested the protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC. The western blot results revealed that the levels of BDNF and p-TrkB were reduced after administration of LPS (Fig. 3A-B, D), and no difference was found in the protein level of TrkB (Fig. 3A, C). Compared to the Control group, the expression of Bax was up-regulated in LPS mice (Fig. 3E-F). At the same time, reduction of Bcl-2 expression was found in the LPS group (Fig. 3E, G). Furthermore, the expression of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 proteins was decreased markedly in LPS mice (Fig. 3H, J-K, M-N, P-Q, S). However, there were no significant differences in the expression of ERK1/2,

CaMK2, CREB and GluR1 proteins (Fig. 3H-I, K-L, N-O, Q-R). These results demonstrate that the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC play a vital role in learning and memory processes.

4. Lipopolysaccharide disturbed the BDNF-TrkB signaling pathway and its downstream cascades in the EC

Next, the protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the EC were also examined using western blot. The results show that a qualitative reduction in the expression of BDNF and p-TrkB was observed in LPS mice (Fig. 4A-B, D). There was no difference in the levels of TrkB protein between Control and LPS groups (Fig. 4A, C). In addition, the protein level of Bax was increased compared to Control mice (Fig. 4E-F), and the expression of Bcl-2 was markedly decreased in LPS mice (Fig. 4E, G). No statistical difference was observed in protein levels of ERK1/2, p-ERK1/2, GluR1 and p-GluR1 between Control and LPS mice (Fig. 4H-J, Q-S). The expression of p-CaMK2 and p-CREB were obviously reduced after using LPS (Fig. 4K, M-N, P). No difference was shown in levels of CaMK2 and CREB (Fig. 4K-L, N-O). These results suggest that, in the EC, the BDNF-TrkB signaling pathway, p-CaMK2 and p-CREB cascades participate in process of learning and memory.

5. 7,8-DHF ameliorated lipopolysaccharide induced learning and memory deficits in mice

7,8-DHF was used to detect the role of the BDNF-TrkB signaling pathway in learning and memory[18]. ANA12 is a small-molecule TrkB antagonist[24], which was used to identify whether 7,8-DHF could reverse learning and memory dysfunction. The results of the novel object recognition test shows that there were no statistical changes in the time spent with identical objects during the training stage (Fig. 5A). In the testing stage, administration of 7,8-DHF increased the time spent on the novel object in LPS mice, but ANA12 reversed the role of 7,8-DHF in LPS mice (Fig. 5B). Further analysis of the discrimination ratio was in line with the result of time spent with an object. 7,8-DHF up-regulated the discrimination ratio in LPS + 7,8-DHF mice, however, the discrimination ratio was reduced in LPS + 7,8-DHF + ANA12 mice (Fig. 5C). These results demonstrate that the preventive use of 7,8-DHF could effectively alleviate the dysfunction of learning and memory caused by LPS, while ANA12 reversed 7,8-DHF therapeutical effects.

6. 7,8-DHF ameliorated lipopolysaccharide induced the BDNF-TrkB signaling pathway and its downstream cascade disorders in the hippocampus

The hippocampus was observed to test changes in protein levels after administration of 7,8-HDF. Western blot results show that 7,8-DHF up-regulated the expression of p-TrkB, Bcl-2, p-ERK1/2, p-CaMK2, p-CREB, p-GluR1, and decreased the protein level of Bax in LPS mice (Fig. 6A, D-H, J-K, M-N, P-Q, S). But ANA12 reversed these changes in 7.8-DHF of LPS + 7,8-DHF + ANA12 mice. There was no significant increase in expression of BDNF after using 7,8-DHF or reduction in the LPS + 7,8-DHF + ANA12 group (Fig. 6A-B). In

addition, there were no significant differences in the expression of TrkB, ERK1/2, CaMK2, CREB and GluR1(Fig. 6A, C, H-I, K-L, N-O, Q-R). These results demonstrate that 7,8-DHF effectively alleviated disorders of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus of the LPS mice, but ANA12 completely reversed the therapeutic effects of 7,8-DHF.

7. 7,8-DHF ameliorated the lipopolysaccharide induced BDNF-TrkB signaling pathway and its downstream cascade disorders in the mPFC

Furthermore, we tested protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC after administration of 7,8-DHF using western blot.

The results are consistent with hippocampal data. The protein levels of p-TrkB, Bcl-2, p-ERK1/2, p-CaMK2, p-CREB, p-GluR1 were increased, and the expression of Bax was reduced in the LPS + 7,8-DHF group (Fig. 7A, D-H, J-K, M-N, P-Q, S). But the effects of 7,8-DHF were reversed by ANA12 in LPS + 7,8-DHF + ANA12 mice. There were no changes in the level of BDNF after using 7,8-DHF or 7,8-DHF + ANA12 in LPS mice (Fig. 7A-B). Furthermore, no statistical difference was observed in the expression of TrkB, ERK1/2, CaMK2, CREB and GluR1(Fig. 7A, C, H-I, K-L, N-O, Q-R). These results confirm that the disorders of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC are alleviated using 7,8-DHF in LPS mice, but ANA12 antagonized the protective effects of 7,8-DHF.

8. 7,8-DHF ameliorated lipopolysaccharide induced the BDNF-TrkB signaling pathway and its downstream cascade disorders in the EC

Finally, the expression changes in related proteins of the EC were detected by western blot. The results show that a qualitative increase in p-TrkB, Bcl-2, p-CaMK2, p-CREB expression and reduction in Bax expression was observed in LPS + 7,8-DHF mice (Fig. 8A, D-G, KC, M-N, P). However, the effects of 7,8-DHF were reversed by ANA12 in LPS + 7,8-DHF + ANA12 mice. Consistent with previous results, 7,8-DHF and ANA12 had no effect on the expression of BDNF protein (Fig. 8A-B). In addition, there were no significant differences in the expression of TrkB, ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 and p-GluR1 proteins (Fig. 8A, C, H-L, N-O, Q-S). These results signify that 7,8-DHF can effectively alleviate disorders of the BDNF-TrkB signaling pathway, p-CaMK2 and p-CREB cascades in the EC of LPS mice, but ANA12 can antagonize the therapeutical effects of 7,8-DHF.

Discussion

Numerous studies have shown that the central neuroinflammatory response is the main mechanism of neurodegenerative diseases, but the specific pathophysiological mechanism is still unclear. In this study, we found that disorders of the BDNF-TrkB signaling pathway and its downstream cascade in mice

participated in learning and memory impairments induced by neuroinflammation, which mainly manifested as reductions in BDNF, p-TrkB, Bcl-2, p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 proteins and upregulation of the expression of Bax protein in different brain regions including the mPFC, hippocampus and EC.

It is widely known that BDNF is the main neurotrophin in the brain[9]. Many researchers have reported that BDNF has important effects on synapses including structural and functional roles in many brain regions[11, 12]. The different effects of BDNF on the brain are related to its diverse forms and downstream receptors that it binds to, such as pro-BDNF and mature-BDNF with the p75 neurotrophin receptor (p75NTR) and TrkB which have opposing effects on synapses[25–27]. In our study, we mainly discuss the roles of mature-BDNF and TrkB receptors on learning and memory deficits induced by neuroinflammation. The expression of BDNF and p-TrkB receptors were reduced in the mPFC, hippocampus and EC regions after application of LPS in mice. This demonstrates that different ways of applying BDNF induced different changes in functions and structures[28]. For example, acute up-regulation of BDNF initiated neurite elongation and spine head enlargement, however, gradual increase in BDNF potentiated dendritic branching and filopodia-like spines. In addition, transient activation of TrkB promoted synaptic transmission in the brain. However, systematic application of BDNF could be catabolized by enzymes in vivo. In this study, we used TrkB agonist 7,8-DHF to simulate physiological actions of BDNF and detected whether the activation of TrkB could alleviate cognitive impairments in LPS mice. As the results show, preventive use of 7,8-DHF effectively alleviated learning and memory dysfunction induced by LPS. At the same time, the level of p-TrkB was increased after administration of 7,8-DHF. However, the application of TrkB antagonist ANA12 completely reversed therapeutic effects of 7,8-DHF, which further indicates that the BDNF-TrkB signaling pathway disorder involved neuroinflammation associated with learning and memory impairments in mice.

Increasing evidence suggests that BDNF exerts its neuroprotective roles by suppressing excitotoxicity of NMDA receptors, promoting regeneration of synapses and inhibiting cell apoptosis[29–31]. Bax is a pro-apoptotic protein which accelerates cell loss including neurons, which lead to memory disorders[32] wheread Bcl-2 is one of anti-apoptotic proteins antagonizing cell apoptosis. Neuroinflammation reduced expression of BDNF and p-TrkB proteins, further disrupting the balance of Bax and Bcl-2 generating cell apoptosis in the mPFC, hippocampus and EC regions. ERK1/2 and CREB play a vital role in learning and memory in the brain which are involved in regulating transcription factors and promoting protein synthesis[33, 34]. In this study, the expression of p-ERK1/2 and p-CREB was decreased in the mPFC and hippocampus of LPS mice, while in the EC, only the level of p-CREB was downregulated and no difference was found in the expression of p-ERK1/2. In addition, CaMK2 is essential for the learning process and synaptic plasticity, and GluR1 is an important component of the postsynaptic density and controls dendrite growth[35, 36]. We found that neuroinflammation reduced the expression of p-CaMK2 and p-GluR1 both in the mPFC and hippocampus, while in the EC, only the level of p-CaMK2 was decreased. These differences between the EC with the hippocampus and mPFC may be related to the mutual regulation of neural circuits.

Previous studies have reported that multiple brain regions are involved in the process of learning and memory, such as the mPFC, hippocampus and EC[37],[38]. Researchers have demonstrated that the mPFC is the most vulnerable among them, and the hippocampus is moderate, whereas the EC is relatively less susceptible to neuroinflammation[39]. But the volume change in the EC is used as the earliest indicator to evaluate preclinical cognitive deficits leading to the development of dementia[40]. The hippocampus is responsible for storing information and is a key region involved in learning and memory. The mPFC has been shown to play a vital role in the process of attention, behavioral flexibility, social and emotional behaviors, and its interactions with the hippocampus are involved in the regulation of learning and memory[41]. The EC is also related to spatial and long-term memory[42]. Neural projections from the mPFC or EC to the hippocampus are known to be involved in the modulation of learning and memory processes[43–45]. Lu et al. found that the $ECII_{PN}$-$CA1_{PV}$ pathway was impaired with spatial learning and memory deficits in Alzheimer's disease mice, and optogenetic activation of $ECII_{PN}$ rescued $ECII_{PN}$-$CA1_{PV}$ pathway defects and alleviated the impairment of spatial learning and memory[46]. In addition, electrical stimulation of the EC alleviated spatial memory deficits in Alzheimer's disease mice infusing amyloid peptides 1–42 into the hippocampus, which suggestes that there is functional projection between the EC and hippocampus. However, it is not clear whether the mutual projection of the mPFC, hippocampus and EC regions and neuroregulation are associated with the metabolic changes in the BDNF-TrkB signaling pathway and its downstream cascade.

There are still many limitations in this study. On the one hand, we only tested protein levels of the BDNF-TrkB signaling pathway and its downstream cascade in different brain regions, and no synaptic related proteins were detected which directly indicates synaptic damages. On the other hand, whether the changes in the BDNF-TrkB signaling pathway and its downstream cascades among the mPFC, hippocampus and EC regions were regulated by the projection of nerve fibers between these regions have not been confirmed and deserves further investigation.

In summary, our research revealed that the BDNF-TrkB signaling pathway and its downstream cascade disorders participated in learning and memory impairments induced by neuroinflammation in mice (Fig. 9). Intraperitoneal injection of the TrkB agonist 7,8-DHF could effectively alleviate cognitive dysfunction in LPS mice. As a result, the BDNF-TrkB signaling pathway and its downstream cascade disorders are a new viewpoint for learning and memory impairments induced by neuroinflammation, and 7,8-DHF might serve as a potential target for preventing or treating cognitive dysfunction induced by neuroinflammation in neurodegenerative diseases.

Abbreviations

BDNF: brain-derived neurotrophic factor; EC: entorhinal cortex; ELISA: Enzyme-linked immunosorbent assay; LPS: lipopolysaccharide; mPFC: medial prefrontal cortex; NORT: novel object recognition test; TrkB: tyrosine kinase B; 7,8-DHF: 7,8- Dihydroxyflavone.

Declarations

Ethics approval and consent to participate

All experiments were approved by the Experimental Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology, and were line with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data and materials

The data and materials supporting the conclusions of this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Wen Zhang conceived the research, carried out the model building, performed the Western blot, coordinated the lab work and drafted the manuscript. Meng-meng Ge and Long-qing Zhang performed the statistical analysis and drafted the manuscript. Xiao-man Yuan and Si-yi Han took care of the NORT and drafted the manuscript. Anne Manyande, Xue-Bi Tian and Yu-Ke Tian participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Figures

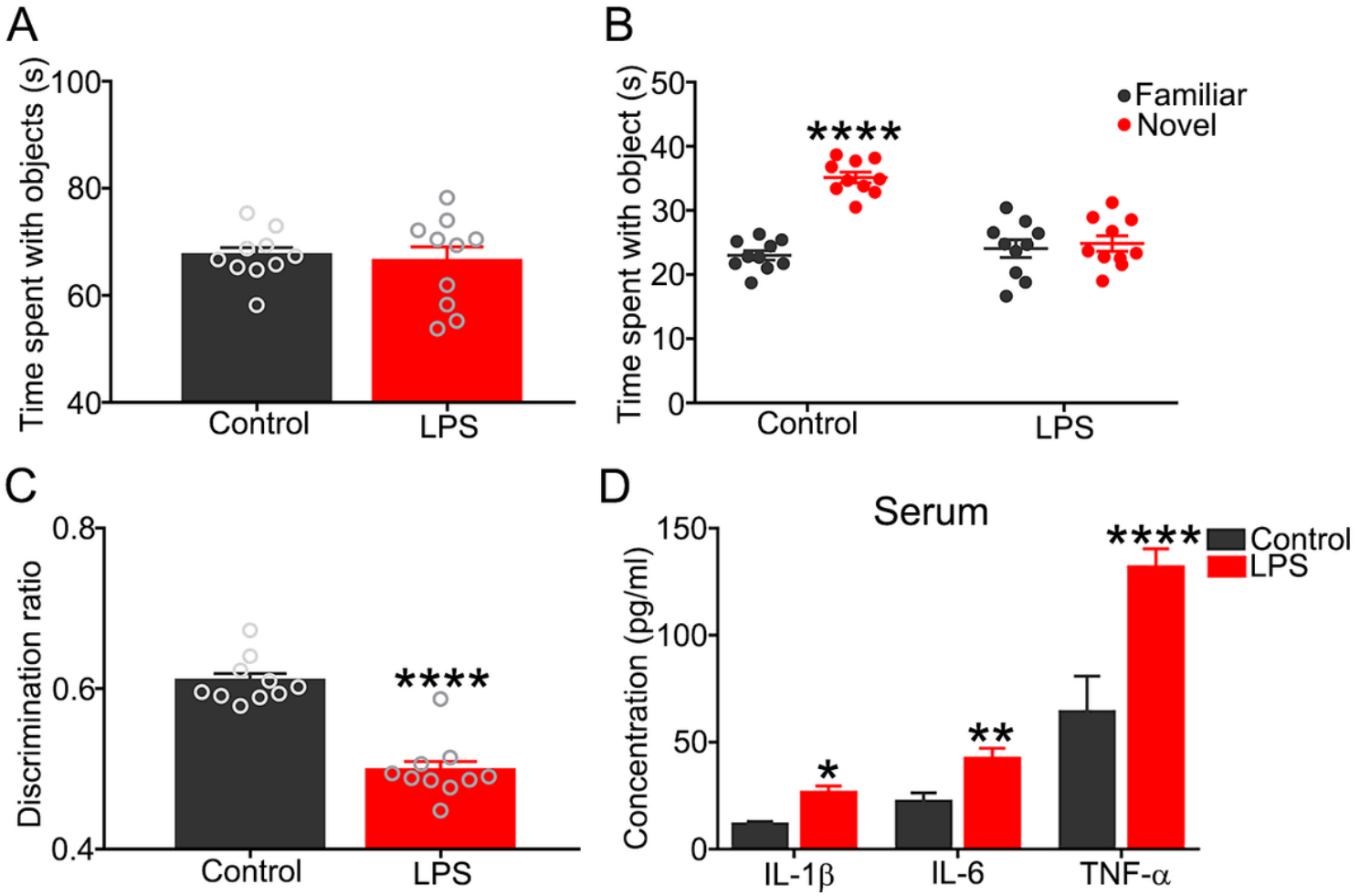

Figure 1

Behavioral tests and pro-inflammation levels in LPS mice. (A-C) There was no significant difference in the time spent in exploring the same objects between the Control and LPS mice during the training phase. In the testing phase, when exposed to the novel object, LPS mice spent less time on the novel object and presented lower discrimination ratio compared with the Control group (n=10). (D) Compared to the Control mice, the concentration of IL-1β, IL-6 and TNF-α serum levels were significantly increased in LPS mice (n=4). Data are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

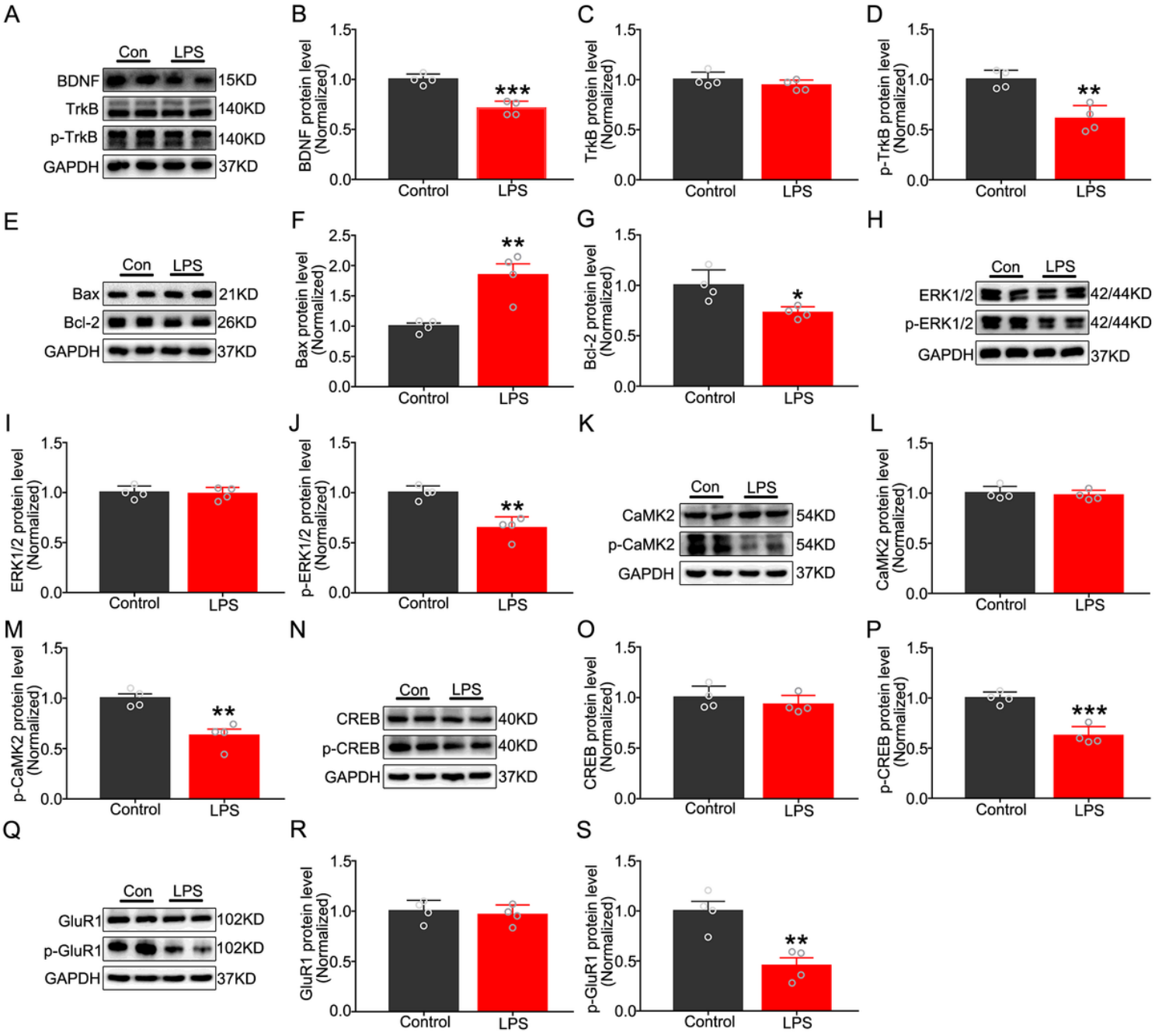

Figure 2

The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus. (A-D) Compared with the Control mice, the levels of BDNF and p-TrkB proteins were decreased in LPS mice, no difference was found in the expression of TrkB (n=4). (E-G) The protein level of Bax was increased and the level of Bcl-2 was reduced in the LPS group compared to the Control group (n=4). (H-S) The protein expression of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice was lower than in Control mice. There was no statistical difference in the levels of ERK1/2, CaMK2, CREB and GluR1 proteins between the Control and LPS groups (n=4). Data are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

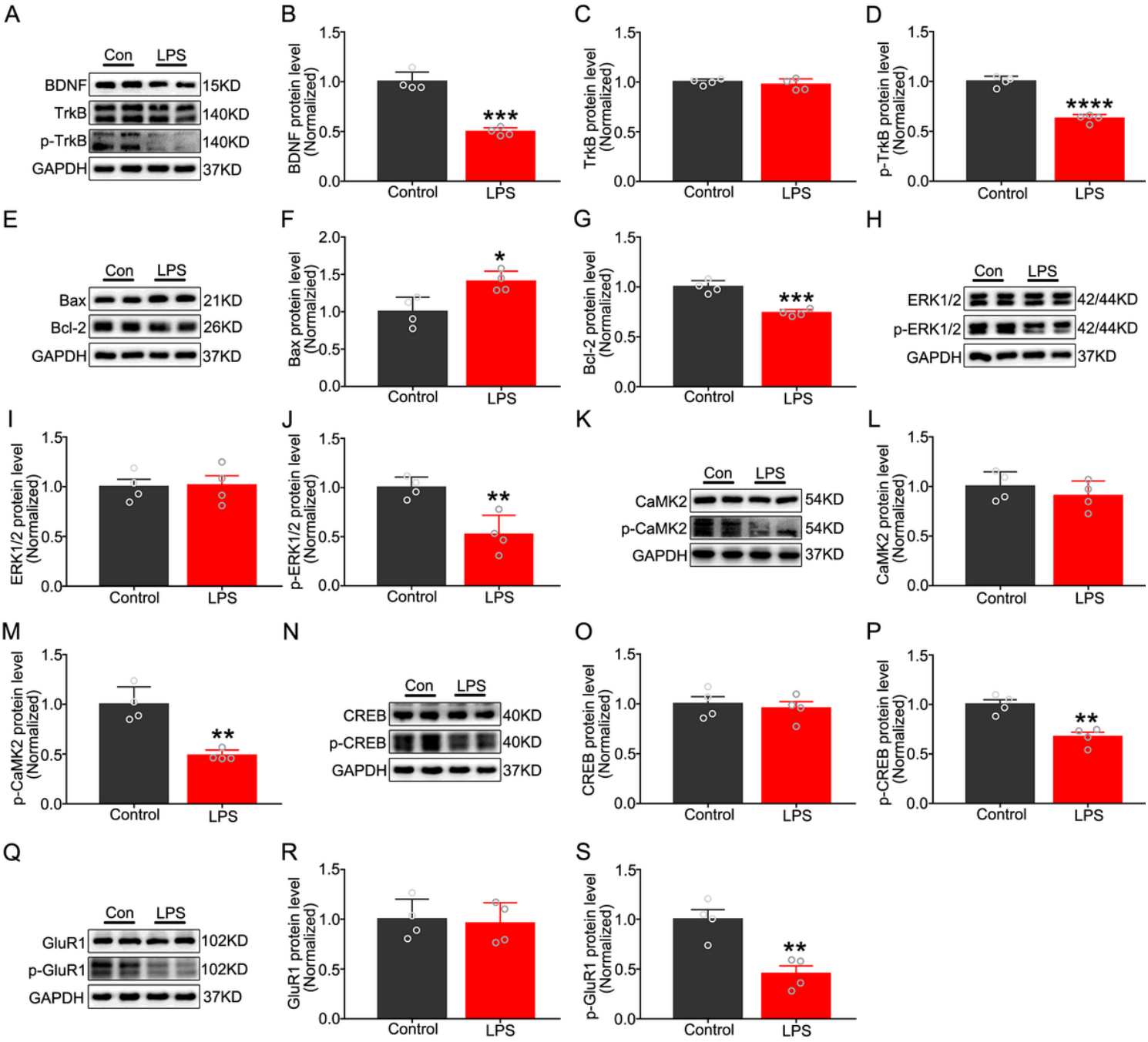

Figure 3

The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC. (A-D) The protein levels of BDNF and p-TrkB were reduced in LPS mice compared to the Control mice, and no difference was found in the level of TrkB between these two groups (n=4). (E-G) Compared with the Control group, the expression of Bax was increased and the level of Bcl-2 was decreased in LPS group (n=4). (H-S) The expression of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice were downregulated. There was no statistical difference in the levels of ERK1/2, CaMK2, CREB and GluR1 proteins between the Control and LPS groups (n=4). Data are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

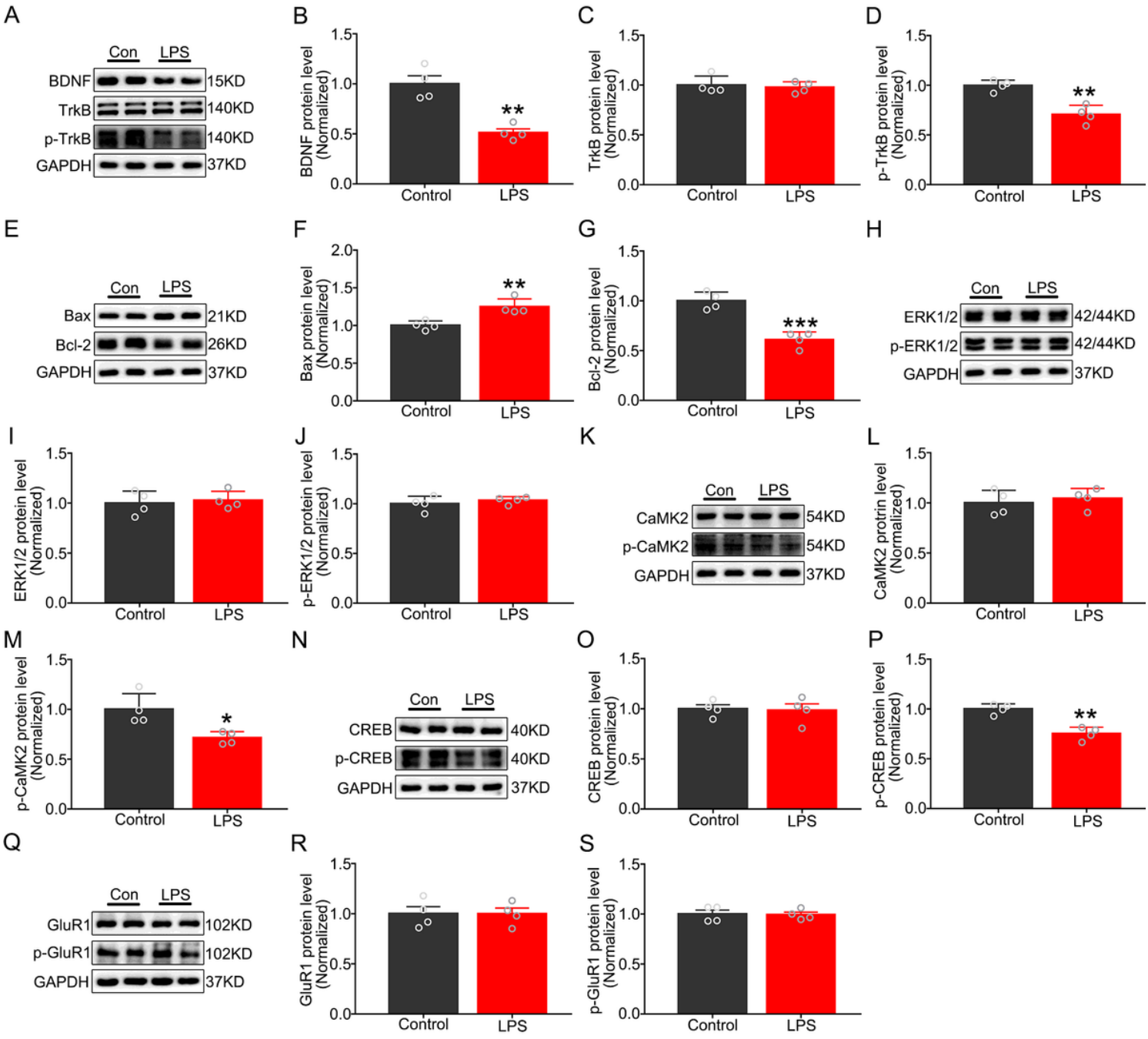

Figure 4

The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the EC. (A-D) The expression of BDNF and p-TrkB were decreased in LPS mice compared to Control mice, and no statistical difference was observed in the expression of TrkB between these two groups (n=4). (E-G) The level of Bax was up-regulated and the level of Bcl-2 was decreased in the LPS group compared to Control mice (n=4). (H-S) The expression of p-CaMK2 and p-CREB was decreased in LPS mice. There was no significant difference in protein levels of ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 and p-GluR1 between the Control and LPS groups (n=4). Data are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$.

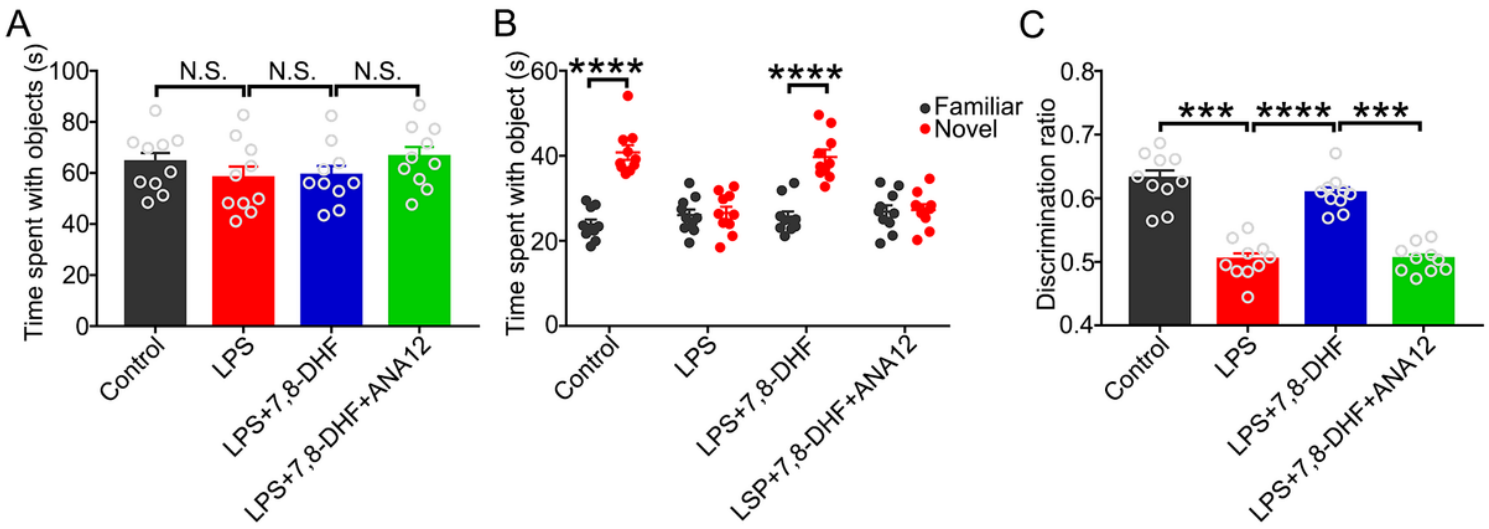

Figure 5

7,8-DHF alleviated LPS-induced learning and memory deficits in the mice. (A-C) During the training phase, there was no statistical difference in the time spent with objects after using 7,8-DHF or ANA12 in LPS mice. In the testing phase, the time spent on the novel object and the discrimination ratio were increased in LPS mice after administration of 7,8-DHF, however, ANA12 completely reversed the effects of 7,8-DHF (n=10). Data are presented as mean ± SEM. ***$P < 0.001$; ****$P < 0.0001$; N.S. $P > 0.05$.

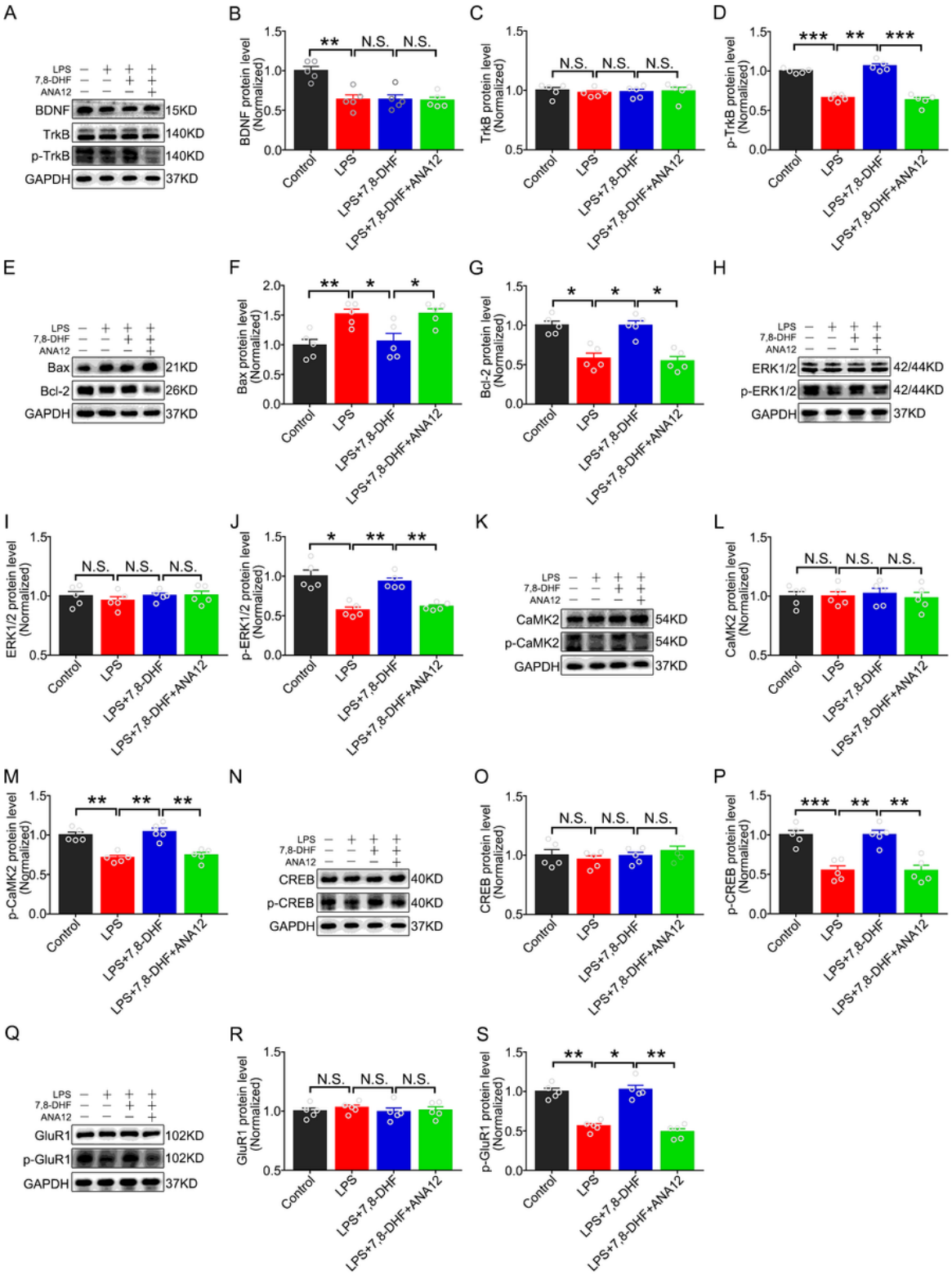

Figure 6

7,8-DHF restored the LPS-induced BDNF-TrkB signaling pathway and its downstream cascade disorders in the hippocampus. (A-D) There were no statistical changes in the expression of BDNF and TrkB in LPS mice after using 7,8-DHF or ANA12. The expression of p-TrkB was increased in LPS mice after using 7,8-DHF, however, ANA12 reversed the effects of 7,8-DHF (n=5). (E-G) 7,8-DHF decreased the expression of Bax and increased the level of Bcl-2 in LPS mice, while ANA12 completely reversed these changes (n=5).

(H-S) The expression of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice was increased after using 7,8-DHF, however, ANA12 reversed the effects of 7,8-DHF. No statistical differences were observed in protein levels of ERK1/2, CaMK2, CREB and GluR1 in LPS groups after using 7,8-DHF or ANA12 (n=5). Data are presented as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; N.S. $P > 0.05$.

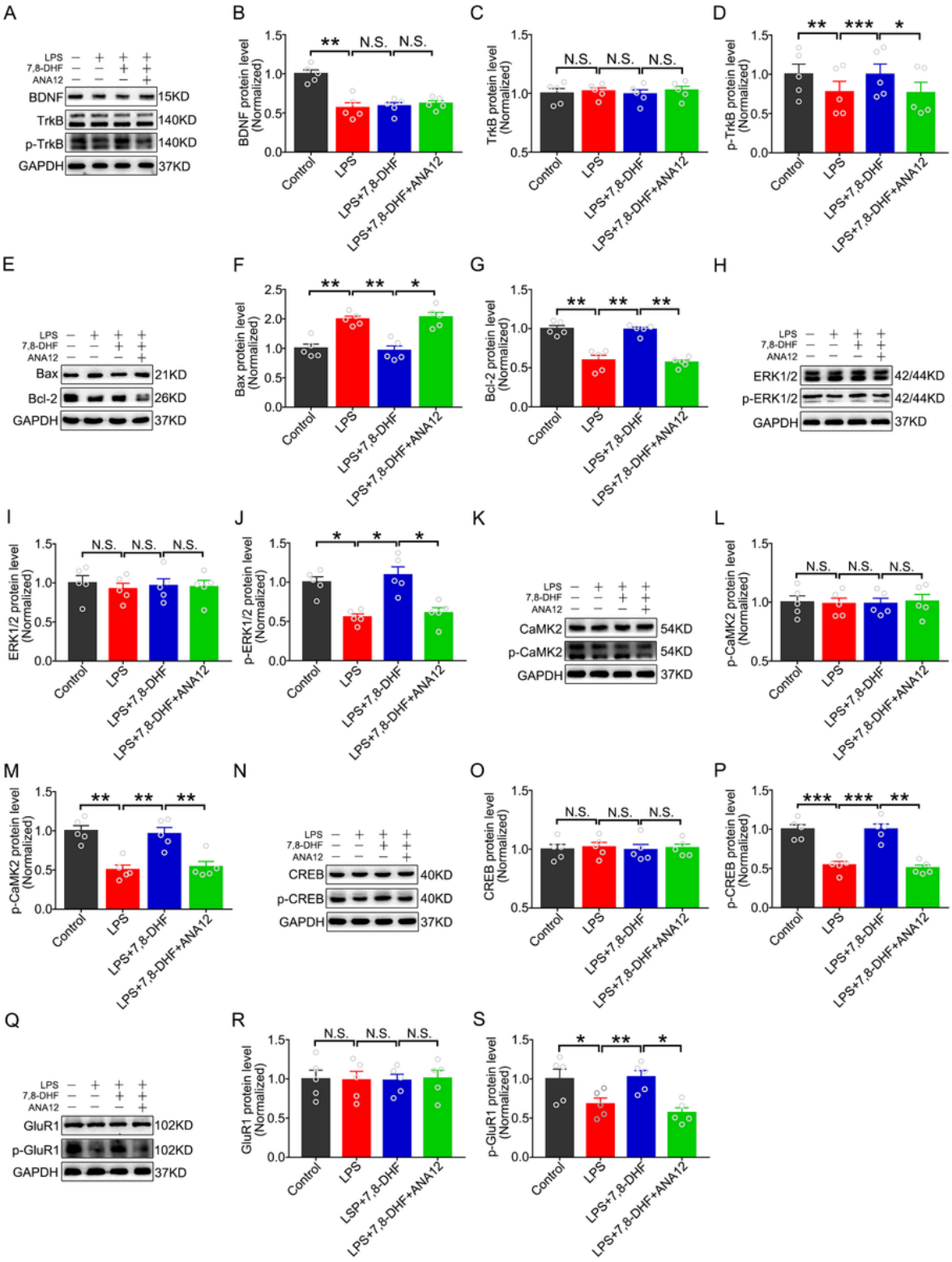

Figure 7

7,8-DHF restored the LPS-induced BDNF-TrkB signaling pathway and its downstream cascades disorder in the mPFC. (A-D) There were no significant differences in protein levels of BDNF and TrkB in LPS mice after administration of 7,8-DHF or ANA12. The level of p-TrkB was increased in LPS mice after administration of 7,8-DHF, however, ANA12 reversed the effects of 7,8-DHF (n=5). (E-G) The level of Bax was reduced and the level of Bcl-2 was increased in LPS mice after administration of 7,8-DHF, while ANA12 completely reversed these changes (n=5). (H-S) The levels of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice were increased after administration of 7,8-DHF, however, ANA12 reversed the effects of 7,8-DHF. There were no statistical differences in protein levels of ERK1/2, CaMK2, CREB and GluR1 in LPS groups after administration of 7,8-DHF or ANA12 (n=5). Data are presented as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 00.5.

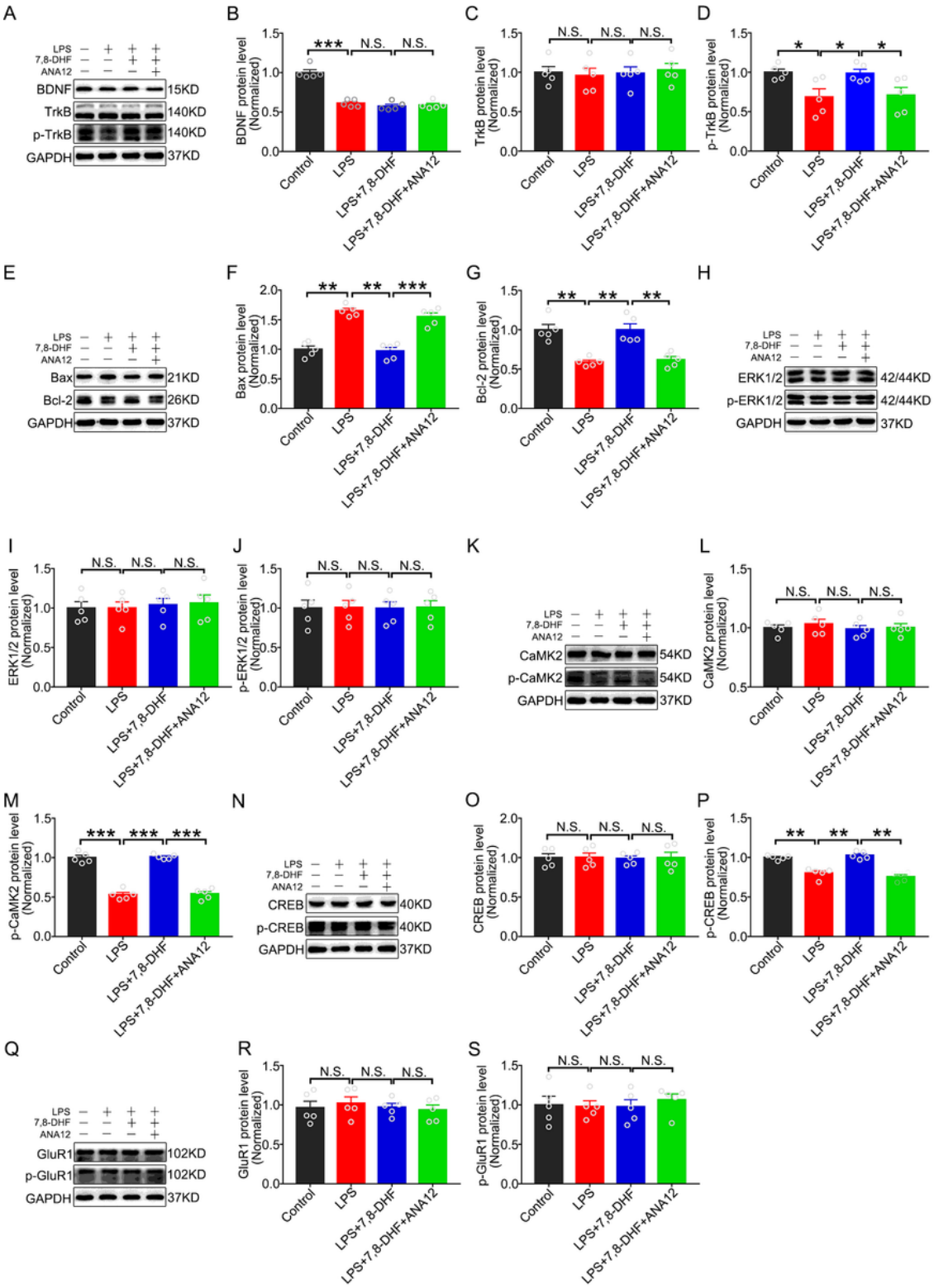

Figure 8

7,8-DHF restored the LPS-induced BDNF-TrkB signaling pathway and its downstream cascade disorder in the EC. (A-D) No statistical difference was observed in the expression of BDNF and TrkB in LPS mice after using 7,8-DHF or ANA12. 7,8-DHF clearly increased the expression of p-TrkB in LPS mice, while ANA12 reversed the therapeutic effects of 7,8-DHF (n=5). (E-G) 7,8-DHF reduced the level of Bax and increased the level of Bcl-2 in the LPS group, however, ANA12 reversed these changes (n=5). (H-S) The expression

of p-CaMK2 and p-CREB was increased in LPS mice after administration of 7.8-HDF, while ANA12 completely reversed the effects of 7,8-DHF. There was no significant difference in protein levels of ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 and p-GluR1 in LPS groups after using 7,8-DHF or ANA12 (n=5). Data are presented as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 00.5.

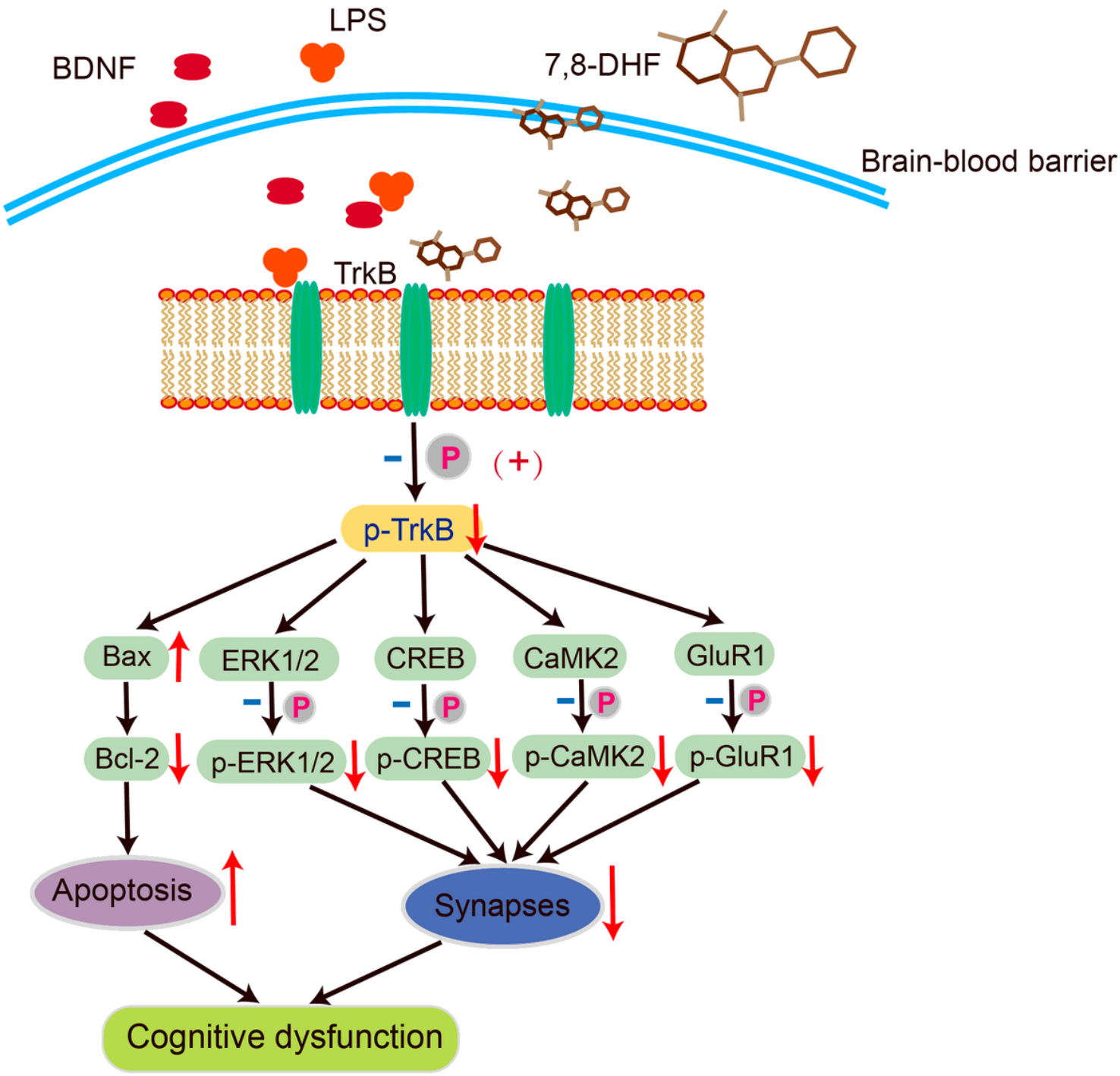

Figure 9

The BDNF-TrkB signaling pathway and its downstream cascade disorders participated in learning and memory impairments. The BDNF-TrkB signaling pathway and its downstream cascades are involved in the synthesis of synapses which then regulates learning and memory. Intraperitoneal injection of LPS

damages the BDNF-TrkB signaling pathway and its downstream cascade leading to cognitive dysfunction. 7,8-DHF can penetrate the blood-brain barrier and activate TrkB receptors, alleviating learning and memory deficits.