NEUROSCIENCE
RESEARCH ARTICLE
W. Zhang et al. / Neuroscience 505 (2022) 21–33

Dysfunction of the Brain-derived Neurotrophic Factor-Tyrosine Kinase B Signaling Pathway Contributes to Learning and Memory Impairments Induced by Neuroinflammation in Mice

Wen Zhang,[a] Meng-meng Ge,[a] Long-Qing Zhang,[a] Xiao-Man Yuan,[a] Si-yi Han,[a] Anne Manyande,[b] Yu-Ke Tian[a] and Xue-Bi Tian[a]*

[a] *Department of Anesthesiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China*

[b] *School of Human and Social Sciences, University of West London, London W5 5RF, United Kingdom*

Abstract—Accumulating evidence suggests that neuroinflammation is the main mechanism in cognitive dysfunction and that brain-derived neurotrophic factor (BDNF) is involved in learning and memory by binding to tyrosine kinase B (TrkB) receptors. Herein, we tested the roles of the BDNF-TrkB signaling pathway and its downstream cascade in lipopolysaccharide (LPS) induced cognitive dysfunction in mice. Mice were treated with LPS (0.25 mg/kg) for 7 days, and learning and memory function was evaluated by the novel object recognition test (NORT). Western blotting was performed to elucidate roles of the BDNF-TrkB signaling pathway and its downstream cascades in LPS mice. The NORT showed that LPS induced learning and memory deficits in mice. The levels of IL-1β, IL-6, and TNF-α in the serum and central nervous system decreased in LPS mice. In addition, LPS reduced the 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 hippocampus and medial prefrontal cortex regions. In the entorhinal cortex, the protein levels of BDNF, p-TrkB, Bcl-2, p-CaMK2 and p-CREB were decreased, and the protein level of Bax was increased in LPS mice. Interestingly, 7,8-DHF alleviated these disorders in LPS mice and improved learning and memory function; however, the TrkB antagonist ANA12 effectively reversed effects of 7,8-DHF. Therefore, we conclude that the BDNF-TrkB signaling pathway and its downstream cascades disorders in different regions are main mechanisms of cognitive dysfunction, and 7,8-DHF maybe useful as a new treatment for preventing or treating cognitive dysfunction induced by neuroinflammation in neurodegenerative diseases.

Key words: brain-derived neurotrophic factor, tyrosine kinase B, learning and memory impairment, lipopolysaccharide, 7,8-dihydroxyflavone.

INTRODUCTION

Numerous studies have revealed that lipopolysaccharide can trigger systemic inflammation by activating Toll-like receptors. Systemic inflammation often promotes central neuroinflammation by upregulating proinflammatory cytokines, such as IL-6 and TNF-α, which increase the permeability of the blood–brain barrier and further cause neuroinflammation in the central nervous system including the hippocampus (Zhan et al., 2018; Adetuyi and Farombi, 2021). This further activates the inflammatory signaling pathway and oxidative stress, which trigger synaptic plasticity changes and neuronal apoptosis, ultimately leading to cognitive dysfunction and neurodegenerative diseases (Zhang et al., 2015, 2020a; Zhao et al., 2019; Li et al., 2020). For example, systemic infection, which occurs with surgery (Wan et al., 2007; Cibelli et al., 2010), can trigger systemic and hippocampal inflammation, resulting in cognitive decline (Barrientos et al., 2009).

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic growth factors that participates in mediating synaptic plasticity and neuronal survival, differentiation, and neurogenesis (Leal et al., 2017; von Bohlen und Halbach and von Bohlen und Halbach, 2018). BDNF exerts its biological functions mainly through binding to tyrosine kinase B (TrkB) receptors (Bekinschtein et al., 2014; Lu et al., 2014), and the BDNF-TrkB signaling pathway is

*Corresponding author.
E-mail addresses: wenzhang@tjh.tjmu.edu.cn (W. Zhang), 962697903@qq.com (M.-m. Ge), 18270881093@163.com (L.-Q. Zhang), yxmyyxjy@163.com (X.-M. Yuan), 1326715355@qq.com (S.-y. Han), Anne.Manyande2@uwl.ac.uk (A. Manyande), yktian@tjh.tjmu.edu.cn (Y.-K. Tian), tianxb@hust.edu.cn (X.-B. Tian).

Abbreviations: BDNF, Brain-derived neurotrophic factor; TrkB, Tyrosine kinase B; LPS, Lipopolysaccharide; NORT, Novel object recognition test; 7,8-DHF, 7,8- Dihydroxyflavone; ELISA, Enzyme-linked immunosorbent assay; p75NTR, p75 neurotrophin receptor; EC, Entorhinal cortex; mPFC, Medial prefrontal cortex.

https://doi.org/10.1016/j.neuroscience.2022.10.003
0306-4522/

involved in the formation of dendritic spines. Furthermore, phosphorylation of TrkB receptors promotes activation of downstream cascades, including ERK1/2, CaMK2, CREB, GluR1 and the mechanism of apoptosis, which are related to regulating learning and memory (Yang et al., 2014). ERK1/2, a member of the mitogen-activated protein kinase superfamily, is involved in regulating cell proliferation, survival, and apoptosis (Morella et al., 2020). CaMK2 is essential for synaptic plasticity and memory formation (Kool et al., 2019). CREB is one of the main downstream transcription factors of ERK1/2 and plays significant roles in neuronal plasticity, learning and memory (Zhang et al., 2020c). GluR1 is also necessary for learning and memory processing and mediates neuronal plasticity (Xiao et al., 2019). Therefore, the reduction in BDNF expression in the brain is associated with impairment of synaptic plasticity and learning and memory failures.

7,8-Dihydroxyflavone (7,8-DHF), a flavonoid derivative, has been identified as an effective agonist of the TrkB receptor that mimics the properties of BDNF (Bollen et al., 2013; Castello et al., 2014; Zhang et al., 2014b). 7,8-DHF can penetrate the blood–brain barrier and plays neuroprotective roles, including increasing dendritic spine density and exerting neurotrophic effects, by activating the BDNF-TrkB signaling pathway and its downstream cascades. ANA12 is a small molecule antagonist of TrkB that crosses the blood–brain barrier to exert its antagonistic effect (Giuliani, 2019). Here, we hypothesized that disorders of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus, medial prefrontal cortex (mPFC) and entorhinal cortex (EC) regions of mice contribute to learning and memory dysfunction induced by LPS.

EXPERIMENTAL PROCEDURES

Animals

In total, 124 male C57BL/6J mice (8 weeks old) were obtained from the Experimental Animal Center of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Mice were raised five per cage under a 12 hour light/dark cycle and given water and food ad libitum. All animal studies were approved by the Experimental Animal Committee of Tongji Hospital 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: the control group, lipopolysaccharide (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) was injected 30 minutes before LPS treatment. In the LPS + 7,8-DHF + ANA12 group, ANA12 (0.5 mg/kg) was injected 30 minutes before 7,8-DHF and LPS. The novel object recognition test was performed to evaluate cognitive function 7 days after treatment.

Novel object recognition test

Seven days after LPS treatment, the novel object recognition test (NORT) was applied to examine cognitive function in mice (Zhang et al., 2020b). Mice were handled 6 days before the test, for 1 minute a day to alleviate stress. Then, they were placed in the box for 5 minutes to acclimatize. Twenty-four hours later, the 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 the mice explored for another 5 minutes. Exploratory behaviours included sniffing, licking and climbing. The discrimination ratio was used to assess learning and memory.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected and centrifuged at 3000*g* for 10 minutes at 4 °C to obtain supernatants. The hippocampus, mPFC and EC were homogenized with saline, and the homogenate was centrifuged at 2500*g* for 10 minutes at 4 °C to obtain the supernatants. The expression levels of IL-6, IL-1β, and TNF-α were determined using commercially available ELISA kits (MD123475, MD6757, MD7125; MDL Biotech, Beijing, China). Concentrations of inflammatory factors were tested according to the manufacturer's instructions and presented as pg/ml of serum protein.

Western blot

Hippocampal tissues were prepared as previously described (Zhang et al., 2020b) and homogenized with radioimmunoprecipitation assay lysis buffer containing protease and phosphatase inhibitors. A commercially available BCA protein assay kit (AR0146, Boster, Wuhan, China) was used to determine the protein concentrations. Then, protein samples were separated using 10% SDS–PAGE and transferred to polyvinylidene fluoride membranes (IPVH00010, 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 membranes were then washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 hours. The primary antibodies used in this research included rabbit anti-BDNF (1:1000; Abclonal, #A11028, Wuhan, China), anti-TrkB (1:1000; Abclonal, #A2099, Wuhan, China), anti-p-TrkB (1:500; Abclonal, #AP0423, Wuhan, China), anti-Bax (1:1000; Abclonal, #A19684, Wuhan, China), anti-Bcl-2 (1:1000; Abclonal, #A19693, Wuhan, China), anti-ERK1/2 (1:1000; Cell Signaling Technology, #9102S, Boston, United States), anti-p-ERK1/2 (1:1000; Cell Signaling Technology, #4370S, Boston, United States), anti-CaMK2 (1:1000; Proteintech, #13730-1-AP, Chicago, United States), anti-p-CaMK2 (1:500; Abclonal, #AP0255, Wuhan, China), anti-CREB (1:1000; Abclonal, #A11064, Wuhan, China), anti-p-CREB (1:1000; Cell Signaling Technology, #9198S, Boston, United States), anti-GluR1 (1:1000; Abclonal, #A7677, Wuhan, China), anti-p-GluR1 (1:500; Abclonal, #AP0356, Wuhan, China), anti-GAPDH (1:1000; Abclo-

nal, #A19056, Wuhan, China), and goat anti-rabbit secondary antibody (1:5000; Abclonal, #AS014, Wuhan, China). 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 analysed using GraphPad Prism version 7.0 and are presented as the means ± SEM. After tests for normality and equal variances, an *F*-test was used to test the statistical significance of the overall relationship between dependent variable and the set of independent variables. For two groups, unpaired Student's *t* test was applied, and comparisons among groups were performed using the one-way ANOVA or two-way ANOVA, followed by Fisher's LSD test or *post-hoc* Tukey test. $P < 0.05$ was considered statistically significant.

RESULTS

Lipopolysaccharide results in learning and memory impairment in mice

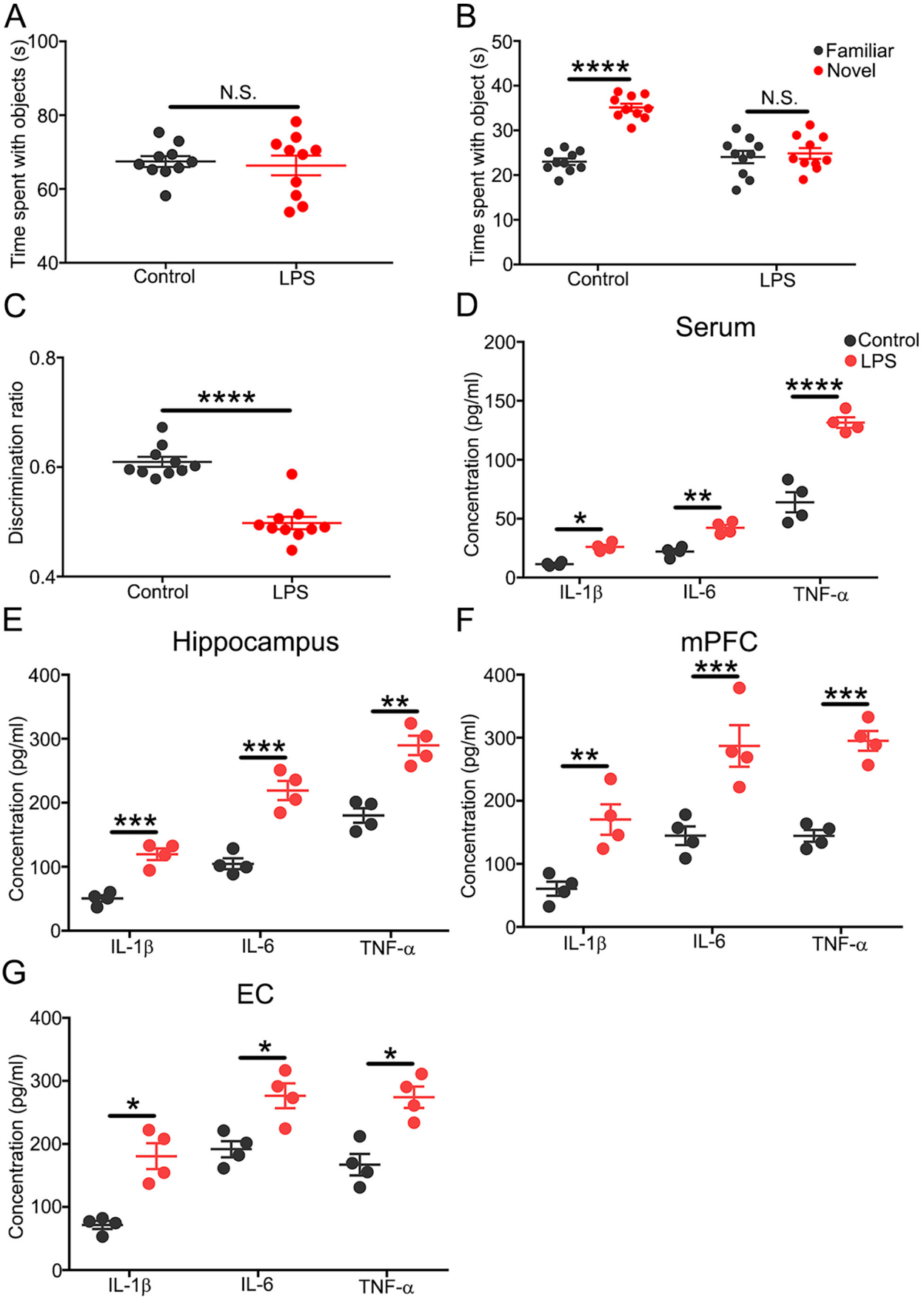
The NORT is widely used to evaluate learning and memory function in mice (Zhang et at., 2020; Zurek et al., 2012). In this study, no significant difference was found in the time spent with objects between the control and LPS mice (Fig. 1A, $t = 0.346$, $P = 0.7333$, Student's *t* test, $n = 10$). In the testing stage, more time was spent on the novel object than on the familiar object in Control mice, but there was no statistically significant difference in LPS mice (Fig. 1B, $F_{(1,9)} = 58.41$, $P < 0.0001$, two-way ANOVA, followed by *pos-hoc* Turkey test, $n = 10$). The result of the discrimination ratio was similar to that in Fig. 1B, which shows that control mice had a greater discrimination ratio than LPS mice (Fig. 1C, $t = 7.697$, $P < 0.0001$, Student's *t* test, $n = 10$). Then, we extracted serum and tissue samples from the hippocampus, mPFC and EC to detect the concentrations of IL-6, IL-1β and TNF-α after LPS treatment. The results showed that the levels of IL-6, IL-1β and TNF-α increased in the LPS group (Fig. 1D, $F_{(2,12)} = 189.8$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 1E, $F_{(2,12)} = 89.1$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 1F, $F_{(2,12)} = 21.87$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 1G, $F_{(2,12)} = 33.37$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$). These findings demonstrate that administration of LPS results in neuroinflammation, which further induces learning and memory impairment in mice.

Lipopolysaccharide reduces the expression levels of the BDNF-TrkB signaling pathway and its downstream cascades in the brains of mice

Then, we detected the protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus, mPFC and EC using western blotting after NORT. The results showed that the protein levels of BDNF and p-TrkB were lower in LPS-treated mice than in control mice (Fig. 2A, $F_{(2,12)} = 9.709$, $P = 0.0031$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 3A, $F_{(2,12)} = 40.96$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 4A, $F_{(2,12)} = 10.17$, $P = 0.0026$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$). No significant difference was found in TrkB protein levels between control and LPS mice. Then, we evaluated the levels of apoptosis-related proteins. Compared with control mice, the level of the proapoptotic protein Bax was distinctly increased, while the level of the antiapoptotic protein Bcl-2 was decreased in LPS mice (Fig. 2B, $F_{(1,6)} = 61.64$, $P = 0.0002$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 3B, $F_{(1,6)} = 56.07$, $P = 0.0003$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 4B, $F_{(1,6)} = 96.59$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$). In addition, the expression levels of downstream components of the BDNF-TrkB signaling pathway were tested. We observed that the protein levels of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 clearly declined in the hippocampus and mPFC of LPS mice (Fig. 2C, $F_{(7,42)} = 12.39$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$; Fig. 3C, $F_{(7,42)} = 7.451$, $P < 0.0001$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$). However, no difference was found in the protein levels of ERK1/2, CaMK2, CREB or GluR1 in the hippocampus and mPFC. Furthermore, in the EC, no statistically significant difference was observed in the protein levels of ERK1/2, p-ERK1/2, GluR1 or p-GluR1 between control and LPS mice (Fig. 4C, $F_{(7.42)} = 4.838$, $P = 0.0005$, two-way ANOVA, followed by Fisher's LSD test, $n = 4$). The expression levels of p-CaMK2 and p-CREB in the EC were markedly decreased after LPS administration, but no difference was shown in the levels of CaMK2 or CREB. These results demonstrate that the hippocampus, mPFC and EC participate in learning and memory dysfunction induced by LPS and that disorders of the BDNF-TrkB signaling pathway and its downstream cascades are the main mechanism of the pathological process.

7,8-DHF ameliorates lipopolysaccharide-induced learning and memory deficits in mice

7,8-DHF was used to further investigate the roles of the BDNF-TrkB signaling pathway in learning and memory (Bollen et al., 2013). ANA12 is a small-molecule TrkB antagonist (Cazorla et al., 2011) that was used to identify whether 7,8-DHF could reverse learning and memory dysfunction. The results of the NORT showed that there were no statistically significant changes in the time spent with identical objects during the training stage (Fig. 5A, $F_{(2.093,18.82)} = 1.044$, $P = 0.3745$, one-way ANOVA, followed by *pos-hoc* Turkey test, $n = 10$). In the testing stage, administration of 7,8-DHF increased the time spent on the novel object in LPS mice, but ANA12 reversed the effect of 7,8-DHF in LPS mice (Fig. 5B, $F_{(3,54)} = 18.37$, $P < 0.0001$, two-way ANOVA, followed by *pos-hoc* Turkey test, $n = 10$). Further analysis of the discrimination ratio is


A
Time spent with objects (s)
100
80
60
40
N.S.
Control
LPS
B
Time spent with object (s)
50
40
30
20
10
0
Familiar
Novel
****
N.S.
Control
LPS
C
Discrimination ratio
0.8
0.6
0.4
****
Control
LPS
D
Serum
Concentration (pg/ml)
200
150
100
50
0
Control
LPS
*
**
****
IL-1β
IL-6
TNF-α
E
Hippocampus
Concentration (pg/ml)
400
300
200
100
0
***
***
**
IL-1β
IL-6
TNF-α
F
mPFC
Concentration (pg/ml)
400
300
200
100
0
**
***
***
IL-1β
IL-6
TNF-α
G
EC
Concentration (pg/ml)
400
300
200
100
0
*
*
*
IL-1β
IL-6
TNF-α

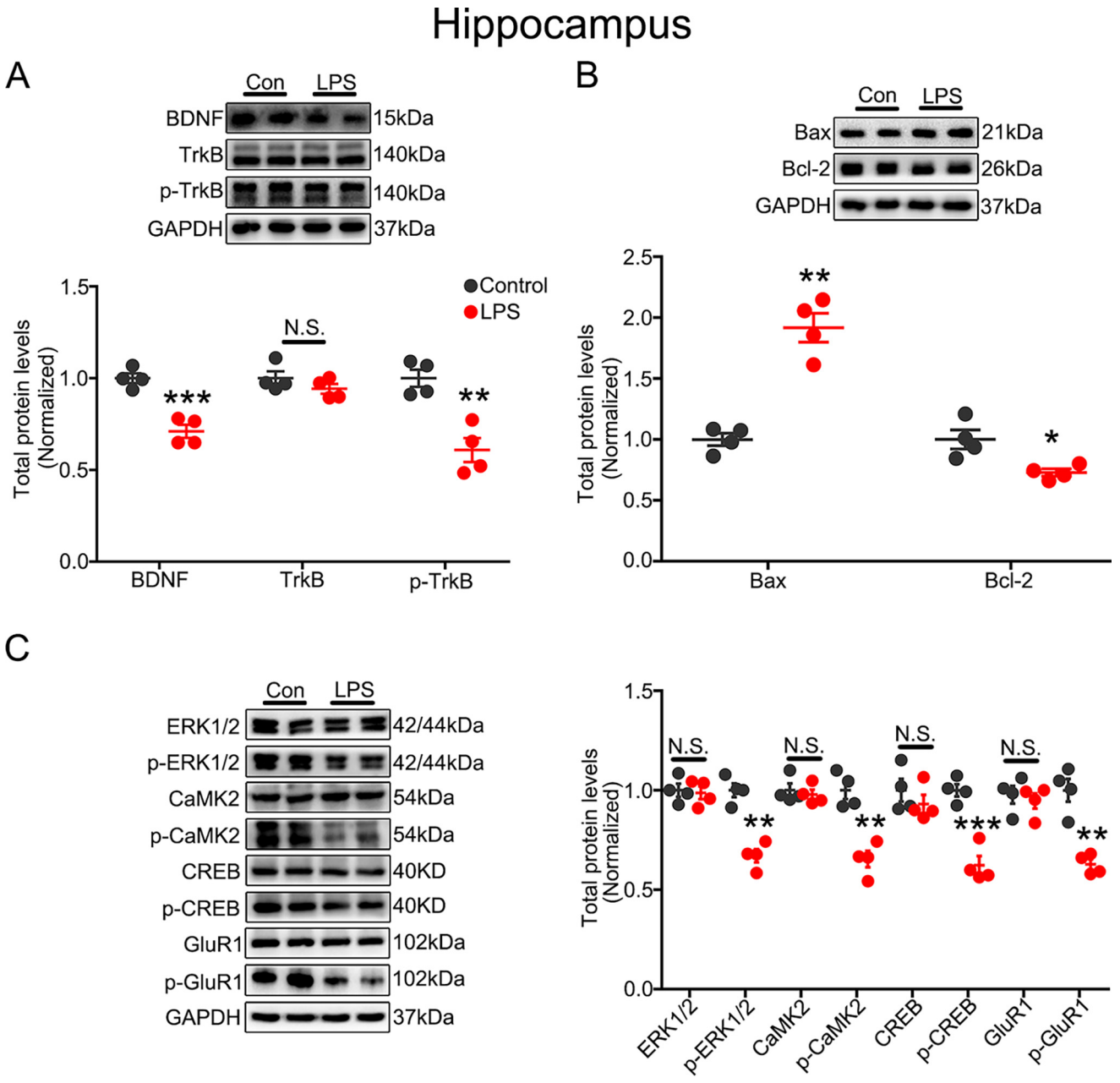


Fig. 2. The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus. (A) Compared with control mice, the levels of BDNF and p-TrkB proteins decreased in LPS mice, and no difference was found in the expression of TrkB (n = 4). **(B)** The protein level of Bax increased and the level of Bcl-2 was reduced in the LPS group compared to the control group (n = 4). **(C)** The protein expression of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice was lower than that in control mice. There was no significant difference in the levels of ERK1/2, CaMK2, CREB or GluR1 proteins between the control and LPS groups (n = 4). Data are presented as the means ± SEM. Two-way ANOVA, followed by Fisher's LSD test; *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 0.05; n = 4.

in line with the result of time spent on the novel object. 7,8-DHF upregulated the discrimination ratio in LPS + 7,8-DHF mice; however, the discrimination ratio decreased in LPS + 7,8-DHF + ANA12 mice (Fig. 5C, $F_{(2.057,18.51)}$ = 38.9, P < 0.0001, one-way ANOVA, followed by *pos-hoc* Turkey test, n = 10). These results demonstrate that preventive administration of 7,8-DHF could effectively alleviate learning and memory deficits caused by LPS, while ANA12 reversed these therapeutic effects of 7,8-DHF.

Fig. 1. Behavioural tests and proinflammatory levels in LPS mice. (A–C) There was no significant difference in the time spent exploring the same objects between 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 a lower discrimination ratio than the control group (n = 10). **(D–G)** Compared to control mice, the levels of IL-1β, IL-6 and TNF-α in the serum, hippocampus, mPFC and EC were significantly increased in LPS mice (n = 4). Data are presented as the means ± SEM. Student's t test; two-way ANOVA, followed by *pos-hoc* Turkey test or two-way ANOVA, followed by Fisher's LSD test; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; N.S. P > 0.05; n = 4–10.

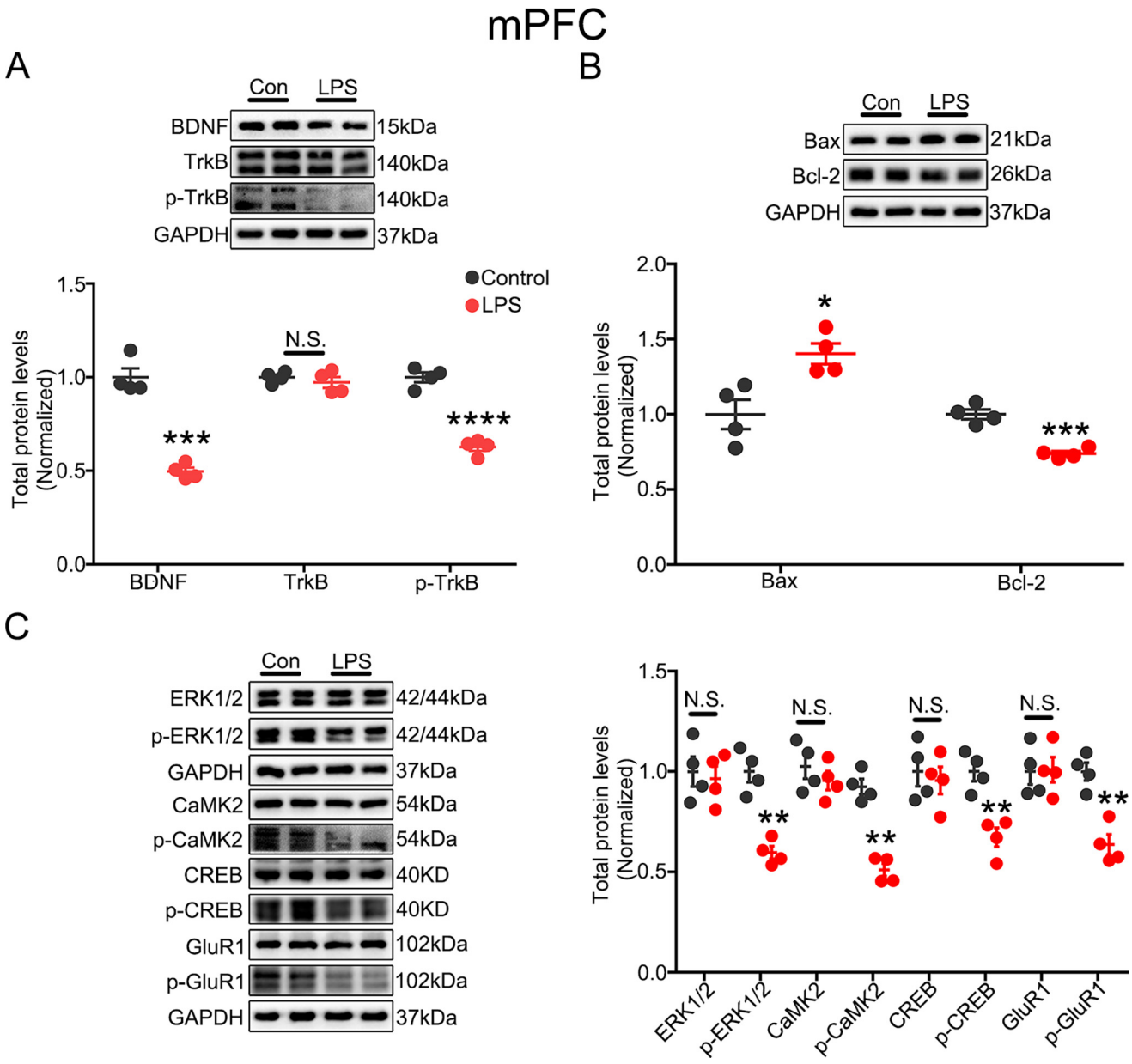


Fig. 3. The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC. (A) The protein levels of BDNF and p-TrkB were reduced in LPS mice compared to control mice, and no difference was found in the level of TrkB between these two groups (n = 4). **(B)** Compared with the control group, the expression of Bax increased and the level of Bcl-2 decreased in the LPS group (n = 4). **(C)** The expressions of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice were downregulated. There was no significant difference in the levels of ERK1/2, CaMK2, CREB or GluR1 proteins between the control and LPS groups (n = 4). Data are presented as the means ± SEM. Two-way ANOVA, followed by Fisher's LSD test; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; N.S. P > 0.05; n = 4.

7,8-DHF ameliorates lipopolysaccharide-induced dysfunctions of the BDNF-TrkB signaling pathway and its downstream cascades in LPS mice

Next, the hippocampus, mPFC and EC were observed to examine changes in protein levels after administration of 7,8-HDF. Western blot results showed that 7,8-DHF increased the expression levels of p-TrkB, Bcl-2, p-ERK1/2, p-CaMK2, p-CREB, and p-GluR1 and decreased the protein level of Bax in the hippocampus and mPFC of LPS mice (Fig. 6A, $F_{(1,16)}$ = 20.04, P = 0.0004, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 6B, $F_{(1,16)}$ = 53.1, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 6C, $F_{(3,48)}$ = 4.553, P = 0.0069, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 7A, $F_{(1,16)}$ = 37.29, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 7B, $F_{(1,16)}$ = 141, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 7C, $F_{(3,48)}$ = 5.811, P = 0.0018, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5). However, the effects of 7,8-DHF were reversed by ANA12 in LPS + 7,8-DHF + ANA12 mice. Interestingly, there were no significant changes in the level of BDNF after administering 7,8-DHF or 7,8-DHF + ANA12 in LPS mice. Furthermore, no

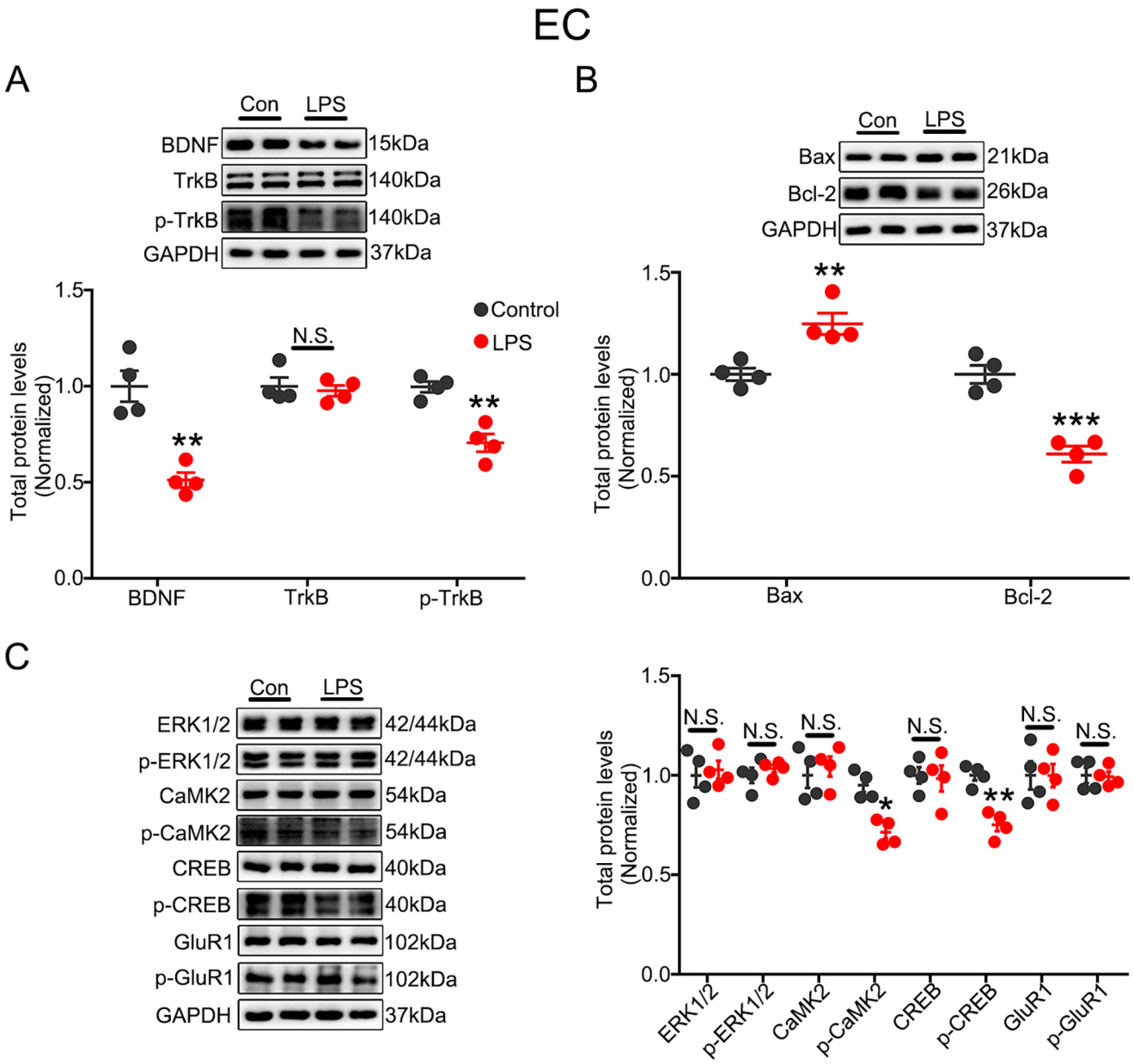


Fig. 4. The protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in the EC. (A) The expressions of BDNF and p-TrkB were decreased in LPS mice compared to control mice, and no significant difference was observed in the expression of TrkB between these two groups (n = 4). **(B)** The level of Bax was upregulated and the level of Bcl-2 was decreased in the LPS group compared to the control group (n = 4). **(C)** The expressions of p-CaMK2 and p-CREB were decreased in LPS mice. There was no significant difference in the protein levels of ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 or p-GluR1 between the control and LPS groups (n = 4). Data are presented as the means ± SEM. Two-way ANOVA, followed by Fisher's LSD test; *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 0.05; n = 4.

statistically significant difference was observed in the expression levels of TrkB, ERK1/2, CaMK2, CREB or GluR1 (Fig. 6D, $F_{(4,64)}$ = 0.1849, P = 0.9454, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 7D, $F_{(4,64)}$ = 0.117, P = 0.9760, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5). In addition, in the EC, a qualitative increase in the expression levels of p-TrkB, Bcl-2, p-CaMK2, and p-CREB and a reduction in Bax protein were observed after administration of 7,8-DHF (Fig. 8A, $F_{(1,16)}$ = 65.81, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 8B, $F_{(1,16)}$ = 113.2, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5; Fig. 8C, $F_{(1,16)}$ = 56.98, P < 0.0001, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5). However, the effects of 7,8-DHF were reversed by ANA12. Consistent with previous results, 7,8-DHF and ANA12 had no effect on the expression level of BDNF protein in the EC of LPS mice. Moreover, there was no significant difference in the protein expression levels of TrkB, ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 or p-GluR1 in the EC (Fig. 8D, $F_{(5,80)}$ = 0.8106, P = 0.5455, two-way ANOVA, followed by *pos-hoc* Turkey test, n = 5). These results demonstrate that 7,8-DHF effectively

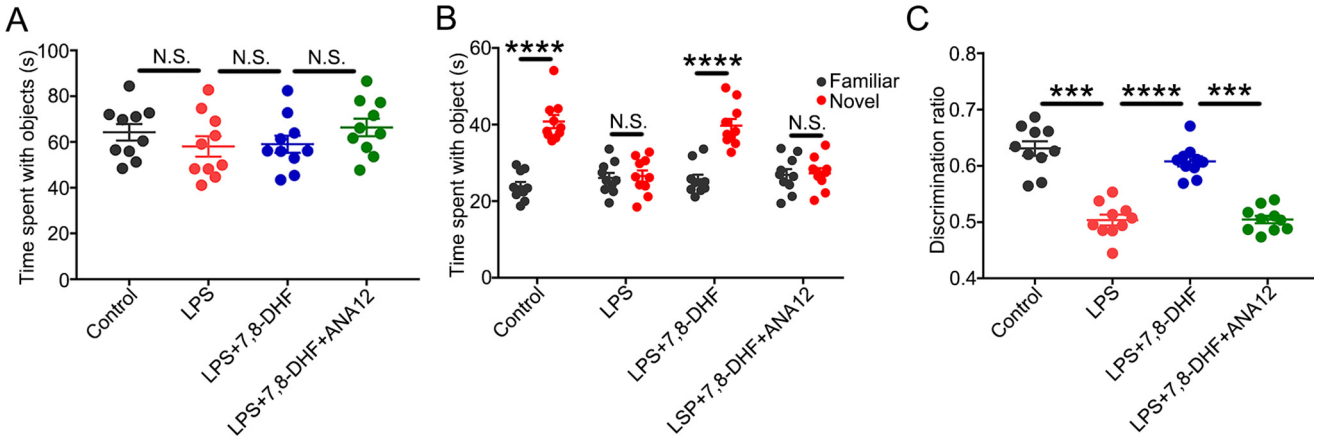


Fig. 5. 7,8-DHF alleviates LPS-induced learning and memory deficits in mice. (A–C) During the training phase, there was no significant difference in the time spent with objects after using 7,8-DHF or ANA12 in LPS mice. In the testing phase, the time spent on the novel object and the discrimination ratio 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 the means ± SEM. One-way ANOVA, followed by *pos-hoc* Turkey test or two-way ANOVA, followed by *pos-hoc* Turkey test; ***$P < 0.001$; ****$P < 0.0001$; N.S. $P > 0.05$; $n = 10$.

alleviated disorders of the BDNF-TrkB signaling pathway and its downstream cascades in LPS mice, but ANA12 completely reversed the therapeutic effects of 7,8-DHF.

DISCUSSION

In this study, we found that disorders of the BDNF-TrkB signaling pathway and its downstream cascades in mice participated in learning and memory impairments induced by neuroinflammation, which mainly manifested as reductions in the levels of 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.

BDNF is the main neurotrophin in the brain (von Bohlen und Halbach and von Bohlen und Halbach, 2018). Many researchers have reported that BDNF has important effects on synapses, including structural and functional roles in many brain regions (Bekinschtein et al., 2014; Lu et al., 2014). The different effects of BDNF on the brain are related to its diverse forms and downstream receptors to which it binds, such as pro-BDNF and mature BDNF with the p75 neurotrophin receptor (p75NTR) and TrkB, which have opposing effects on synapses (Fobian et al., 2009; Kowiański et al., 2017; Sasi et al., 2017). In our study, we mainly discuss the roles of mature BDNF and TrkB receptors on learning and memory deficits induced by neuroinflammation. The expressions of BDNF and p-TrkB receptors were reduced in the mPFC, hippocampus and EC after application of LPS in mice. LPS induced elevations of IL-1β, IL-6, TNF-α and iNOS by activating the Toll-like receptor 4, which further reduced the expressions of BDNF protein and TrkB phosphorylation.

In addition, different ways of administering BDNF induced different changes in functions and structures (Ji et al., 2010). For example, acute upregulation of BDNF initiated neurite elongation and spine head enlargement, but a gradual increase in BDNF potentiated dendritic branching and filopodia-like spines. Furthermore, transient activation of TrkB may promote synaptic transmission in the brain. Since BDNF does not cross the blood–brain barrier, we used the TrkB agonist 7,8-DHF to simulate the 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 increased after administration of 7,8-DHF. However, the application of the TrkB antagonist ANA12 completely reversed the therapeutic effects of 7,8-DHF, which further indicates that reductions in the BDNF-TrkB signaling pathway are associated with learning and memory impairments induced by neuroinflammation in mice. Interestingly, Zhang and colleagues found that the expression of BDNF protein increased in the nucleus accumbens of LPS mice, which is similar to a report that showed that direct infusion of ANA-12 into the nucleus accumbens completely blocked the ability of phasic stimulation to induce social avoidance (Zhang et al., 2014a). These differences may be due to the different roles of BDNF in depression-like behaviour.

Increasing evidence suggests that BDNF exerts its neuroprotective effects by suppressing excitotoxicity of NMDA receptors, promoting regeneration of synapses and inhibiting cell apoptosis (Yamada and Nabeshima, 2004; Ren and Dubner, 2007; Miranda et al., 2018). Bax is a proapoptotic protein that accelerates cell loss, including neurons, and is involved in learning and memory disorders (Sun et al., 2021). Bcl-2 is an antiapoptotic protein that antagonizes cell apoptosis. In our study, neuroinflammation reduced the protein expression levels of BDNF and p-TrkB, which disrupted the balance of Bax and Bcl-2, eventually leading to cell apoptosis in the mPFC, hippocampus and EC regions. ERK1/2 and CREB play vital roles in learning and memory in the brain and are involved in regulating transcription factors and promoting protein synthesis (Cao et al., 2013; Zheng et al., 2020). In this study, the expression of p-ERK1/2 and p-CREB decreased in the mPFC and hippocampus of LPS mice,

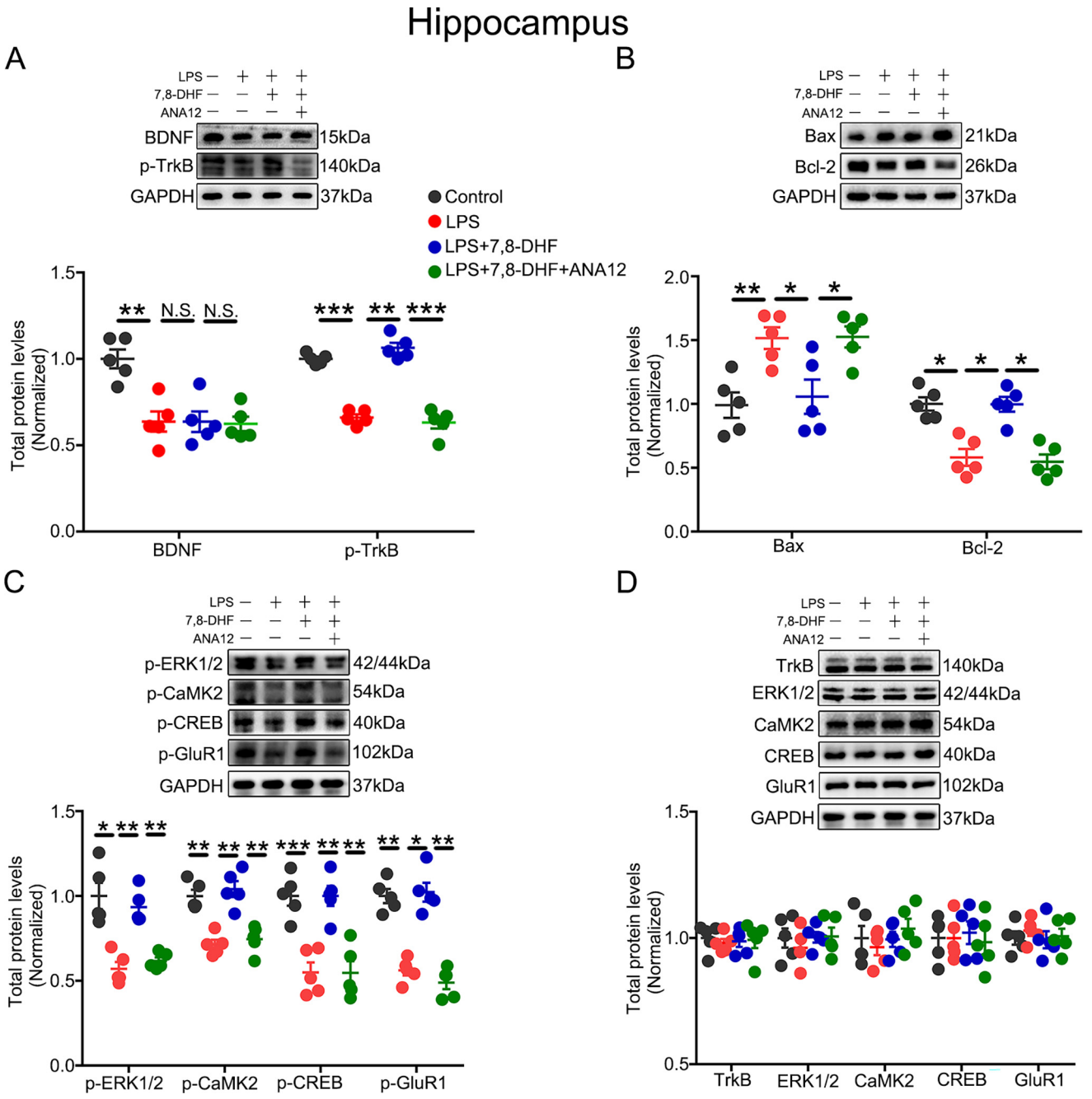


Fig. 6. 7,8-DHF restores LPS-induced disorders of the BDNF-TrkB signaling pathway and its downstream cascades in the hippocampus. **(A)** There were no significant changes in the expression of BDNF in LPS mice after treatment with 7,8-DHF or ANA12. The expression of p-TrkB increased in LPS mice after administration of 7,8-DHF; however, ANA12 reversed the effects of 7,8-DHF (n = 5). (B) 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). **(C)** The expressions of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice increased after 7,8-DHF treatment; however, ANA12 reversed the effects of 7,8-DHF (n = 5). **(D)** No significant differences were observed in the protein levels of TrkB, ERK1/2, CaMK2, CREB or GluR1 in the LPS groups after 7,8-DHF or ANA12 treatment (n = 5). Data are presented as the means ± SEM. Two-way ANOVA, followed by *pos-hoc* Turkey test; *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 0.05; n = 5.

while only the level of p-CREB was downregulated in the EC, 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 (Sanderson et al., 2008; Vigil and Giese, 2018). We found that neuroinflammation reduced the expressions of p-CaMK2 and p-GluR1 both in the mPFC and hippocampus, while in the EC, only the level of p-CaMK2 decreased. These differences between the EC and 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 (Opitz, 2014; Tanimizu et al., 2017). Researchers have

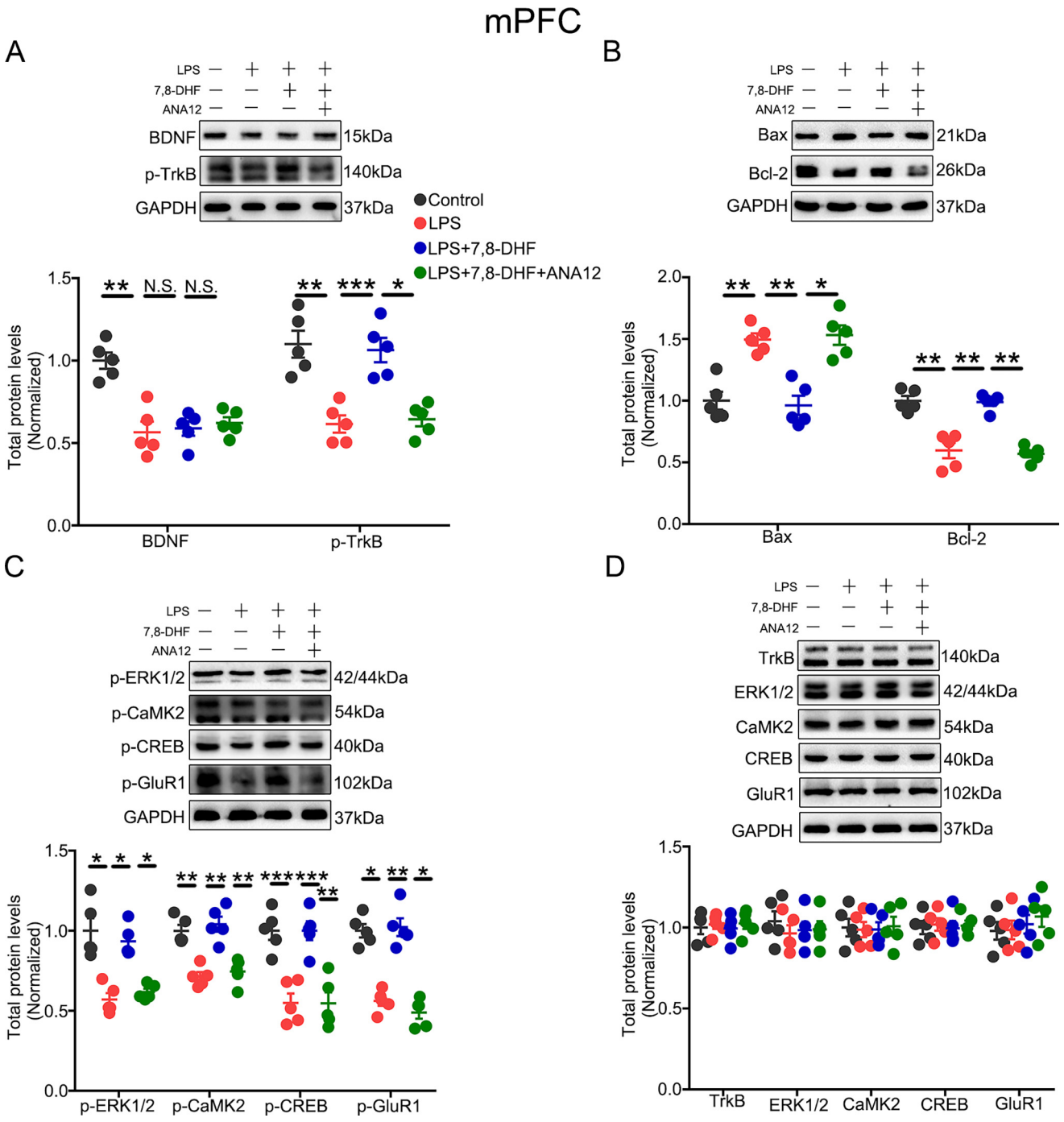


Fig. 7. 7,8-DHF restores LPS-induced disoeders of the BDNF-TrkB signaling pathway and its downstream cascades in the mPFC. (A) There were no significant differences in the protein levels of BDNF in LPS mice after administration of 7,8-DHF or ANA12. The level of p-TrkB increased in LPS mice after 7,8-DHF treatment, however, ANA12 reversed the effects of 7,8-DHF (n = 5). **(B)** The level of Bax declined and the level of Bcl-2 increased in LPS mice after administration of 7,8-DHF, while ANA12 completely reversed these changes (n = 5). **(C)** The levels of p-ERK1/2, p-CaMK2, p-CREB and p-GluR1 in LPS mice increased after administration of 7,8-DHF; however, ANA12 reversed the effects of 7,8-DHF (n = 5). **(D)** There were no significant differences in the protein levels of TrkB, ERK1/2, CaMK2, CREB or GluR1 in the LPS groups after administration of 7,8-DHF or ANA12 (n = 5). Data are presented as the means ± SEM. Two-way ANOVA, followed by *pos-hoc* Turkey test; *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 00.5; n = 5.

demonstrated that the mPFC is the most vulnerable among them, with the hippocampus moderately vulnerable and the EC relatively less susceptible to neuroinflammation (Maiti et al., 2008). However, the volume change in the EC is used as the earliest indicator to evaluate preclinical cognitive deficits leading to the development of dementia (Rodrigue and Raz, 2004). The hippocampus is responsible for storing information and is a key region engaged in learning and memory. The mPFC has been shown to play a vital role in the process of attention, behavioural flexibility, social and emotional behaviours, and its interactions with the hippocampus are implicated

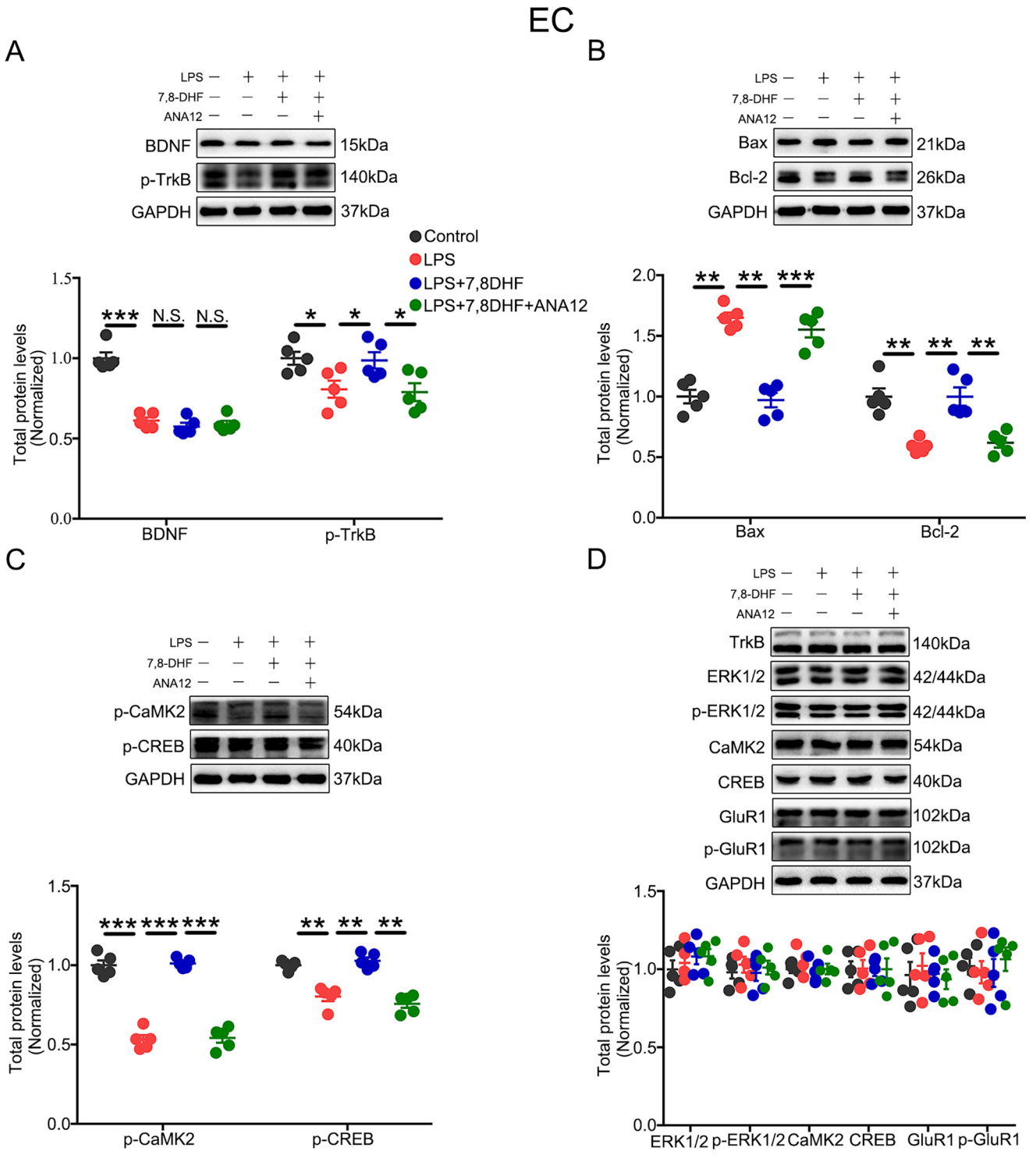


Fig. 8. 7,8-DHF restores LPS-induced disorders of the BDNF-TrkB signaling pathway and its downstream cascades in the EC. (A) No significant difference was observed in the expression of BDNF in LPS mice after treatment with 7,8-DHF or ANA12. 7,8-DHF clearly increased the expression of p-TrkB in LPS mice, while ANA12 reversed the therapeutic effects of 7,8-DHF (n = 5). **(B)** 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). **(C)** The expressions of p-CaMK2 and p-CREB increased in LPS mice after administration of 7.8-HDF, while ANA12 completely reversed the effects of 7,8-DHF (n = 5). **(D)** There was no significant difference in the protein levels of TrkB, ERK1/2, p-ERK1/2, CaMK2, CREB, GluR1 or p-GluR1 in the LPS groups after treatment with 7,8-DHF or ANA12 (n = 5). Data are presented as the means ± SEM. Two-way ANOVA, followed by *pos-hoc* Turkey test; *P < 0.05; **P < 0.01; ***P < 0.001; N.S. P > 00.5; n = 5.

in the regulation of learning and memory (Euston David et al., 2012). EC is also related to spatial and long-term memory (Garcia and Buffalo, 2020). Neural projections from the mPFC or EC to the hippocampus are known to be involved in the modulation of learning and memory processes (Ladurelle et al., 2011; Vertes, 2015; Chao et al.,

2020). 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 (Yang et al., 2018). In addition, electrical stimulation of the EC alleviated spatial memory deficits in Alzheimer's disease mice infused with amyloid peptides 1–42 into the hippocampus, which suggests that there is functional projection between the EC and hippocampus. However, it is unclear whether the mutual projection of the mPFC, hippocampus and EC regions and neuroregulation are associated with metabolic changes in the BDNF-TrkB signaling pathway and its downstream cascades.

There are several limitations in this study. On the one hand, we only tested the protein levels of the BDNF-TrkB signaling pathway and its downstream cascades in different brain regions, and no synaptic-related proteins were detected that directly indicated synaptic damage. On the other hand, whether the changes in the BDNF-TrkB signaling pathway and its downstream cascadess among the mPFC, hippocampus and EC regions are regulated by the projection of nerve fibres between these regions has not been confirmed and deserves further investigation.

In summary, our research revealed that the BDNF-TrkB signaling pathway and its downstream cascades disorders participated in learning and memory impairments induced by neuroinflammation in mice (graphical abstract). The hippocampus, mPFC and EC regions play crucial roles in learning and memory. Intraperitoneal injection of the TrkB agonist 7,8-DHF effectively alleviated cognitive dysfunction in LPS mice. As a result, the BDNF-TrkB signaling pathway and its downstream cascades disorders in different regions 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.

ACKNOWLEDGEMENTS

The authors would like to thank Professor Yuke Tian for providing a critical appraisal of the study.

FUNDING

This work was financially supported by grants from the National Natural ScienceFoundation of China 81571053 and 81974170.

AVAILABILITY OF DATA AND MATERIALS.

The database used and/or analysed during this study is available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

ZW conceived the research, carried out the model building, performed the Western blot, coordinated the lab's work and drafted the manuscript. GMM and ZLQ performed the statistical analysis and drafted the manuscript. YXM and HSY implemented the NORT and drafted the manuscript. AM, TXB and TYK participated in the study design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All protocols were approved by the Ethics Committee for Experimental Animal Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (approval no. TJH-202007008). All procedures using mice were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Adetuyi BO, Farombi EO (2021) 6-Gingerol, an active constituent of ginger, attenuates lipopolysaccharide-induced oxidation, inflammation, cognitive deficits, neuroplasticity, and amyloidogenesis in rat. J Food Biochem:45.

Barrientos RM, Frank MG, Hein AM, Higgins EA, Watkins LR, Rudy JW, Maier SF (2009) Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection. Brain Behav Immun 23:46–54.

Bekinschtein P, Cammarota M, Medina JH (2014) BDNF and memory processing. Neuropharmacology 76(Pt C):677–683.

Bollen E, Vanmierlo T, Akkerman S, Wouters C, Steinbusch HM, Prickaerts J (2013) 7,8-Dihydroxyflavone improves memory consolidation processes in rats and mice. Behav Brain Res 257:8–12.

Cao G, Zhu J, Zhong Q, Shi C, Dang Y, Han W, Liu X, Xu M, et al. (2013) Distinct roles of methamphetamine in modulating spatial memory consolidation, retrieval, reconsolidation and the accompanying changes of ERK and CREB activation in hippocampus and prefrontal cortex. Neuropharmacology 67:144–154.

Castello NA, Nguyen MH, Tran JD, Cheng D, Green KN, LaFerla FM (2014) 7,8-Dihydroxyflavone, a small molecule TrkB agonist, improves spatial memory and increases thin spine density in a mouse model of Alzheimer disease-like neuronal loss. PLoS ONE 9:e91453.

Cazorla M, Premont J, Mann A, Girard N, Kellendonk C, Rognan D (2011) Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. J Clin Invest 121:1846–1857.

Chao OY, de Souza Silva MA, Yang Y-M, Huston JP (2020) The medial prefrontal cortex - hippocampus circuit that integrates information of object, place and time to construct episodic memory in rodents: Behavioral, anatomical and neurochemical properties. Neurosci Biobehav Rev 113:373–407.

Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, et al. (2010) Role of interleukin-1beta in postoperative cognitive dysfunction. Ann Neurol 68:360–368.

Euston David R, Gruber Aaron J, McNaughton Bruce L (2012) The role of medial prefrontal cortex in memory and decision making. Neuron 76:1057–1070.

Fobian K, Owczarek S, Budtz C, Bock E, Berezin V, Pedersen MV (2009) Peptides derived from the solvent-exposed loops 3 and 4 of BDNF bind TrkB and p75NTRreceptors and stimulate neurite outgrowth and survival. J Neurosci Res.
Garcia AD, Buffalo EA (2020) Anatomy and function of the primate entorhinal cortex. Annu Rev Vision Sci 6:411–432.
Giuliani C (2019) The flavonoid quercetin induces AP-1 activation in FRTL-5 thyroid cells. Antioxidants 8.
Ji Y, Lu Y, Yang F, Shen W, Tang TT, Feng L, Duan S, Lu B (2010) Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci 13:302–309.
Kool MJ, Proietti Onori M, Borgesius NZ, van de Bree JE, Elgersma-Hooisma M, Nio E, Bezstarosti K, Buitendijk GHS, et al. (2019) CAMK2-dependent signaling in neurons is essential for survival. J Neurosci 39:5424–5439.
Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J (2017) BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. Cell Mol Neurobiol 38:579–593.
Ladurelle N, Gabriel C, Viggiano A, Mocaër E, Baulieu EE, Bianchi M (2011) Agomelatine (S20098) modulates the expression of cytoskeletal microtubular proteins, synaptic markers and BDNF in the rat hippocampus, amygdala and PFC. Psychopharmacology 221:493–509.
Leal G, Bramham CR, Duarte CB (2017) BDNF and hippocampal synaptic plasticity. Neurotrophins:153–195.
Li Y, Liu T, Li Y, Han D, Hong J, Yang N, He J, Peng R, et al. (2020) Baicalin ameliorates cognitive impairment and protects microglia from LPS-induced neuroinflammation via the SIRT1/HMGB1 pathway. Oxid Med Cell Longevity 2020:1–16.
Lu B, Nagappan G, Lu Y (2014) BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol 220:223–250.
Maiti P, Muthuraju S, Ilavazhagan G, Singh SB (2008) Hypobaric hypoxia induces dendritic plasticity in cortical and hippocampal pyramidal neurons in rat brain. Behav Brain Res 189:233–243.
Miranda M, Kent BA, Morici JF, Gallo F, Saksida LM, Bussey TJ, Weisstaub N, Bekinschtein P (2018) NMDA receptors and BDNF are necessary for discrimination of overlapping spatial and non-spatial memories in perirhinal cortex and hippocampus. Neurobiol Learn Mem 155:337–343.
Morella I, Hallum H, Brambilla R (2020) Dopamine D1 and glutamate receptors co-operate with brain-derived neurotrophic factor (BDNF) and TrkB to modulate ERK signaling in adult striatal slices. Front Cell Neurosci 14.
Opitz B (2014) Memory function and the hippocampus. In: Hippocampus in Clinical Neuroscience. p. 51–59.
Ren K, Dubner R (2007) Pain facilitation and activity-dependent plasticity in pain modulatory circuitry: role of BDNF-TrkB signaling and NMDA receptors. Mol Neurobiol 35:224–235.
Rodrigue KM, Raz N (2004) Shrinkage of the entorhinal cortex over five years predicts memory performance in healthy adults. J Neurosci: Off J Soc Neurosci 24:956–963.
Sanderson DJ, Good MA, Seeburg PH, Sprengel R, Rawlins JN, Bannerman DM (2008) The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. Prog Brain Res 169:159–178.
Sasi M, Vignoli B, Canossa M, Blum R (2017) Neurobiology of local and intercellular BDNF signaling. Pflügers Arch Eur J Physiol 469:593–610.
Sun XL, Zhang JB, Guo YX, Xia TS, Xu LC, Rahmand K, Wang GP, Li XJ, et al. (2021) Xanthohumol ameliorates memory impairment and reduces the deposition of beta-amyloid in APP/PS1 mice via regulating the mTOR/LC3II and Bax/Bcl-2 signalling pathways. J Pharm Pharmacol..
Tanimizu T, Kenney JW, Okano E, Kadoma K, Frankland PW, Kida S (2017) Functional connectivity of multiple brain regions required for the consolidation of social recognition memory. J Neurosci 37:4103–4116.
Vertes RP (2015) Major diencephalic inputs to the hippocampus. In: The Connected Hippocampus. p. 121–144.
Vigil FA, Giese KP (2018) Calcium/calmodulin-dependent kinase II and memory destabilization: a new role in memory maintenance. J Neurochem 147:12–23.
von Bohlen und Halbach O, von Bohlen und Halbach V (2018) BDNF effects on dendritic spine morphology and hippocampal function. Cell Tissue Res 373:729–741.
Wan Y, Xu J, Ma D, Zeng Y, Cibelli M, Maze M (2007) Postoperative impairment of cognitive function in rats: a possible role for cytokine-mediated inflammation in the hippocampus. Anesthesiology 106:436–443.
Xiao D, Liu L, Li Y, Ruan J, Wang H (2019) Licorisoflavan A exerts antidepressant-like effect in mice: Involvement of BDNF-TrkB pathway and AMPA receptors. Neurochem Res 44:2044–2056.
Yamada K, Nabeshima T (2004) Interaction of BDNF/TrkB signaling with NMDA receptor in learning and memory. Drug News Perspect 17:435–438.
Yang YJ, Li YK, Wang W, Wan JG, Yu B, Wang MZ, Hu B (2014) Small-molecule TrkB agonist 7,8-dihydroxyflavone reverses cognitive and synaptic plasticity deficits in a rat model of schizophrenia. Pharmacol Biochem Behav 122:30–36.
Yang X, Yao C, Tian T, Li X, Yan H, Wu J, Li H, Pei L, et al. (2018) A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal-CA1 synapses. Mol Psychiatry 23:199–210.
Zhan X, Stamova B, Sharp FR (2018) Lipopolysaccharide associates with amyloid plaques, neurons and oligodendrocytes in Alzheimer's disease brain: A review. Front Aging Neurosci 10.
Zhang X-Y, Cao J-B, Zhang L-M, Li Y-F, Mi W-D (2015) Deferoxamine attenuates lipopolysaccharide-induced neuroinflammation and memory impairment in mice. J Neuroinflamm 12.
Zhang Z, Liu X, Schroeder JP, Chan CB, Song M, Yu SP, Weinshenker D, Ye K (2014a) 7,8-dihydroxyflavone prevents synaptic loss and memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacol: Off Publ Am College Neuropsychopharmacol 39:638–650.
Zhang H, Ma L, Guo W-Z, Jiao L-B, Zhao H-Y, Ma Y-Q, Hao X-M (2020a) TSPO ligand etifoxine attenuates LPS-induced cognitive dysfunction in mice. Brain Res Bull 165:178–184.
Zhang JC, Wu J, Fujita YK, Yao W, Ren Q, Yang C, Li SX, Shirayama YK, Hashiomto KJ (2014b) Antidepressant effects of TrkB ligands on depression-like behavior and dendertic changes in mice after inflammation. Int J Neuropsychopharmacol.
Zhang W, Xiong BR, Zhang LQ, Huang X, Zhou WC, Zou Q, Manyande A, Wang J, et al. (2020b) Disruption of the GABAergic system contributes to the development of perioperative neurocognitive disorders after anesthesia and surgery in aged mice. CNS Neurosci Ther.
Zhang B, Zhao J, Wang Z, Xu L, Liu A, Du G (2020c) DL0410 attenuates oxidative stress and neuroinflammation via BDNF/TrkB/ERK/CREB and Nrf2/HO-1 activation. Int Immunopharmacol 86.
Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J, Lu D, Wei W, et al. (2019) Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Sci Rep 9.
Zheng XX, Zhang KY, Li YC, Chen YW, Yue YS, Xia SZ, Li Y, Deng HH, et al. (2020) Imperatorin ameliorates learning and memory deficits through BDNF/TrkB and ERK/CaMKIIalpha/CREB signaling in prenatally-stressed female offspring. Phytother Res 34:2408–2418.
Zurek AA, Bridgwater EM, Orser BA (2012) Inhibition of alpha5 gamma-Aminobutyric acid type A receptors restores recognition memory after general anesthesia. Anesth Analg 114:845–855.

(Received 22 June 2022, Accepted 3 October 2022)
(Available online 18 October 2022)