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ORIGINAL PAPER

Disruption of Striatal-Enriched Protein Tyrosine Phosphatase Signaling Might Contribute to Memory Impairment in a Mouse Model of Sepsis-Associated Encephalopathy

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Abstract

Sepsis-associated encephalopathy (SAE) is a potentially irreversible acute cognitive dysfunction with unclear mechanism. Striatal-enriched protein tyrosine phosphatase (STEP) is a brain-specific phosphatase which normally opposes synaptic strengthening by regulating key signaling molecules involved in synaptic plasticity and neuronal function. Thus, we hypothesized that abnormal STEP signaling pathway was involved in sepsis-induced cognitive impairment evoked by lipopolysaccharides (LPS) injection. The levels of STEP, phosphorylation of GluN2B (pGluN2B), the kinases extracellular signal-regulated kinase 1/2 (pERK), cAMP-response element binding protein (CREB), synaptophysin, brain derived neurotrophic factor (BDNF), and post-synaptic density protein 95 (PSD95) in the hippocampus, prefrontal cortex, and striatum were determined at the indicated time points. In the present study, we found that STEP levels were significantly increased in the hippocampus, prefrontal cortex, and striatum following LPS injection, which might resulted from the disruption of the ubiquitin–proteasome system. Notably, a STEP inhibitor TC-2153 treatment alleviated sepsis-induced memory impairment by increasing phosphorylation of GluN2B and ERK1/2, CREB/BDNF, and PSD95. In summary, our results support the key role of STEP in sepsis-induced memory impairment in a mouse model of SAE, whereas inhibition of STEP may provide a novel therapeutic approach for this disorder and possible other neurodegenerative diseases.

Keywords Striatal-enriched protein tyrosine phosphatase · LPS · Cognition · Synaptic plasticity

Introduction

Sepsis-associated encephalopathy (SAE) is a potentially irreversible acute cognitive dysfunction invoked by the immune response to bacterial lipopolysaccharide (LPS) or other endotoxic bacterial cell wall components in the absence of direct brain infection [1], which is clinically characterized by slowing speed of information processing, impaired attention, memory dysfunction, delirium, or coma [2, 3]. Unfortunately, there is no effective therapy for SAE, in part because of our limited knowledge of its underlying pathophysiological mechanisms. Thus, deeper understanding of its etiological mechanisms is necessary for seeking novel and more effective therapeutic strategies.

Striatal-enriched protein tyrosine phosphatase (STEP, also known as "PTPN5") is a brain-specific tyrosine phosphatase enriched in the striatum, cortex, and hippocampus [4]. STEP has two major alternatively spliced isoforms, a membrane-associated STEP61 and a cytosolic STEP46. Both isoforms are enriched in the striatum, whereas STEP61

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is only expressed in neurons of the hippocampus, cortex, and spinal dorsal horn. In addition, STEP61 and STEP46 contain a signature consensus PTP sequence essential for catalytic activity and a kinase interacting motif required for substrate binding. STEP normally negatively regulates synaptic strengthening by dephosphorylating and inactivating its substrates including extracellular signal-regulated kinases 1 and 2 (ERK1/2), proline-rich tyrosine kinase 2, and Src family tyrosine kinase Fyn, by promoting internalization of surface *N*-methyl-D-aspartate receptors (NMDARs) and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors [5–7]. In line with this notion, high STEP level disrupts synaptic function and contributes to the cognitive deficits in several neurodegenerative disease [8–10]. By contrast, STEP KO mice show strengthened synaptic plasticity, and enhanced hippocampal-and amygdala-dependent memory consolidation [11]. STEP also dephosphorylates ERK1/2, which subsequently inactivates its downstream signaling pathway the cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF), two main molecules implicated in memory consolidation [12].

Given STEP is associated with multiple mechanisms linking to memory and learning impairment, we hypothesized that abnormal STEP signaling pathway might contribute to synaptic and cognitive impairment in an animal model of SAE induced by LPS.

Materials and Methods

Mice

Ninety male C57BL/6 mice (3–4 months, 22–25 g) were obtained from the Animal Center of Jinling Hospital, Nanjing, China. All animal experiments were performed according to the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, MD, USA), and approved by the Ethics Committee of Jinling Hospital, Nanjing University, China. Mice were reared with free access to food and water in standard conditions maintained at 22–25 °C with stable humidity.

Drugs Administration and Experimental Design

Experiment 1: To determine the time course of STEP and related proteins, mice were euthanized at following time points: 12 h, 24 h, 48 h, and 168 h after LPS injection (3 mg/kg, Sigma-Aldrich, St Louis, MO, USA).

Experiment 2: To test whether STEP inhibition could reverse the biochemical and cognitive impairment induced by LPS, a selective STEP inhibitor 8-(trifluoromethyl)-1,2,3,4,5-benzo pentathiepin-6-amine hydro-chloride (TC-2153) was used. Mice were injected *i.p.* with vehicle (Veh, 2% DMSO in saline) or TC-2153 (10 mg/kg in 2% DMSO, Sigma, St Louise, MO, USA) after LPS injection (3 mg/kg, *i.p.*). The dose of TC-2153 were chosen based on one previous study [13]. The experimental design was shown in Fig. 1a.

Behavioral Tests

All behavioral procedures were performed in a sound-isolated room, and data were recorded by the same investigator who was blinded to the grouping of mice as described by our previous study [14].

Open Field Test

Forty-eight hours following LPS injection, the spontaneous locomotor activity of the mice in the open field test was tracked and recorded by a photobeam activity system and software (XR-XZ301, Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China). The apparatus consists of a white polyester resin chamber (40 cm × 60 cm × 50 cm). The mice were placed in the center of the arena and allowed to explore for 5 min, and the total distance moved and the time spent in the center were recorded. The chamber was cleaned with 75% ethanol after each test.

Y-Maze

Four hours after open field test, we used the Y-maze to assess spontaneous alternation for spatial working memory [15]. The Y-maze consisted of three arms at 120° angle that were labeled A, B, and C. Each arm was 14 cm in width, 30 cm in length, and 15 cm in height. Each mouse was placed in the far end of one same arm and allowed to move freely across the apparatus for 8 min. The total number and sequence of arm entries were recorded. If the mouse consecutively entered three different arms, it was counted as a spontaneous alternation performance. The score of alternation was calculated using the formula: Score = number of alternating triads/(total number of arms entries − 2) × 100%. The chamber was thoroughly cleaned with 75% ethanol after each trial.

Western Blot Analysis

The hippocampus, prefrontal cortex, and striatum were obtained for western blot analysis at the indicated time points. Each sample was homogenized in the RIPA lysis buffer mixed with 1% protease inhibitor cocktail and 1% PMSF. After centrifuging at 13,000×*g* for 10 min at 4 °C, the supernatant was collected and its protein concentration was measured by Bradford assay, 30 μg of proteins per lane

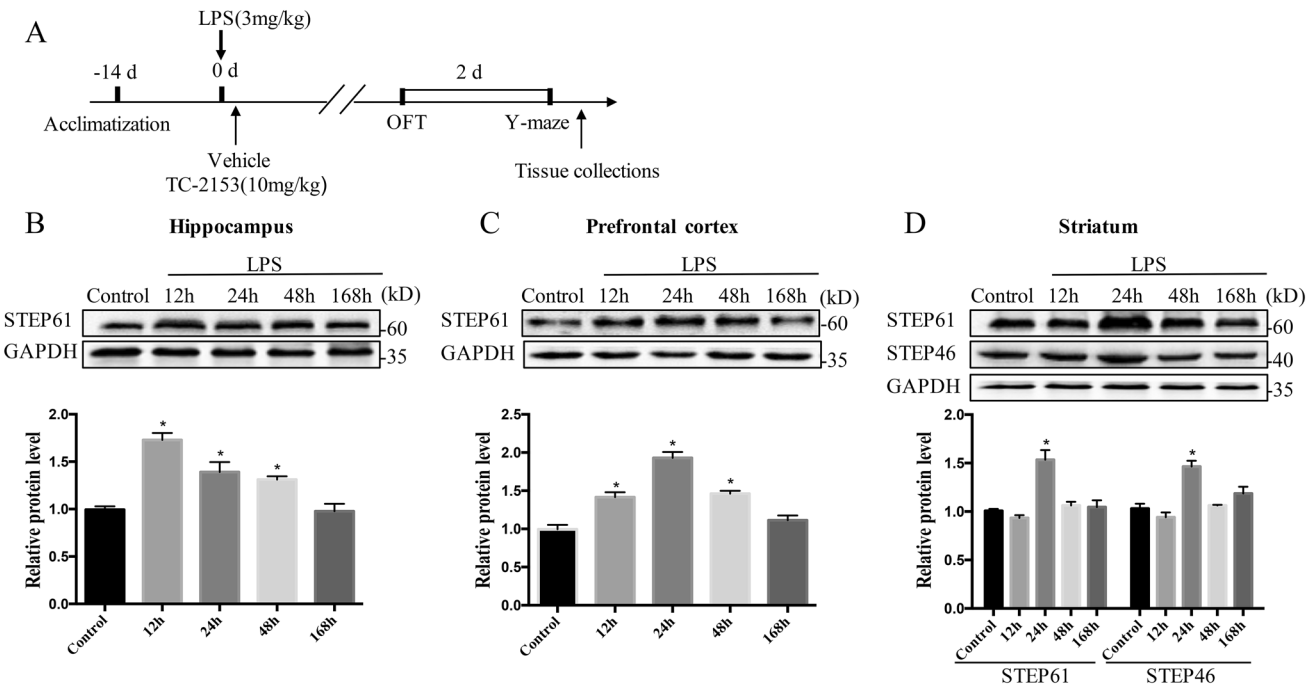

Fig. 1 Time course of STEP protein expression after LPS injection. **a** Schematic timeline of the experimental procedures. STEP levels were analyzed by western blot of protein extracts obtained from the hippocampus (**b**), prefrontal cortex (**c**), and striatum (**d**) of mice treated with saline or LPS. Representative western blot bands of STEP and quantitative analysis of the relative protein levels were shown. Data are expressed as mean ± SEM and statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$, $n = 4$ mice/group)

were loaded on SDS-PAGE gels and then was transferred to the nitrocellulose blotting membranes. After blocking with 5% non-fat milk in Tris-Buffered Saline with Tween (TBST), membranes were incubated overnight at 4 °C in each primary antibody. The primary antibodies used were anti-STEP, anti-pCREB, and anti-CREB (all from Cell Signaling Technologies, Danvers, MA, USA); anti-NMDAR2B, anti-pERK1/2, anti-ERK1/2, anti-brain-derived neurotrophic factor (BDNF), anti-postsynaptic density 95 (PSD95), anti-synaptophysin (all from Abcam, Cambridge, UK); anti-NMDAR2B (phosphoTyr1472) (pGluN2B) (Gene Tex, San Antonio, USA), anti-ubiquitin (Santa Cruz Biotechnology, Dallas, USA) and anti-GAPDH (Bioworld, St. Louis Park, MN, USA). After washing in TBST for three times, the membranes were incubated with appropriate secondary antibodies (goat anti-rabbit, or goat anti-mouse; Bioworld, St. Louis Park, MN, USA). Chemiluminescence method was used to detect the protein bands and the band intensity were analyzed with Image J Software (Wayne Rasband, National Institute of Health, USA).

Immunoprecipitation Assay

The hippocampal, prefrontal cortical, and striatal protein extracts obtained from saline or LPS-treated mice at 24 h after LPS administration were diluted in 400 µl ice-cold immunoprecipitation buffer (20 mM Tris–HCl, pH 8, 137 mM NaCl, 2 mM EDTA, 1% NP-40, 10 mM *N*-ethylmaleimide, 10% glycerol with complete protease inhibitor). Equal amount of supernatants (400 µg) were precleared with Dynabeads™ Sheep anti-Mouse IgG (Thermo Scientific, Waltman, MA, USA) to minimize non-specific binding according to the manufacturer's product description. The anti-STEP antibody was used to pull-down STEP. Ubiquitinated STEP species were visualized by probing with anti-ubiquitin antibody.

Statistical Analysis

Statistical analyses were performed by Prism 6.0 (GraphPad Software, La Jolla, CA, USA). All data are presented as mean ± SEM. Differences among multiple groups were assessed by one-or two-way analysis of variance (ANOVA) with post hoc Tukey's test. The survival rate was analyzed by the Kaplan–Meier method and compared by the log-rank test. *P* value < 0.05 was considered significant.

Results

STEP Expression Increased Significantly After LPS Injection

To characterize STEP expression after LPS injection, we performed western blot of hippocampal, prefrontal cortical, and striatal protein extracts obtained from LPS-injected mice at 12 h, 24 h, 48 h, and 168 h post-LPS. In the hippocampus, STEP61 was increased at 12 h and then progressively decreased to normal level at 168 h post-LPS ($F_{4,15} = 19.47$, $p < 0.0001$; Fig. 1b). Similarly, in the prefrontal cortex, STEP61 level was significantly upregulated at 12 h, and this upregulation persisted until 48 h post-LPS ($F_{4,15} = 34.89$, $p < 0.0001$; Fig. 1c). However, we only observed increased STEP61 and STEP46 levels at 24 h post-LPS in the striatum (STEP61: $F_{4,15} = 15.88$, $p < 0.0001$; STEP46: $F_{4,15} = 14.98$, $p < 0.0001$; Fig. 1d).

Tyrosine Phosphorylation of STEP Substrates Decreased Markedly After LPS Injection

STEP dephosphorylates NMDAR subunit GluN2B at Tyr^{1472}, leading to internalization of GluN2B-containing NMDAR. In the hippocampus, we found a significantly reduced Tyr^{1472}-phosphorylated GluN2B (pGluN2B) compared to control group from 12 to 48 h following LPS injection ($F_{4,15} = 13.14$, $p < 0.0001$; Fig. 2a). Likewise, in the prefrontal cortex, pGluN2B levels was dramatically downregulated at 24 h and 48 h post-LPS ($F_{4,15} = 24.39$, $p < 0.0001$; Fig. 2b). In the striatum, pGluN2B levels only decreased at 24 h following LPS injection ($F_{4,15} = 3.517$, $p = 0.032$; Fig. 2c).

STEP-mediated dephosphorylation of ERK1/2 at $Tyr^{204/187}$ inactivates ERK1/2, opposing synaptic strengthening during LTP. Thus, we next tested whether increased STEP level would affect the $Tyr^{204/187}$-phosphorylation of ERK1/2 (pERK). In the hippocampus, there was a significantly decreased pERK compared to control group from 12 to 48 h after LPS injection ($F_{4,15} = 22.22$, $p < 0.0001$; Fig. 2a). Similarly, in the prefrontal cortex, pERK level was dramatically reduced at 24 and 48 h post-LPS ($F_{4,15} = 20.43$, $p < 0.0001$; Fig. 2b). In the striatum, pERK level was downregulated at 24 h following LPS ($F_{4,15} = 5.622$, $p = 0.006$; Fig. 2c).

We then analyzed the phosphorylation state of CREB and BDNF levels in the hippocampus, prefrontal cortex, and striatum. Both are downstream targets of ERK1/2 and have been demonstrated to be involved in memory consolidation [2]. In the hippocampus and prefrontal cortex, LPS significantly decreased the levels of pCREB (hippocampus: $F_{4,15} = 8.579$, $p < 0.001$; prefrontal cortex: $F_{4,15} = 8.423$, $p < 0.001$; Fig. 3a, b) and BDNF (hippocampus: $F_{4,15} = 9.650$, $p < 0.001$; prefrontal cortex: $F_{4,15} = 9.925$, $p < 0.001$; Fig. 3a, b) at 24 h and 48 h following LPS. However, in the striatum, we only observed a significantly decrease of pCREB at 24 h and BDNF at 48 h (pCREB: $F_{4,15} = 5.629$, $p = 0.06$; BDNF: $F_{4,15} = 4.118$, $p = 0.019$; Fig. 3c) when STEP61 and STEP46 levels were only enhanced at 24 h post-LPS.

As shown in Fig. 3, the level of PSD95 in the hippocampus was significantly decreased from 24 h and persisted until 168 h following LPS ($F_{4,15} = 26.22$, $p < 0.0001$; Fig. 3a). In the prefrontal cortex, PSD95 level was dramatically reduced at 24 and 48 h post-LPS ($F_{4,15} = 11.75$, $p < 0.001$; Fig. 3b).

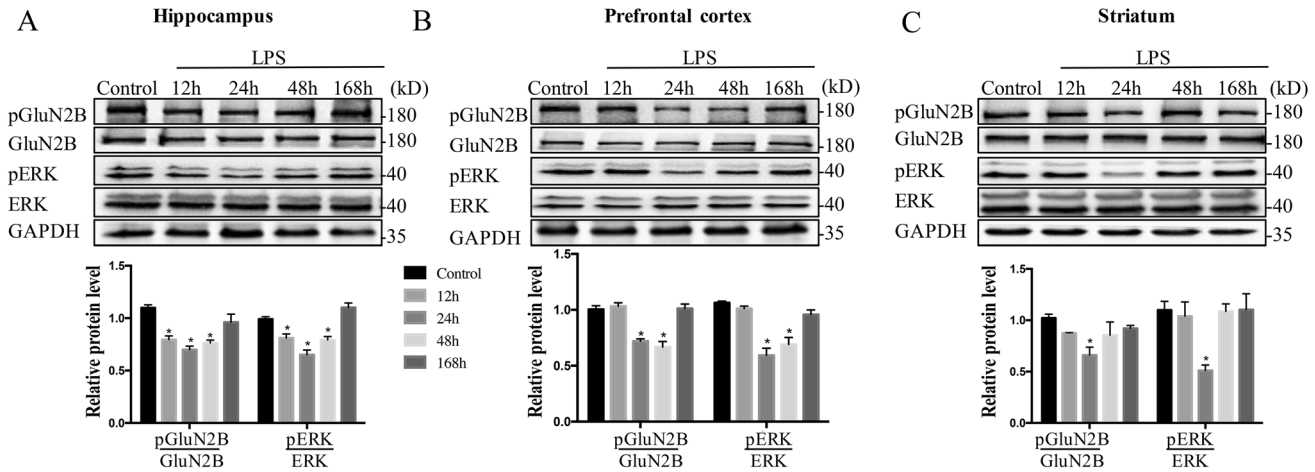

Fig. 2 Time course of tyrosine phosphorylation of STEP substrates GluN2B and ERK1/2 after LPS injection. Representative western blot bands of pGluN2B and pERK1/2 in the hippocampus (**a**), prefrontal cortex (**b**), and striatum (**c**) and quantitative analysis of the relative protein levels were shown. Data are expressed as mean ± SEM and statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$, $n = 4$ mice/group)

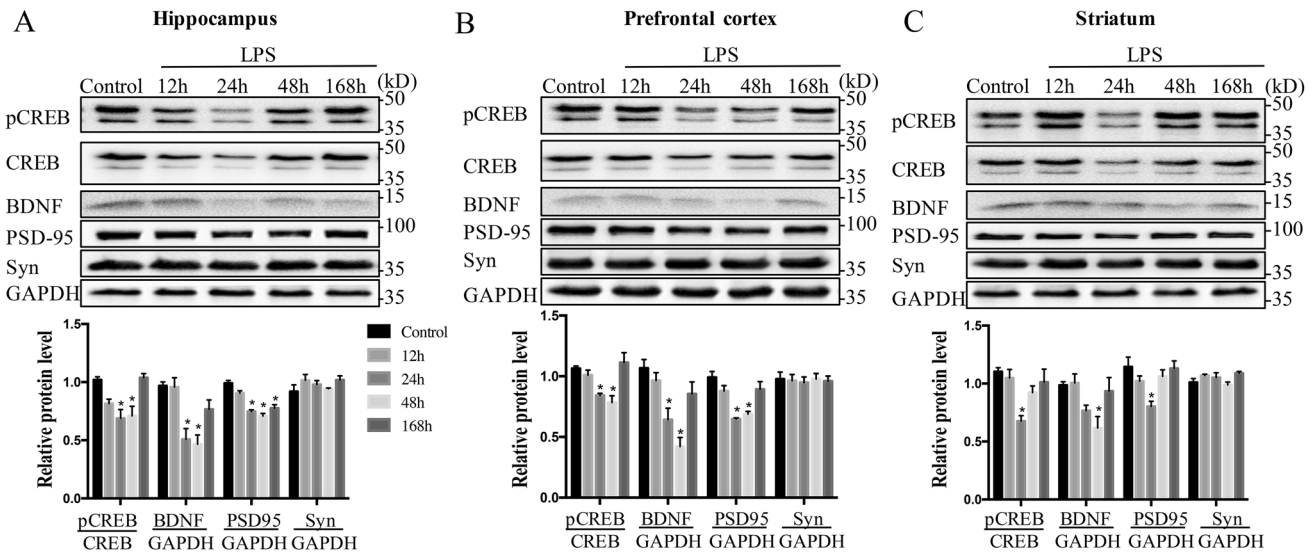

Fig. 3 Time course of CREB/BDNF, PSD95, and synaptophysin after LPS injection. Representative western blot bands of pCREB, BDNF, PSD95, and synaptophysin in the hippocampus (**a**), prefrontal cortex (**b**), and striatum (**c**) and quantitative analysis of the relative protein levels were shown. Data are expressed as mean ± SEM and statistical significance was determined using one-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$, $n = 4$ mice/group)

In the striatum, PSD95 was downregulated at 24 h following LPS ($F_{4,15} = 5.202$, $p = 0.008$; Fig. 3c). However, the level of the presynaptic membrane protein synaptophysin was not altered in the hippocampus, prefrontal cortex or striatum following LPS injection.

STEP Accumulation Involved the Ubiquitin–Proteasome System

It has been demonstrated that STEP is normally ubiquitinated and degraded by the proteasome [16]. To test whether increase in STEP resulted from the disruption of the ubiquitin–proteasome system (UPS) in LPS-treated mice, we immunoprecipitated STEP and performed western blot against ubiquitin at 24 h after LPS injection, when STEP levels changed significantly in all these brain regions. We found that the levels of ubiquitin-conjugated STEP were lower in the hippocampus, prefrontal cortex, and striatum of LPS-treated mice compared with control group (Fig. 4), suggesting disruption of STEP degradation might contribute to its accumulation upon LPS injection.

STEP Inhibition Reversed LPS-Induced Memory Impairment

The open field test was performed at 48 h following LPS to determine whether LPS or TC-2153 treatment influence the locomotor activity of mice. There was no significant difference in the total distance ($F_{1,35} = 0.080$, $p = 0.779$; Fig. 5b) and time spent in the center of arena ($F_{1,35} = 0.032$, $p = 0.860$; Fig. 5c) among the four groups.

Next, we used the Y-maze to assess spontaneous alternation for spatial working memory, a time point that STEP level was significantly increased following LPS injection in the hippocampus and prefrontal cortex. Two-way ANOVA analysis of alternation behavior showed that the interaction between LPS and TC-2153 treatment was significant ($F_{1,35} = 4.404$, $p = 0.043$). Post hoc comparisons revealed that the alternation score was significantly decreased in the LPS + Veh group compared with the control + Veh group ($p < 0.001$), which was significantly ameliorated in the LPS + TC-2153 group ($p < 0.05$; Fig. 5d). This suggested that TC-2153 attenuated the spatial working memory impairment induced by LPS injection, although it did not improve survival in mice treated with LPS (Fig. 5a). In addition, the total number of entries was counted as an indicator of animal's locomotor activity and the possible effect of LPS and TC-2153 treatment. Results showed that there existed no significant difference among the four groups ($F_{1,35} = 0.052$, $p = 0.821$; Fig. 5e).

Inhibition of STEP Increased Phosphorylation of STEP Substrates

We next investigated whether TC-2153 was able to reverse the biochemical deficits induced by LPS. As previously reported, TC-2153 potently inhibited STEP activity by forming a covalent bond with the active site Cys472 required for activity, without changing total or phosphorylation levels of

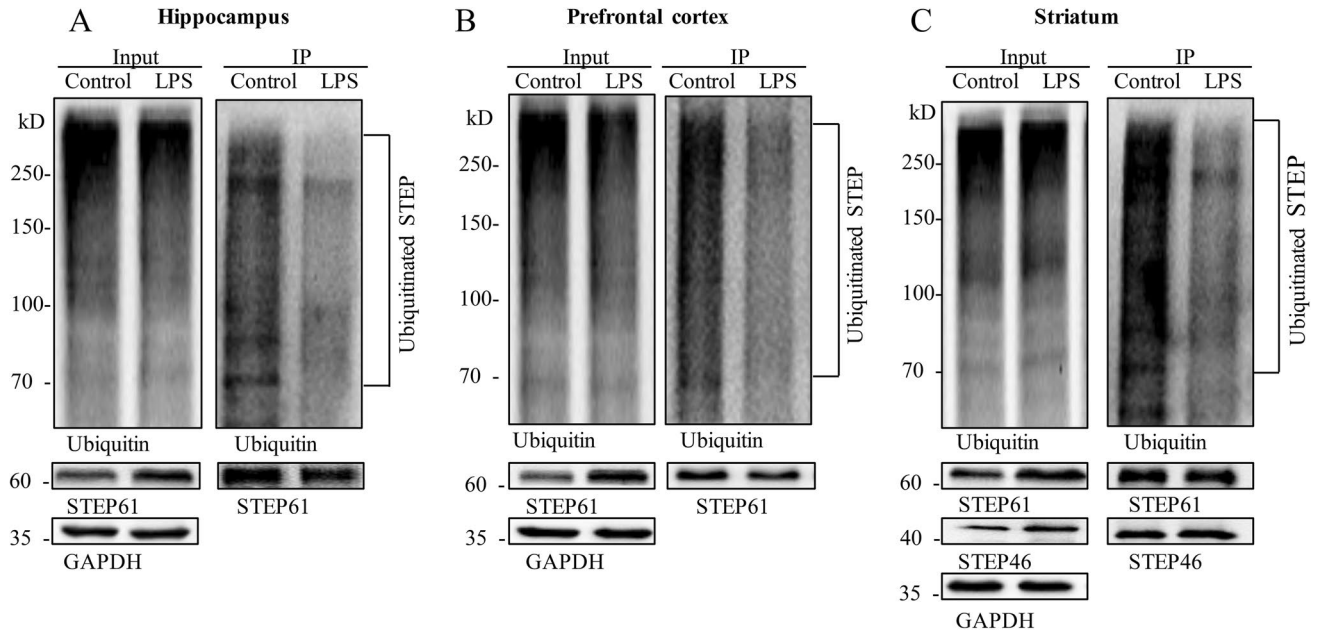

Fig. 4 STEP accumulation involved the ubiquitin–proteasome system in LPS-treated mice. The levels of STEP-ubiquitin conjugates in the hippocampus (**a**), prefrontal cortex (**b**), and striatum (**c**) were determined by performing immunoprecipitation of STEP and western blot against ubiquitin and STEP. Representative immunoblots are shown

STEP [17]. Consistently, we showed that TC-2153 treatment did not change STEP level in the hippocampus, prefrontal cortex or striatum (Fig. 6).

In the hippocampus, there was no interaction between LPS and TC-2153 treatment for pGluN2B and pERK following LPS (interaction effect, pGluN2B: $F_{1,8}=3.816$, $p=0.087$; pERK: $F_{1,8}=4.867$, $p=0.058$; Fig. 6a). Post hoc comparisons showed that LPS significantly decreased the phosphorylation of GluN2B and ERK (pGluN2B: $p<0.05$; pERK: $p<0.01$), which were significantly reversed by TC-2153 treatment (all $p<0.05$). In the prefrontal cortex, the interaction between LPS and TC-2153 treatment was significant for pGluN2B and pERK (interaction effect, pGluN2B: $F_{1,8}=7.109$, $p=0.029$; pERK: $F_{1,8}=11.85$, $p=0.009$; Fig. 6b). Post hoc comparisons showed that LPS significantly downregulated the phosphorylation of GluN2B and ERK (pGluN2B: $p<0.05$; pERK: $p<0.01$), which were markedly improved by TC-2153 (pGluN2B: $p<0.05$; pERK: $p<0.01$). However, no change in pGluN2B and pERK was observed among the four groups in the striatum (Fig. 6c).

Inhibition of STEP Increased CREB/BDNF Signaling and PSD95 Expression

We then analyzed the effect of TC-2153 on the levels of CREB and BDNF, which are downstream targets of ERK1/2. As shown in Fig. 7, the interaction between LPS and TC-2153 treatment was significant for pCREB and BDNF (hippocampus: interaction effect, pCREB: $F_{1,8}=9.346$, $p=0.017$; BDNF: $F_{1,8}=7.375$, $p=0.026$; prefrontal cortex: interaction effect, pCREB: $F_{1,8}=26.13$, $p<0.001$, BDNF: $F_{1,8}=17.140$, $p=0.003$). Post hoc comparisons showed that LPS significantly decreased the expressions of pCREB and BDNF (hippocampus: pCREB: $p<0.01$, BDNF: $p<0.05$; prefrontal cortex: pCREB: $p<0.0001$, BDNF: $p<0.001$), which were reversed by TC-2153 treatment (hippocampus: all $p<0.05$; prefrontal cortex: all $p<0.01$). In the striatum, the interaction between LPS and TC-2153 was significant for BDNF (interaction effect, $F_{1,8}=10.68$, $p=0.011$). Post hoc comparisons showed that TC-2153 treatment alleviated the decreased BDNF induced by LPS ($p<0.05$). Again, no change in pCREB was observed among the four groups in the striatum.

In addition, the interaction between LPS and TC-2153 treatment was significant for PSD95 in both hippocampus and prefrontal cortex (interaction effect, the hippocampus: $F_{1,8}=7.913$, $p=0.023$; prefrontal cortex: $F_{1,8}=14.59$, $p=0.005$; Fig. 7a, b). Post hoc comparisons showed that TC-2153 treatment reversed the decreased PSD95 evoked by LPS (the hippocampus: $p<0.05$; prefrontal cortex: $p<0.01$). However, no change in PSD95 was observed among the four groups in the striatum.

Discussion

In this study, we found increased STEP levels in the hippocampus, prefrontal cortex, and striatum in an animal model of SAE, which might attribute to the disruption of

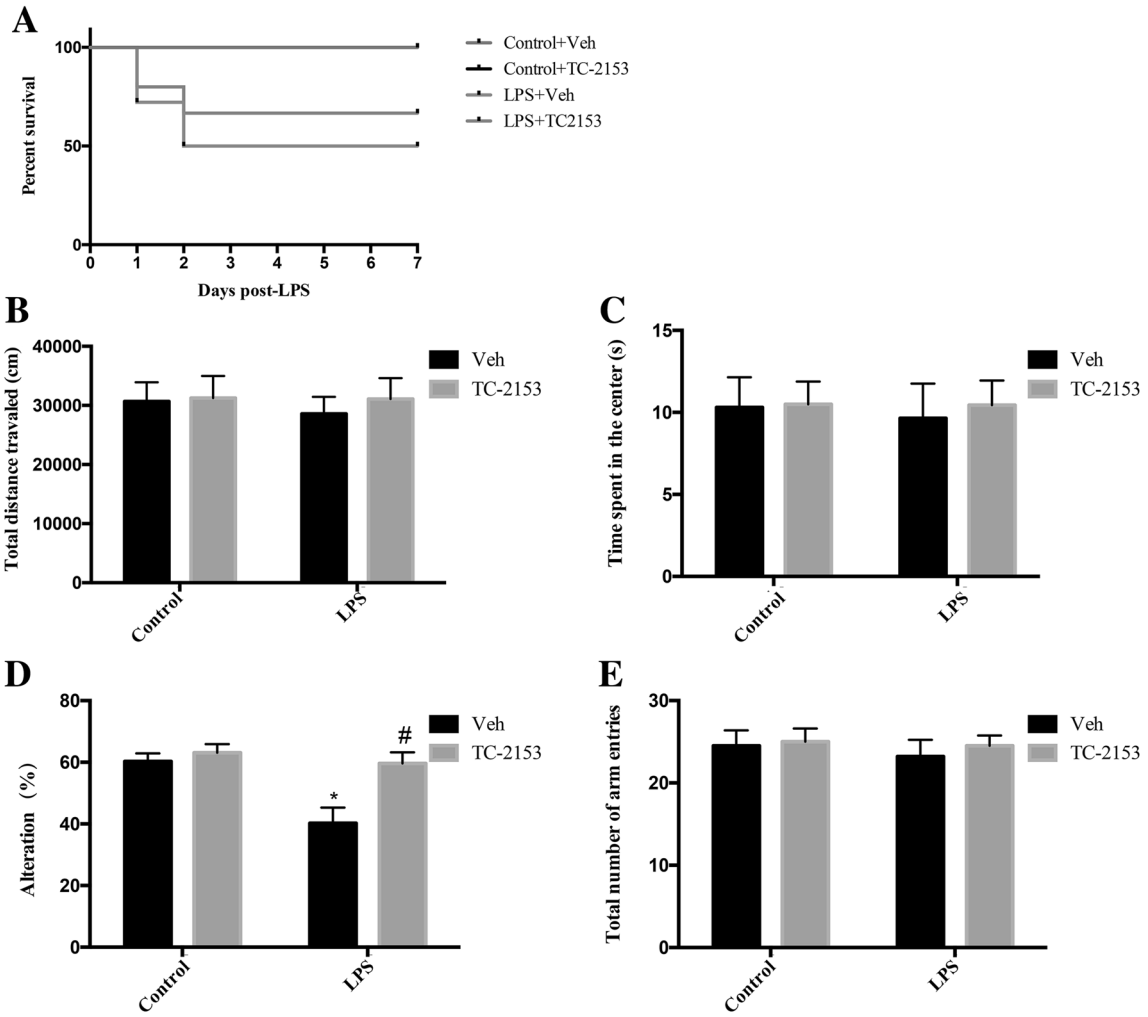

Fig. 5 STEP inhibition reversed LPS-induced cognitive impairment. There was no significant difference at the mortality rate (**a**), ambulatory distance (**b**), and time spent in the center (**c**) in the open field test among the four groups at 48 h following LPS. The alternation score was significantly decreased in the LPS + Veh group compared with the control group, while TC-2153 administration significantly increased the alternation performance (**d**). No significant difference was detected in total number of entries (**e**) among the four groups. All data are expressed as mean ± SEM and statistical significance was determined using two-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$ compared with control + Veh group; $^{\#}p < 0.05$, compared with LPS + Veh group, n = 9–10 mice/group)

the ubiquitin–proteasome system. Notably, STEP inhibitor TC-2153 alleviated SAE possibly by increasing phosphorylation of GluN2B and ERK1/2, CREB/BDNF, and PSD95. To the best of our knowledge, this is the first report regarding the change profile of STEP and its role in an animal model of SAE.

Up until now, several potential mechanisms, such as inflammation, oxidative stress, mitochondrial dysfunction, neurotransmission disturbance, and cell death, have been proposed to be implicated in SAE [18, 19]. However, the precise mechanism remains largely to be elucidated. Accumulating evidence has suggested that STEP dysfunction has been implicated in the pathogenesis of multiple neuropsychiatric disorders. STEP is elevated in postmortem anterior cingulate cortex and dorsolateral prefrontal cortex in schizophrenia (SZ) patients [20]. In addition, it is reported that genetic reduction or pharmacological inhibition of STEP increases the phosphorylation of STEP targets, thus prevents the loss of NMDARs from synaptic membranes, alters neuronal activity and reverses behavioral deficits in an animal model of SZ [13]. It has also been demonstrated that amyloid β can trigger dephosphorylation STEP at Ser^{221} by activating protein phosphatase 2B/calcineurin and protein phosphatase 1 leading to dephosphorylation of GluN2B Tyr^{1472} and internalization of GluN2B-containing NMDARs [21]. Moreover, Amyloid β can also decrease degradation of STEP via Aβ-mediated inhibition of the UPS [22]. These findings raise the possibility that STEP dysfunction might also play a key role in SAE. In the current study, we showed STEP level was

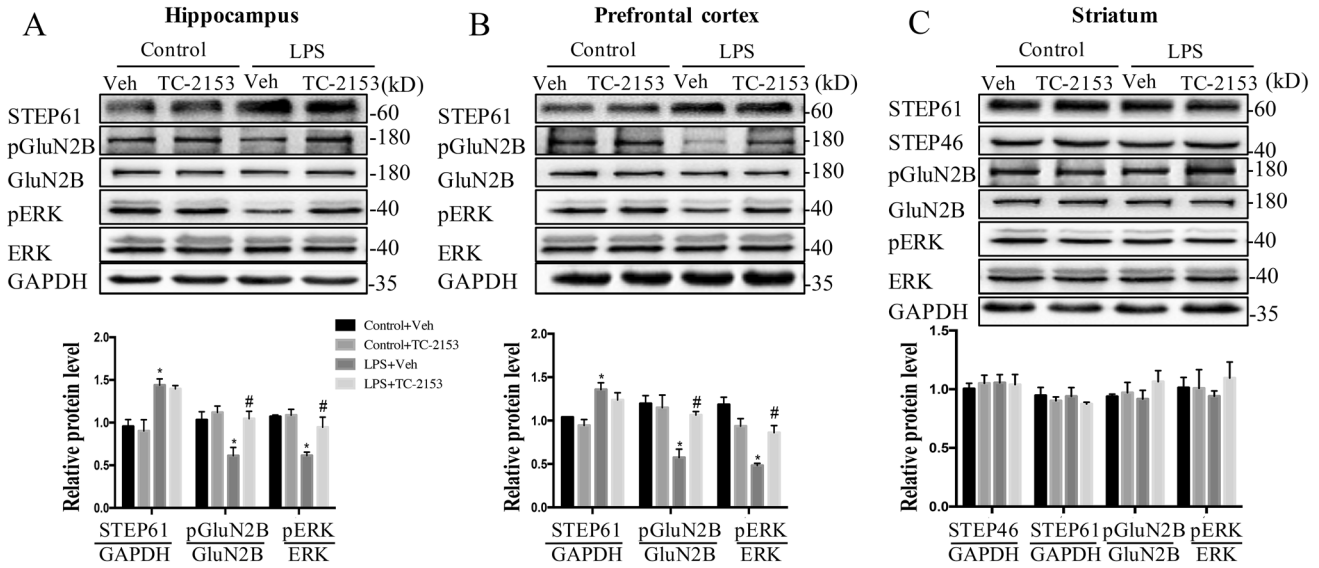

Fig. 6 Inhibition of STEP by TC-2153 increased phosphorylation of GluN2B and ERK1/2 level. Representative western blot bands of STEP, pGluN2B, pERK in the hippocampus (**a**), prefrontal cortex (**b**) and striatum (**c**) and quantitative analysis of the relative protein levels were shown. Mice data are expressed as mean ± SEM and statistical significance was determined by two-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$, compared with control + Veh group; $^{\#}p < 0.05$, compared with LPS + Veh group, n = 3 mice/group)

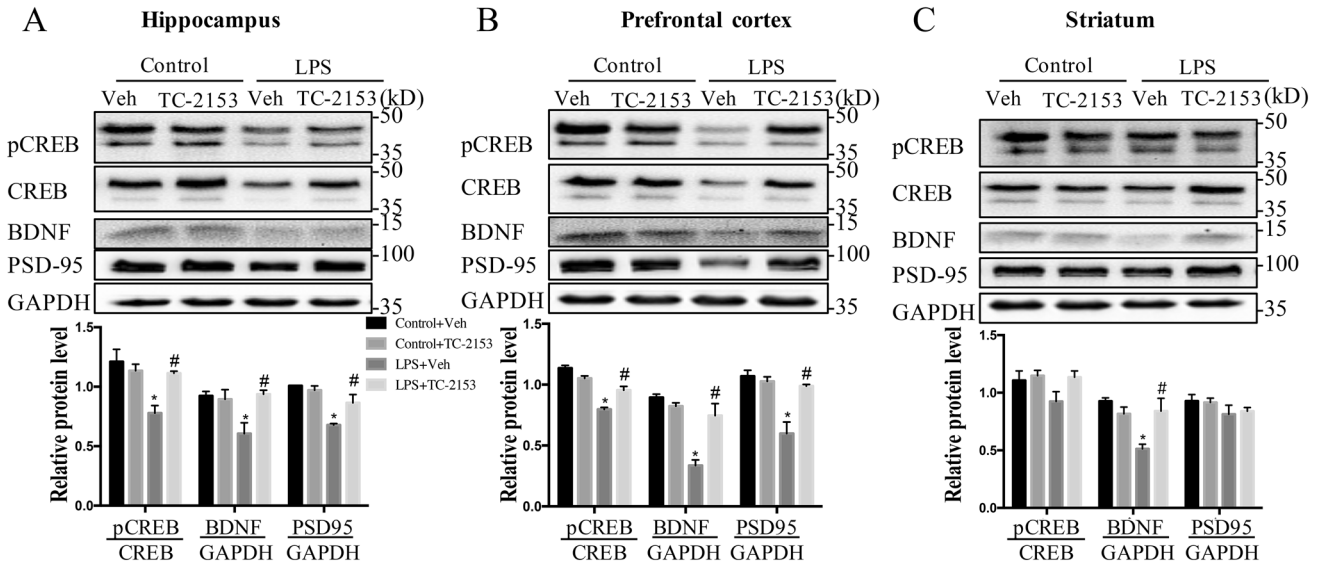

Fig. 7 Inhibition of STEP by TC-2153 increased CREB/BDNF and PSD95 expression. Representative western blot bands of CREB/BDNF and PSD95 in the hippocampus (**a**), prefrontal cortex (**b**) and striatum (**c**) and quantitative analysis of the relative protein levels were shown. Mice data are expressed as mean ± SEM. and statistical significance was determined using two-way analysis of variance (ANOVA) with Tukey's test (*$p < 0.05$, compared with control + Veh group; $^{\#}p < 0.05$, compared with LPS + Veh group, n = 3 mice/group)

increased at 12 h and persisted until 48 h following LPS injection in the hippocampus and prefrontal cortex, which coincided with a time point when mice had significant memory impairment. Notably, STEP inhibitor TC-2153 was able to reverse LPS-induced memory impairment, suggesting STEP signaling plays a causal role in LPS-induced memory impairment. However, the mechanism by which increased STEP level leads to memory impairment induced by LPS remains unclear.

The STEP normally opposes the development of synaptic strengthening by dephosphorylation of regulatory tyrosine residues on their substrates, including the GluN2B, the kinases ERK1/2, Fyn, and proline-rich tyrosine kinase 2, thereby inactivating them and controlling the

duration of their signal [23, 24]. Consistently, we found the increased STEP expression was associated with more pronounced dephosphorylation of the STEP substrates GluN2B and ERK1/2. In addition, the changes of STEP and its substrates in hippocampus were more prolonged than those in the striatum and prefrontal cortex, suggesting the hippocampus is more susceptible to sepsis insult, which is consistent with previous study [25]. However, STEP inhibition with TC-2153 restored phosphorylation of both proteins and also reversed the memory impairment, which further suggested that abnormal STEP and its downstream substrates contributed to LPS-induced memory impairment. However, our study did not exclude the possibility that other substrates of STEP could also be implicated in LPS-related memory impairment.

CREB is a downstream target of ERK1/2, and when activated, it subsequently facilitates the transcription of key proteins required for activity-dependent plasticity, especially BDNF [26, 27]. The later one is a small dimer protein that has been demonstrated to modulate brain development, synaptogenesis, and neuroplasticity [27, 28]. BDNF and STEP have opposing functions in the brain, with BDNF supporting and STEP opposing synaptic strengthening [29]. Moreover, BDNF and STEP expressions are regulated by a reciprocal negative feedback mechanism [30]. For example, BDNF can induce a rapid ubiquitin-mediated degradation of STEP, whereas STEP could modulate BDNF transcription through ERK1/2-CREB pathway [31]. In our study, we showed that the levels of pCREB and BDNF decreased substantially after LPS injection in the hippocampus, prefrontal cortex, and striatum, whereas inhibiting STEP increased both BDNF and pCREB expressions. Given the vital role of pCREB and BDNF in the regulation of memory and learning, our results provided one possible mechanism by which elevated STEP activity exerted its detrimental effects in sepsis-induced memory impairment.

In addition, synapses are particularly vulnerable in neurodegenerative conditions, and LPS can destroy synaptic proteins and contribute to the memory deficit [32]. Specifically, PSD95 is a key player at the postsynaptic density by scaffolding specialized large membrane complexes composed of NMDA receptors [1]. The present study suggested that sepsis decreased PSD95 levels in various brain regions, with the hippocampus being most affected. Indeed, mice lacking PSD95 showed a severely destroyed spatial memory performance [33]. Surprisingly, the presynaptic protein synaptophysin was not affected by LPS injection. However, another study has demonstrated significantly decreased levels of both synaptophysin and PSD95 in the hippocampus of sepsis mice at 24 h and 3 days following cecal ligation and perforation [34]. The discrepancy could be explained by different animal models used and time of protein determination.

The UPS is a major degradation pathway that regulates STEP levels [35, 36], and growing evidence shows that the dysfunction of this pathway results in the accumulation of STEP, which consequently leads to many central nervous system diseases [21, 29, 37]. To confirm this possibility, we immunoprecipitated STEP and performed western blot against ubiquitin and showed disruption of STEP ubiquitination decreased STEP degradation, which consequently contributes to its accumulation in LPS-treated mice. Since UPS dysfunctions can also impact the accumulation of other proteins, it is possible that decreased degradation of additional proteins might also be involved in SAE. However, we did not detect the mRNA levels of STEP, partly because the STEP's expression and ability to bind to and dephosphorylate its substrates is regulated mainly by the ubiquitin–proteasome system, which is a post-translational modification at the protein levels [38, 39].

In conclusion, our study provides one possibility that sepsis disrupted STEP ubiquitination resulting in the accumulation of STEP, which subsequently increased dephosphorylation of its substrates GluN2B and ERK1/2, induced CREB inactivation and a decline in BDNF, ultimately leads to memory impairment (Fig. 8). Pending further studies, STEP represents a promising neuroprotective approach to treat SAE.

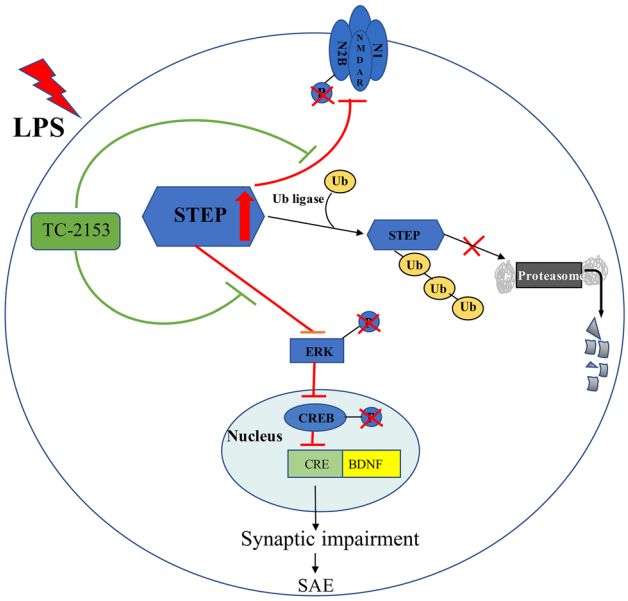

Fig. 8 Schematic model of disruption in STEP associated with SAE. Sepsis disrupted STEP ubiquitination resulting in the accumulation of STEP, which subsequently increased dephosphorylation of its substrates GluN2B and ERK1/2, induced CREB inactivation, and a decline in BDNF, ultimately leading to cognitive impairment

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Compliance with Ethical Standards

Conflict of interest We declare that we have no competing interests.

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