ORIGINAL RESEARCH PAPER

Fluoxetine alleviates postoperative cognitive dysfunction by attenuating TLR4/MyD88/NF-κB signaling pathway activation in aged mice

Yusheng Yao[1,3] · Daoyi Lin[1] · Yuzhi Chen[2] · Linwei Liu[1] · Yushang Wu[1] · Xiaochun Zheng[1,3]

Abstract

Objective and design Postoperative cognitive dysfunction (POCD) is a common complication following surgery among elderly patients. Emerging evidence demonstrates that neuroinflammation plays a pivotal role in the pathogenesis of POCD. This study tested the hypothesis that fluoxetine can protect against POCD by suppressing hippocampal neuroinflammation through attenuating TLR4/MyD88/NF-κB signaling pathway activation.

Subjects Aged C57BL/6 J male mice (18 months old) were studied.

Treatment Aged mice were intraperitoneally injected with fluoxetine (10 mg/kg) or saline for seven days before splenectomy. In addition, aged mice received an intracerebroventricular injection of a TLR4 agonist or saline seven days before splenectomy in the rescue experiment.

Methods On postoperative days 1, 3, and 7, we assessed hippocampus-dependent memory, microglial activation status, proinflammatory cytokine levels, protein levels related to the TLR4/MyD88/NF-κB signaling pathway, and hippocampal neural apoptosis in our aged mouse model.

Results Splenectomy induced a decline in spatial cognition, paralleled by parameters indicating exacerbation of hippocampal neuroinflammation. Fluoxetine pretreatment partially restored the deteriorated cognitive function, downregulated proinflammatory cytokine levels, restrained microglial activation, alleviated neural apoptosis, and suppressed the increase in TLR4, MyD88, and p-NF-κB p65 in microglia. Intracerebroventricular injection of LPS (1 μg, 0.5 μg/μL) before surgery weakened the effect of fluoxetine.

Conclusion Fluoxetine pretreatment suppressed hippocampal neuroinflammation and mitigated POCD by inhibiting microglial TLR4/MyD88/NF-κB pathway activation in aged mice.

Keywords Microglia · Neuroinflammation · Postoperative cognitive dysfunction · Fluoxetine · Toll-like receptor 4

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Introduction

Postoperative cognitive dysfunction (POCD) is increasingly recognized as a complication following intensive surgical procedures, particularly affecting elderly individuals [1]. POCD is defined as a decline in cognitive function at varying postoperative intervals, up to 3 months–7.5 years after surgery, manifested by memory impairment, deficiency in daily function, psychomotor retardation, and so on [2, 3]. As life expectancy prolongs and patients aged 65 years or older account for a tremendously expanding proportion of major operation consumers, growth in the number of patients susceptible to postoperative neurocognitive disorders is inevitable. Unfortunately, no medication has been approved for the prevention of POCD.

Although the mechanism for POCD remains elusive, mounting evidence has suggested the critical role of neuroinflammation, mainly labeled by microglial activation, in the pathological process of POCD [4]. Neuroinflammation increases neuronal apoptosis, reduces neurogenesis, and alters synaptic plasticity [5, 6]. These events may damage neural function, eventually resulted in POCD. The TLR4/MyD88/NF-κB signaling pathway was shown to facilitate neuroinflammation in the pathophysiology of neurodegenerative diseases, including POCD [7–9]. Thus, targeting the TLR4/MyD88/NF-κB signaling pathway to alleviate neuroinflammation may be instructive in exploring a prophylactic medication for POCD.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is prescribed widely as an antidepressant [10]. Preclinical experiments have indicated that fluoxetine exhibits neuroprotective profiles due to its anti-neuroinflammatory properties [11]. Evidence that fluoxetine exerts an anti-neuroinflammatory effect through TLR4-mediated signaling pathways has been elaborated in several studies [12, 13]. Moreover, fluoxetine has been reported to protect cognitive function in neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases [14, 15]. However, the effect of fluoxetine pretreatment on POCD has not been explored. Therefore, we tested the hypothesis that fluoxetine pretreatment might benefit POCD protection by suppressing TLR4/MyD88/NF-κB signaling pathway activation and attenuating neuroinflammation.

Materials and methods

Animals

C57BL/6 J male mice (18 months old, weighing 28–36 g) were obtained from the Model Animal Research Center of Fujian Medical University. We conducted the experiments according to the Care and Use of Laboratory Animals guidelines published by the US National Institutes of Health. All protocols were approved by the Animal Care and Use Committee of Fujian Medical University (No. FJMUIACUC2021-0430).

Experimental design

A schematic diagram of the experimental procedure is depicted in Fig. 1. In part A, mice were randomly allocated to four different groups: saline-pretreated control group (C + S), fluoxetine-pretreated control group (C + F), saline-pretreated surgery group (S + S), and fluoxetine-pretreated surgery group (S + F). Fluoxetine hydrochloride (F132, Sigma, USA) was dissolved in 0.9% saline and intraperitoneally injected (10 mg/kg) once daily for seven consecutive days in the C + F and S + F groups [16]. Meanwhile, mice in the C + S and S + S groups were administered saline at an equivalent volume. In the S + S and S + F groups, splenectomy was performed to establish the POCD model after fluoxetine or saline pretreatment. The hippocampus was harvested for further analyses after completing the behavioral experiment at 1, 3, and 7 days postoperatively. In part B, mice were randomly divided as follows: saline-pretreated control group (C + S), saline-pretreated surgery group

Fig. 1 Schematic diagram of the experimental program. Part A: The C + F and S + F groups were administered 10 mg/kg intraperitoneal fluoxetine every day for 7 days before surgery. Splenectomy was carried out on day 0 for the S + S and S + F groups. The Morris water maze trial was performed for 5 consecutive days before surgery for training and on postoperative days 1, 3, and 7 for testing. Hippocampal tissues were harvested for further analyses after the behavioral tests. Part B: The TLR4 agonist LPS was microinjected into the lateral ventricle 7 days before surgery in the S + F + LPS group
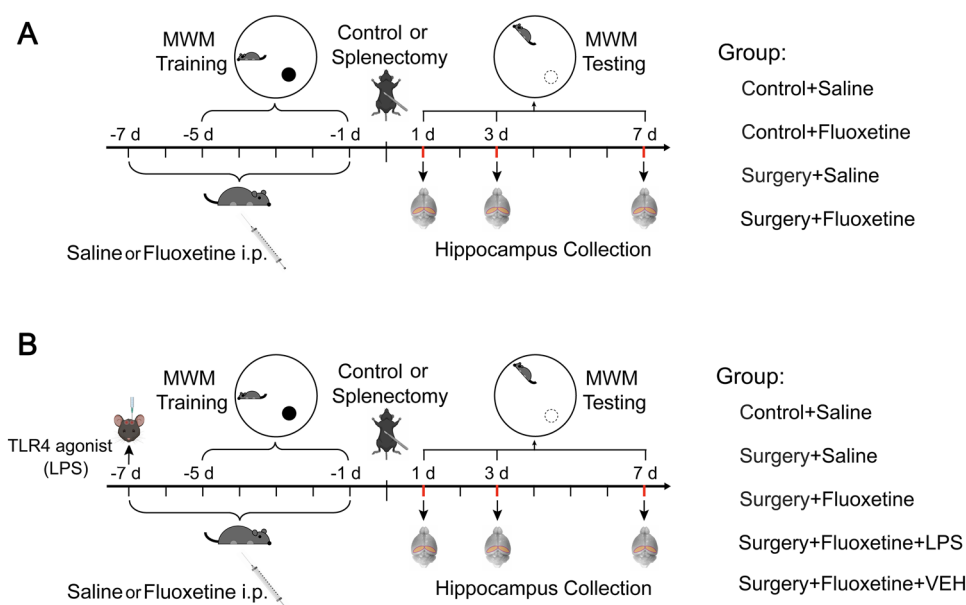
(S + S), fluoxetine-pretreated surgery group (S + F), fluoxetine-pretreated surgery group with intracerebroventricular lipopolysaccharide (LPS) injection (S + F + LPS), and fluoxetine-pretreated surgery group with an intracerebroventricular injection of vehicle (S + F + VEH). LPS (055: B5, Sigma, USA) was dissolved in sterile 0.9% saline and infused into the right lateral ventricle (1 μg, 0.5 μg/μL) seven days before surgery in the S + F + LPS group. An equivalent volume of vehicle (0.9% saline) was intracerebroventricularly injected seven days before surgery in the S + F + VEH group. The other interventions were identical to those described in Part A.

Splenectomy

Mice were anesthetized with 1% pentobarbital sodium (40 mg/kg, i.p.) and fixed on the operating platform in the right lateral decubitus position. The vital signs of mice, including heart rate, blood oxygen saturation, and respiratory rate, were continuously monitored throughout the surgery. Heat management was conducted with a rectal temperature probe and maintained at 36.0−37.0 °C with a thermal blanket (WB-1, Shanghai Yuyan Instruments Co., Ltd. China). After an adequate depth of anesthesia was achieved, a 1 cm incision was performed in the abdominal wall. Then, the spleen was gently exposed, and the blood vessels were ligated, followed by removal of the spleen. After the surgical procedures, the wound was infiltrated with 0.15% ropivacaine for analgesia. All mice were allowed to resume normal activities, diet, and water after recovery.

Stereotaxic injection

Mice were anesthetized using intraperitoneal injection with 1% pentobarbital sodium (40 mg/kg) and transferred to a brain stereotaxic apparatus (71000; RWD Life Science Co., Ltd., China). The TLR4 agonist LPS (1 μg, 0.5 μg/μL) was administered into the right lateral ventricle (from bregma, AP − 0.3 mm, ML 1.0 mm, DV − 2.5 mm) by a Hamilton syringe (80630, Hamilton Co., USA) with a microinjection pump (KDS LEGATO 130, RWD Life Science Co., Ltd., China) [17]. The infusion rate was 0.2 μL/min, and the needle was removed after 10 min of additional residence time to prevent reflux. All infusions of LPS were conducted 30 min before fluoxetine or saline injection.

Cognitive function assessment

We used the Morris water maze (MWM) to assess hippocampus-related spatial learning and memory abilities on postoperative days 1, 3, and 7. In the initial training phase, mice underwent training to use visual cues to swim toward a hidden platform that was submerged 1 cm under the water's surface. At the end of each trial, mice remained on the platform (if they could not find the platform within 60 s, we guided them toward it) for 10 s. After five days of training, splenectomy was performed, and a single 60-s probe trial was scheduled 1, 3, and 7 days after the operation, with the platform removed. The swimming track, escape latency, swimming speed, time spent in the target quadrant, and crossing times were acquired and analyzed by the MWM video analysis system (Zhenghua Biological Instrument Equipment Co., Ltd, China).

Enzyme-linked immunosorbent assay (ELISA)

Hippocampal IL-1β, IL-6, and TNF-α were assayed using ELISA kits (Biosource, Invitrogen, BMS6002, BMS603-2, BMS607-3, USA) on postoperative days 1, 3, and 7. All experimental protocols were conducted according to the manufacturer's instructions. The concentrations of IL-1β, IL-6, and TNF-α were calculated following the standard curve and are presented as pg/mg protein.

Western blot

The western blot protocol was conducted as previously described [18]. Briefly, the protein concentration of hippocampal samples on postoperative day 1 was determined using a BCA protein assay kit (P0010, Beyotime, China). After blocking with 5% nonfat dry milk in TBS-Tween 20 (0.05%) (TBST), the membranes were incubated overnight at 4 ℃ with the appropriate primary antibodies and then incubated for 60 min at 25 ℃ with secondary antibodies after three washes with TBST. The primary and secondary antibodies are listed in Supplementary Table 1. Blots were developed with an enhanced chemiluminescence reagent (AS1059, ASPEN, China) and detected using X-ray film (XBT-1, Kodak, USA). The band intensity was quantified by densitometric analysis using ImageJ for Windows (V 1.8.0, NIH, USA). Relative protein expression levels were obtained by normalization to β-actin.

Nissl staining

On postoperative day 1, brain tissues were harvested and processed with paraformaldehyde (4%) for fixation, embedded in paraffin, and sectioned into 4 μm slices using a sliding microtome (RM2235, Leica, Germany). The sections were stained with toluidine blue (89640, Sigma, USA) and observed using an automated acquisition system (TissueFAXS Confocal Plus SL, Austria). Six high-power domains were randomly selected from the same part of the hippocampus, and the number of normal neurons per domain was determined.

Immunofluorescence and TUNEL staining

Mice were perfusion-fixed, and their brains were removed and sectioned (thickness of 4 μm) on postoperative days 1 and 7. Immunofluorescence staining was performed following routine immunostaining protocols [19]. The primary and secondary antibodies used are listed in Supplementary Table 2. TUNEL staining on postoperative day 7 was performed using a TUNEL assay kit (11684795910, Roche, USA). Finally, the slices were covered with Fluoroshield mounting medium with DAPI (P-36931, Invitrogen, USA) and observed using an automated acquisition system (TissueFAXS Confocal Plus SL, Austria). We randomly selected six high-power areas from different mice to quantify positive cells. The numbers from these fields were averaged and expressed as positive cells per area for each mouse. Tissue sections were analyzed by an observer blinded to the experimental cohorts.

Statistical analysis

We performed statistical analysis using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., USA). Data are presented as the mean ± standard deviation (SD) and were compared using the independent-samples t test or one-way analysis of variance followed by the Bonferroni post hoc test. Differences were considered significant if the two-tailed P value was less than 0.05.

Results

Fluoxetine partially restored neurocognitive function after splenectomy in aged mice

The MWM test was conducted to assess spatial learning and memory capacity before and after splenectomy. During the five consecutive preoperative days of the MWM training phase, the four groups displayed similar spatial learning abilities, as demonstrated by the variation tendency of the escape latency. (Fig. 2A, B). A diminished percentage of time in the target quadrant (Fig. 2C) and platform crossing number (Fig. 2D) were observed in the S + S group compared to the C + S group during the MWM testing phase. However, compared to the S + S group, the S + F group mice displayed a higher percentage of time spent in the target quadrant (Fig. 2C) and an increased number of platform crossings (Fig. 2D). Given that splenectomy alone and fluoxetine treatment did not change swimming speed (Fig. 2E), the above results indicate that

fluoxetine pretreatment can preserve learning and memory and potentially mitigate POCD.

Fluoxetine downregulated neuroinflammation in the hippocampus of aged mice following surgery

We measured the levels of hippocampal TNF-α, IL-6, and IL-1β on postoperative days 1, 3, and 7. The proinflammatory cytokines in the S + S group within three days after surgery were above the levels of the control group, yet fluoxetine treatment depressed surgery-induced inflammation, as shown in Fig. 3. Intriguingly, following steep increases in cytokine levels on postoperative day 1, the surgical groups with or without fluoxetine treatment presented decreases in the protein levels of the three cytokines. This result implies that fluoxetine moderately curbs the upregulation of proinflammatory cytokines.

Fluoxetine suppressed hippocampal microglial activation in aged mice following surgery

Microglial activation promotes neuroinflammation [20]. Hence, we explored whether the anti-inflammatory effect of fluoxetine was associated with the inhibition of microglial activation. In the S + S group, the numbers of IBA-1-positive cells in the hippocampal CA1, CA3, DG, and HF regions increased at 24 h after splenectomy (Fig. 4). Notably, fluoxetine treatment remarkably restrained this increase, restoring the IBA-1-positive cell count to approximately normal levels in the hippocampal CA1, CA3, DG, and HF regions, as shown in Fig. 4. These results further reveal the possible anti-inflammatory mechanism of fluoxetine, inhibiting microglial activation behind its cognitive restorative effect.

Fluoxetine attenuated hippocampal neuron apoptosis in aged mice following surgery

Neural apoptosis is associated with neuroinflammation and is a crucial pathological factor of cognitive dysfunction [21]. Therefore, we applied Nissl staining, western blotting, and confocal immunofluorescence analysis to examine the extent of hippocampal neuronal apoptosis. The number of surviving neurons on postoperative day 1 in the Nissl staining experiment decreased in the S + S group (Fig. 5A, B). In contrast, in the S + F group, this decrease was mitigated (Fig. 5A, B). The expression of the proapoptotic proteins Bax and Caspase 3 dramatically increased in the S + S group after surgery. In contrast, the apoptosis-inhibiting protein Bcl-2 decreased, which was reversed by fluoxetine treatment (Fig. 5C–F). As shown in the confocal immunofluorescence analysis on postoperative day 7, the percentage of apoptotic neurons labeled by TUNEL and NeuN was markedly increased in the S + S group, and the increase was alleviated
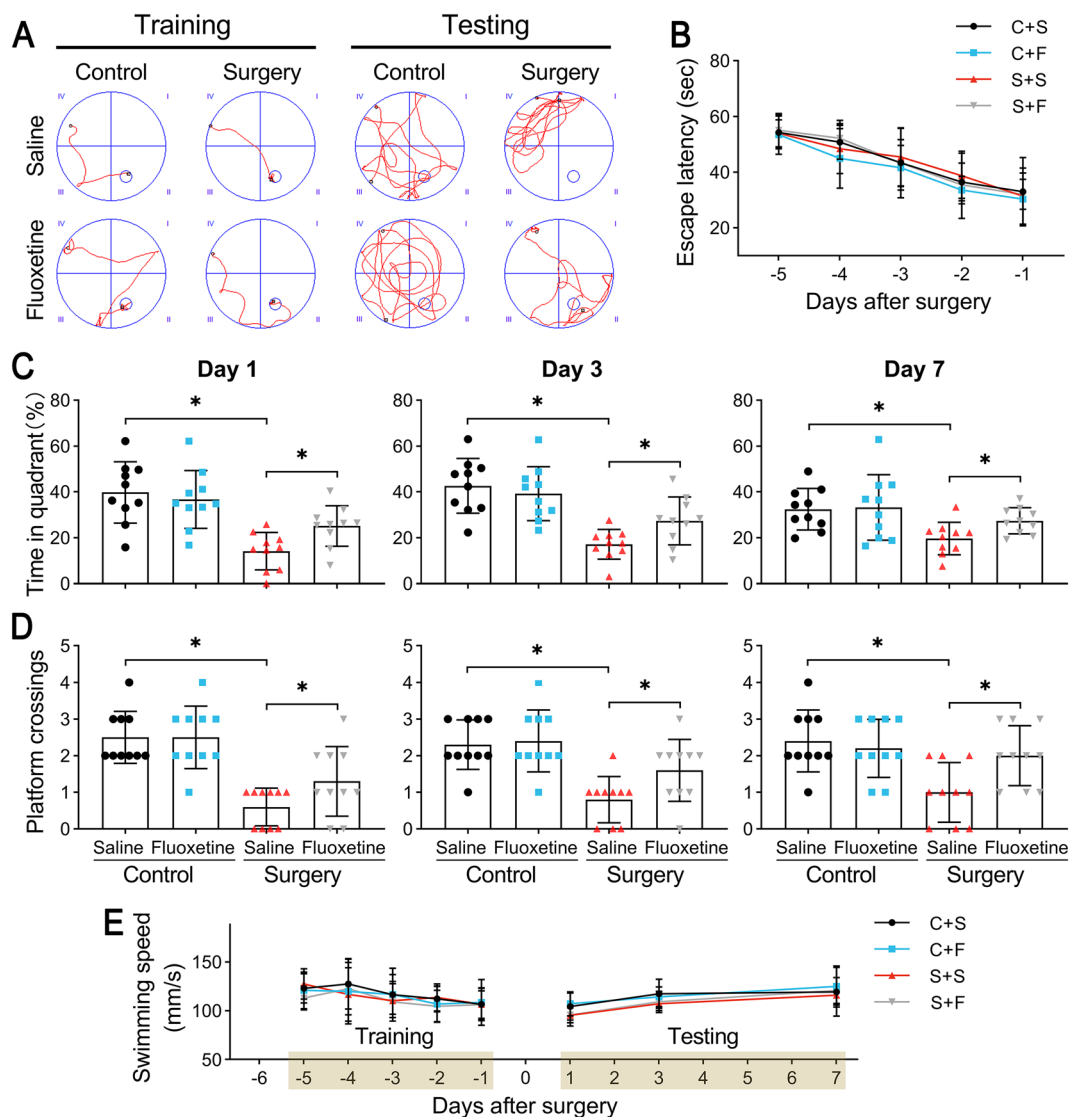
Fig. 2 Fluoxetine relieved cognitive impairment after surgery. **A** Representative trajectories of mice during the MWM training and testing phases. **B** The line chart shows the escape latency represented by the time elapsed before the mice found the platform during the MWM training phase. **C** The bar charts represent the percentages of the time taken to swim in the target quadrant during the MWM testing stage at 1, 3, and 7 days postoperatively. **D** The bar charts represent the crossings over the target platform during the MWM testing stage at 1, 3, and 7 days postoperatively. **E** The line chart shows the swimming speed of the mice during the MWM training and testing phases. $N = 10$ for each group; data are presented as the mean $\pm$ SD, *$P < 0.05$

in the S + F group (Fig. 5G, H). The above results illustrate that fluoxetine can mitigate neural apoptosis, possibly relieving POCD.

Fluoxetine inhibited TLR4/MyD88/NF-κB pathway activation in microglia of aged mice following surgery

To evaluate whether treatment with fluoxetine could alleviate surgery-induced activation of the TLR4/MyD88/NF-κB pathway in microglia, we performed colocalization of TLR4 and IBA-1 fluorescence immunostaining

and detected protein expression. In the confocal immunofluorescence analysis, the number of TLR4^{+}/IBA-1^{+} cells increased in the S + S group. In contrast, the increase in the S + F group was milder, preliminarily implicating the suppressive effect of fluoxetine on the TLR4/MyD88/NF-κB pathway of activated microglia (Fig. 6A, B). Then, we further quantified the levels of IBA-1, TLR4, MyD88, and phosphorylated NF-κB p65 (p-NF-κB p65). The levels of IBA-1, TLR4, MyD88, and p-NF-κB p65 were elevated in the S + S group, whereas this elevating trend was attenuated in the S + F group (Fig. 6C–E). These results

Fig. 3 Fluoxetine attenuated the temporary increase in proin-flammatory cytokine levels after surgery. ELISA kits were used to quantify the levels of TNF-α, IL-6, and IL-1β at 1, 3, and 7 days postoperatively. The bar charts show the proinflam-matory cytokine levels on these three days. $N = 6$ for each group, data are presented as the mean $\pm$ SD, $*P < 0.05$
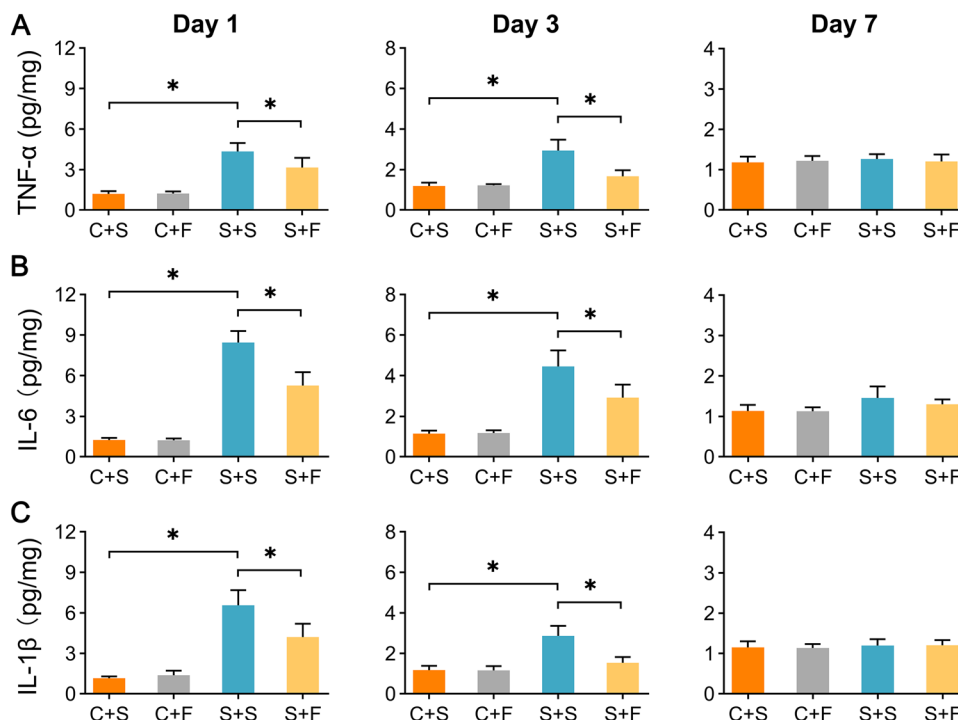
indicated the suppressive effect of fluoxetine on the TLR4/MyD88/NF-κB pathway in microglia.

The protective potency of fluoxetine against POCD is related to suppressing TLR4/MyD88/NF-κB signaling pathway activation in microglia

To investigate the possible relationship between fluoxetine's suppressive effect on the TLR4/MyD88/NF-κB signaling pathway and its alleviating effect on POCD in aged mice, we injected the TLR4 agonist LPS (1 μg, 0.5 μg/μL) into the lateral ventricle of aged mice to overexpress TLR4 before splenectomy (Fig. 7A–C). Compared with the S + F group, the S + F + LPS group displayed a shorter time in the target quadrant (Fig. 7D) and fewer target crossings (Fig. 7E), similar to the S + S group. Correspondingly, TUNEL experiments showed that the percentage of apop-totic neurons labeled by NeuN markedly increased in the S + S and S + F + LPS groups, whereas neuronal apoptosis was reduced in the S + F and S + F + VEH groups (Fig. 7F, G). Meanwhile, the ELISA results indicated that the expres-sion of TNF-α, IL-6, and IL-1β on postoperative day 1 in the S + F + LPS group was far above that in the S + F group (Fig. 8A).

Then, to elucidate the effect of fluoxetine on TLR4 on microglia, we performed immunofluorescence colocaliza-tion of IBA-1 and TLR4, where the expression of TLR4 on microglia in the S + F + LPS group was above that in the S + F group (Fig. 8B, C). Furthermore, we obtained consistent results for the expression levels of TLR4, MyD88, and p-NF-κB p65 (Fig. 8D, E). These results suggested that fluoxetine may prevent neuroinflammation, attenuate neural apoptosis, and alleviate POCD by inhibiting the activation of the TLR4/MyD88/NF-κB pathway in microglia.

Discussion

In the current study, we demonstrated that preoperative application of fluoxetine protected against cognitive decline in aged mice following surgery, which was associated with alleviating hippocampal neuroinflammation via the inhi-bition of the TLR4/MyD88/NF-κB pathway in microglia. Fluoxetine pretreatment is promising as a candidate inter-vention for POCD.

Impairment of hippocampus-dependent spatial memory is the typical manifestation of POCD [22–24]. As a robust and reliable test sensitive to hippocampus-dependent cog-nitive dysfunction, the MWM test was chosen to evalu-ate the establishment of the POCD model in our study [25, 26]. The impaired water maze performance of mice following splenectomy indicated the successful establish-ment of the POCD model and was improved by fluox-etine pretreatment. Additionally, fluoxetine effectively prevented the acute aggravation of neuroinflammation induced by splenectomy in the hippocampus, which was correlated with the inhibition of microglial activation, in line with a previous study [27]. Intriguingly, following
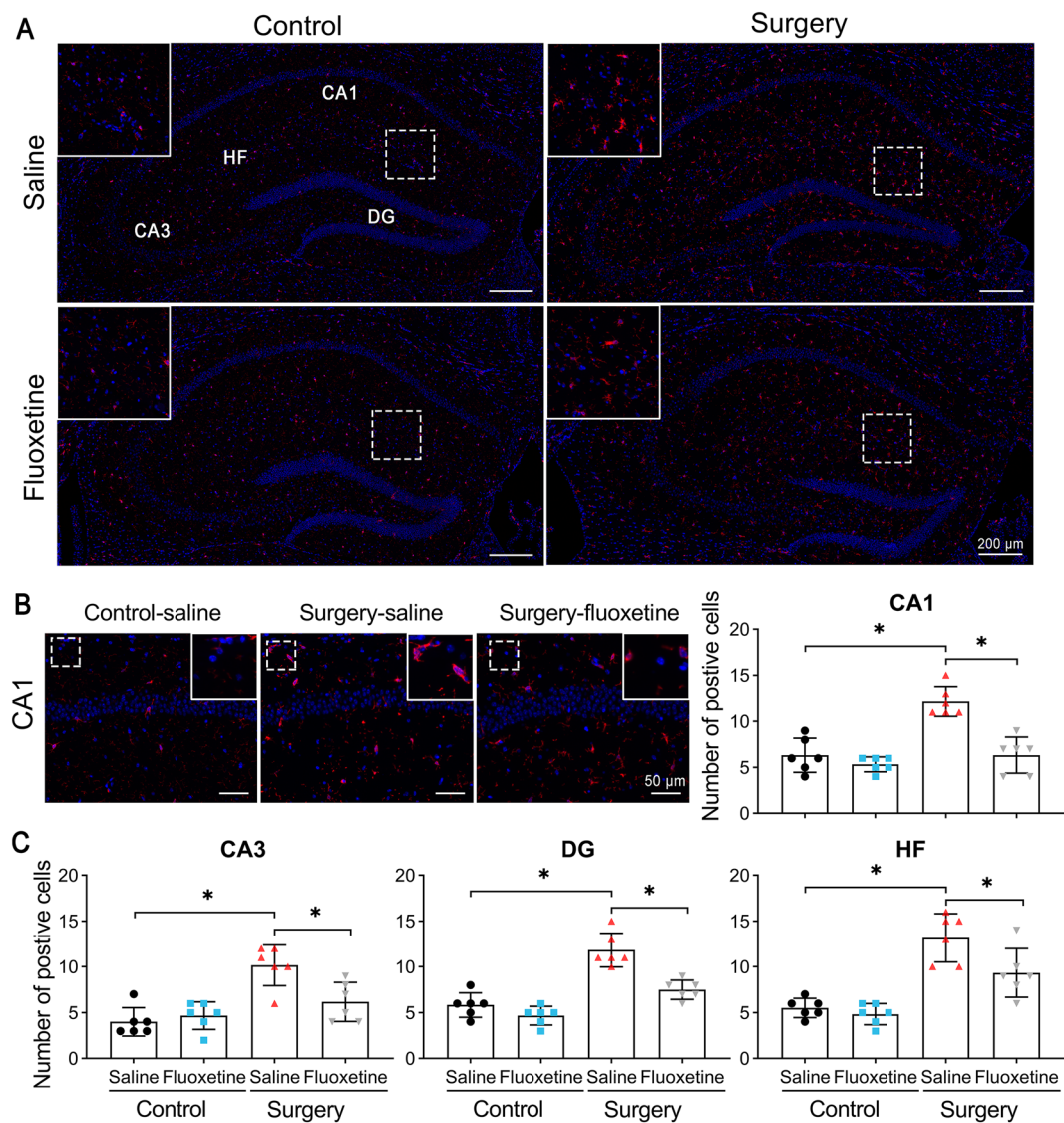
Fig. 4 Fluoxetine reduced the number of IBA-1-positive microglia in the hippocampus after surgery. **A** Representative fluorescence images show the immunoreactivity of IBA-1-positive microglia 24 h after surgery. **B** Regional magnification of the hippocampal CA1 field highlights fluoxetine's effect on IBA-1-positive microglial increments. **C** The bar graphs represent IBA-1-positive microglial counts in the hippocampal CA1, CA3, DG, and HF regions. $N=6$ for each group; data are presented as the mean $\pm$ SD, $*P < 0.05$

steep increases in cytokine levels on postoperative day 1, surgical mice presented decreases in the protein levels of the three cytokines. These results suggest that the rage of proinflammatory cytokines may be transient and that profound detrimental impacts on cognition may result from more extensive homeostatic or functional changes in the hippocampus triggered by proinflammatory cytokines, such as neuronal apoptosis or synaptic plasticity alteration [21, 28]. To test this hypothesis, we detected the apoptotic neuron rate. We found that neural apoptosis of surgical mice in the hippocampus was still observed along with cognitive decline on postoperative day 7, although

proinflammatory cytokine levels had returned to normal. Although we discovered that neuronal apoptosis was curbed by fluoxetine, whether fluoxetine directly affects neurons is still unclear and worth exploring in vitro. At this point, the neuroprotective effect of fluoxetine was preliminarily clarified, which was associated with its anti-neuroinflammatory properties.

Of all TLR family members, TLR4 is the key receptor that activates microglia, which play a vital role in neuroinflammation [7]. Activated microglia cause an inflammatory cascade reaction, which may result in neurological dysfunction, including POCD. A preclinical study of
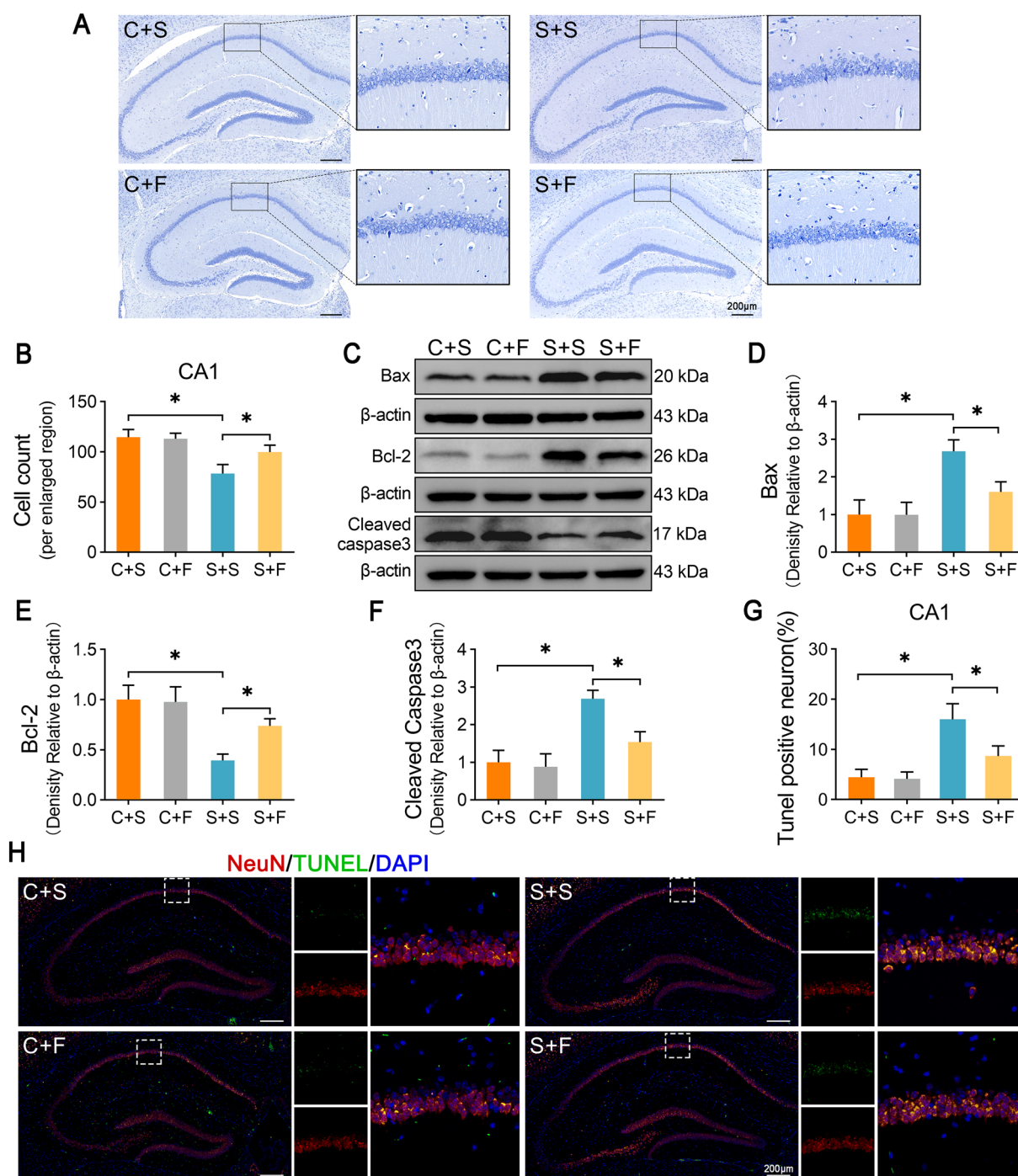
Fig. 5 Fluoxetine inhibited neural apoptosis after surgery. **A**, **B** Nissl staining shows the extent of neural survival in the hippocampus on postoperative day 1. **C–F** Representative western blot bands for apoptosis-related proteins are presented in bar graphs. **G**, **H** Fluorescence images show NeuN (red) and TUNEL (green) coexpression in the hippocampus on postoperative day 7, and the statistics are represented in bar graphs. $N = 5$–6 for each group; data are presented as the mean $\pm$ SD, $*P < 0.05$

cerebral ischemia–reperfusion injury indicated that upregulated TLR4, activated microglia, and elevated inflammatory cytokine levels might induce a neuroinflammatory response and reduce neuron viability [29]. Likewise, we found TLR4/MyD88/NF-κB signaling pathway activation in microglia of POCD mice, which was inhibited by fluoxetine pretreatment. More importantly, we used an intracerebroventricular injection of LPS, a TLR4 signaling
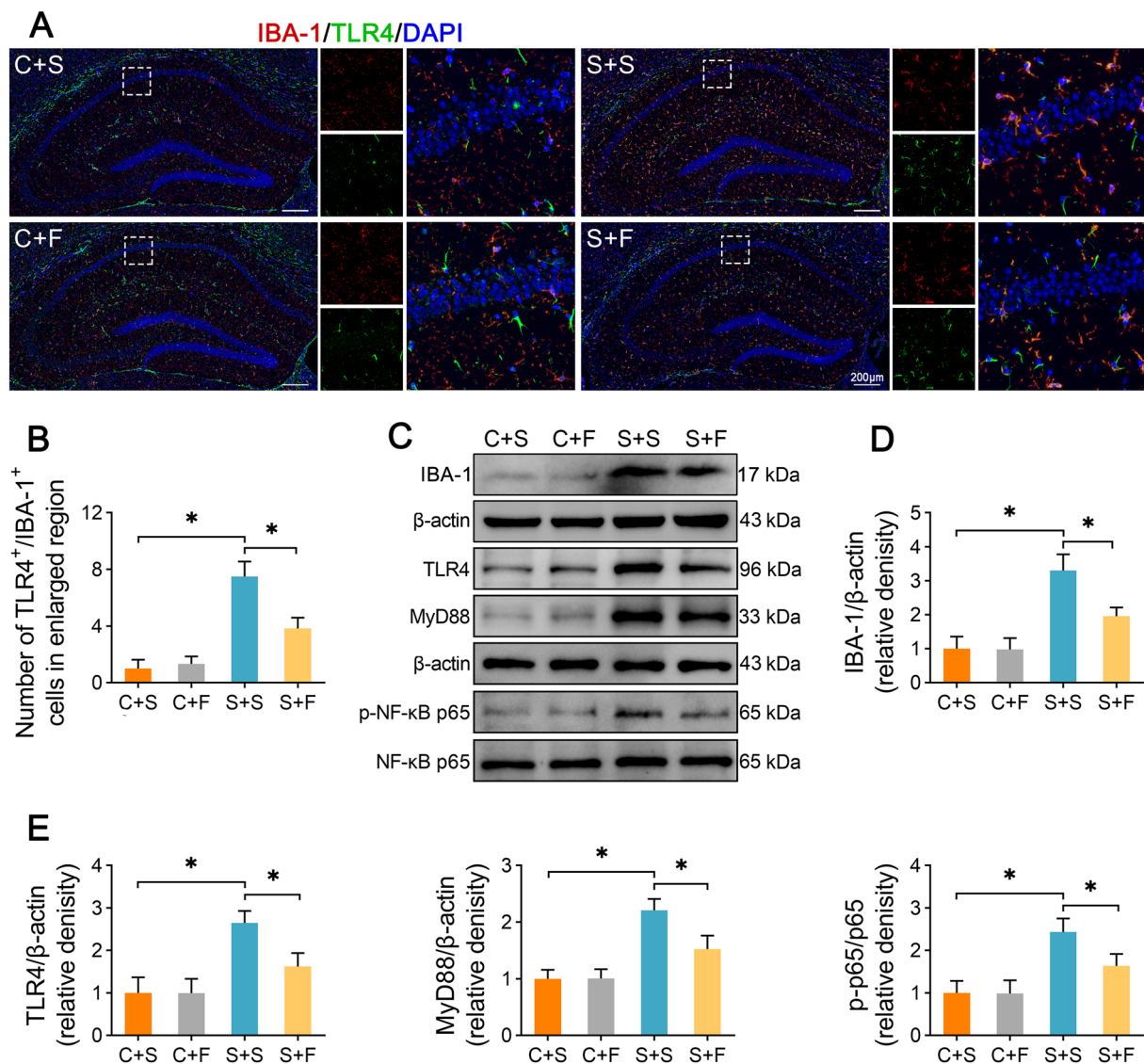
Fig. 6 Fluoxetine repressed the hippocampal TLR4/MyD88/NF-κB signaling pathway. **A**, **B** Fluorescence images show IBA-1 (red) and TLR4 (green) coexpression, and the statistics are displayed in a bar graph. **C–E** Representative western blot bands for IBA-1, TLR4, MyD88, p-NF-κB p65, and total NF-κB p65, with statistics presented in bar graphs. $N = 5–6$ for each group, data are presented as the mean $\pm$ SD, and $*P < 0.05$

pathway activator [17], to trigger overactivation of the TLR4/MyD88/NF-κB signaling pathway and found that the protective effect of fluoxetine was reversed. The above evidence elucidates TLR4/MyD88/NF-κB signaling pathway involved, at least in part, in POCD and the protective mechanisms of fluoxetine on POCD in aged mice, as shown in Fig. 9. Briefly, TLR4 and MyD88 are elevated in activated microglia induced by surgery, inducing NF-κB p65 phosphorylation and nuclear translocation, which upregulates the levels of inflammatory cytokines, promotes neuroinflammation, and induces neuronal apoptosis in the hippocampus. Fluoxetine ameliorates the neuroinflammatory

response and mitigates apoptosis, which is closely related to inhibiting TLR4/MyD88/NF-κB pathway activation in microglia, thus presenting a potential protective effect on POCD.

Our study indicated that the development of drugs targeting the TLR4/MyD88/NF-κB signaling pathway might be a candidate protective intervention for POCD. However, the development of new medications suffers from multiple challenges, including long development cycles, high costs, and undefined side effects, especially in elderly individuals [30, 31]. Searching for a drug commonly prescribed in clinical practice with a neuroprotective effect may be an alternative

Fig. 7 Activation of TLR4 abolished the effects of fluoxetine in restoring cognitive function and mitigating neural apoptosis. **A–C** Microinjection of the TLR4 agonist LPS into the right lateral ventricle. Western blot analysis confirmed the activation of TLR4 in the hippocampus in the S+LPS group. **D**, **E** The bar charts represent the percentages of the time taken to swim in the target quadrant and platform crossings during the MWM testing phase on postoperative days 1, 3, and 7. F, G Fluorescence images show NeuN (red) and TUNEL (green) hippocampal coexpression on postoperative day 7, and the statistics are represented in bar graphs. $N = 5$–6 for each group; data are presented as the mean±SD, *$P < 0.05$
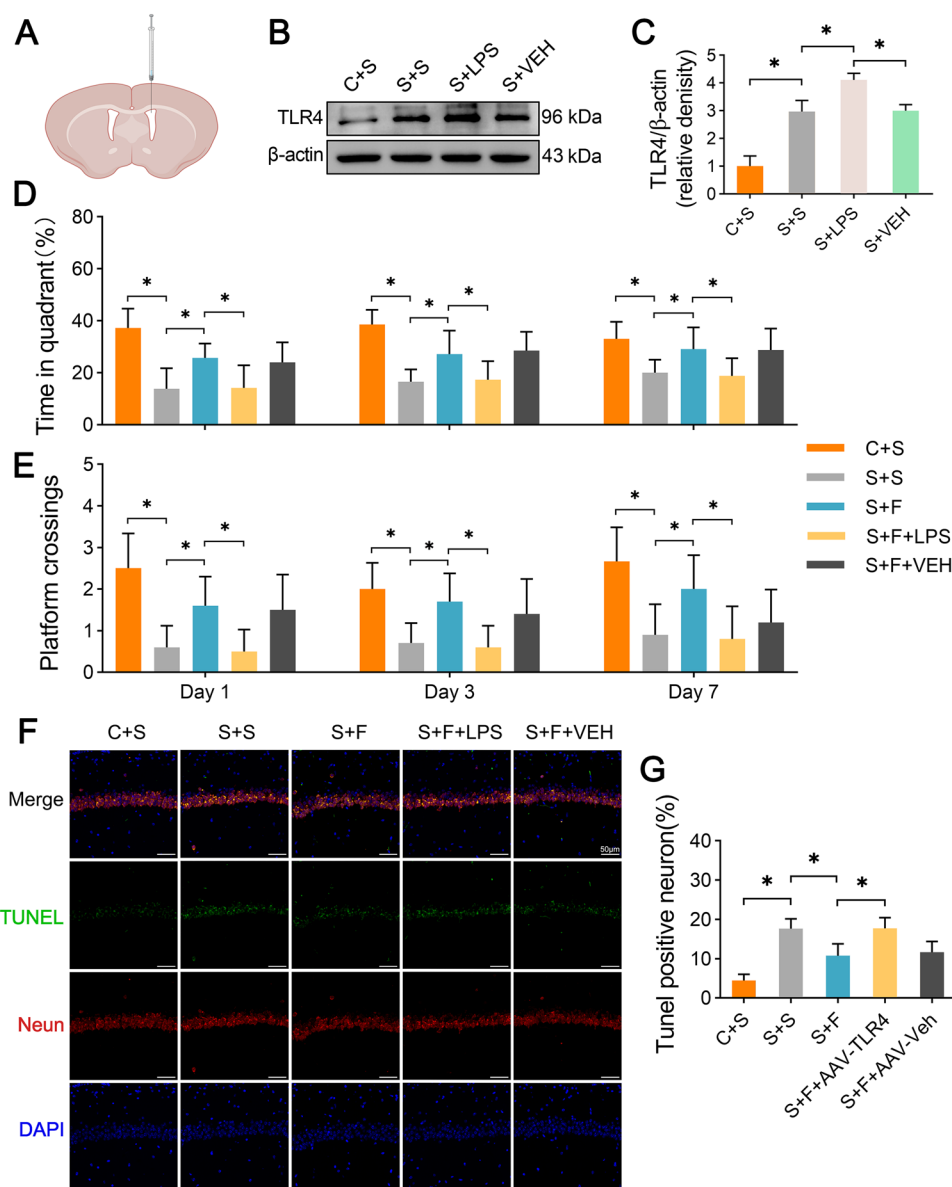
to prevent POCD. This study confirmed the effectiveness of fluoxetine pretreatment for POCD in aged mice. In addition, given the proven protective effects in treating neurological diseases [32–34] and the relatively high safety profile [35], fluoxetine may be a promising pharmaceutical intervention for POCD. We have registered and are conducting a randomized controlled trial [36] to verify the clinical efficacy of fluoxetine for POCD.

The following limitations of our study should be addressed. First, we only investigated hippocampus-dependent spatial learning function using the MWM test. However, POCD is a syndrome of multiple neurological disorders, including fear memory formation and mood disorders,

possibly involving several brain regions in addition to the hippocampus [37]. The changes in neurological function in a single brain region may not comprehensively reflect the protective effect of fluoxetine on POCD. Second, the current study mainly focuses on the impact of fluoxetine in the hippocampus, yet there is a lack of experimental data to validate this hypothesis in peripheral blood. Third, we cannot exclude the influence of antidepressant effects on memory improvement in this study. Further studies are necessary to explore the correction between antidepressant and anti-neuroinflammation in the setting of fluoxetine for reducing POCD.
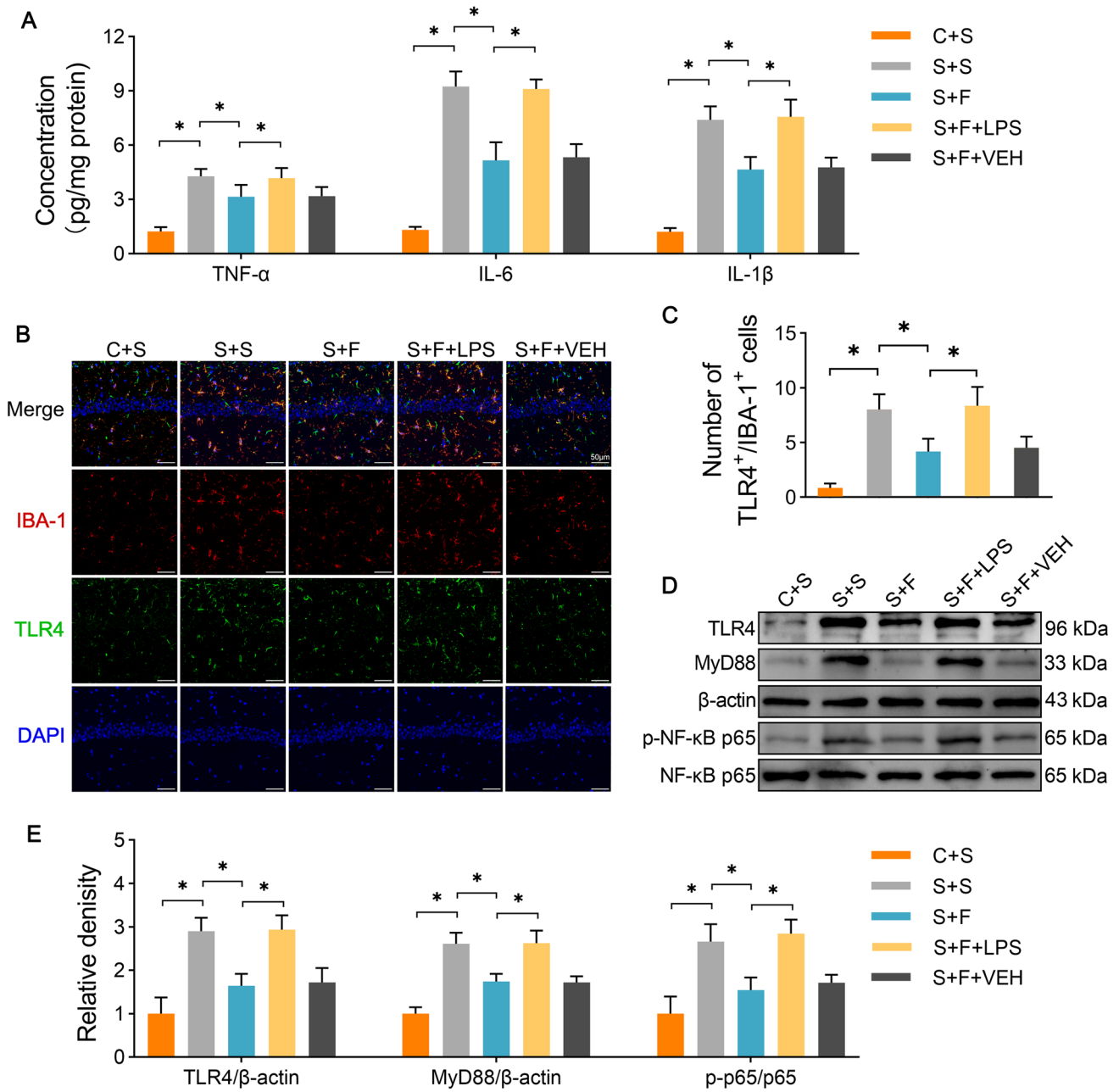
Fig. 8 Activation of the TLR4/MyD88/NF-κB pathway reversed the anti-inflammatory effect of fluoxetine. **A** The bar charts show the expression of TNF-α, IL-6, and IL-1β at 1 day postoperatively. **B**, **C** Fluorescence images show IBA-1 (red) and TLR4 (green) coexpression, and the statistics are displayed in a bar graph. **D**, **E** Representative western blot bands for TLR4, MyD88, p-NF-κB p65, and total NF-κB p65, with statistics presented in bar graphs. $N = 5$–6 for each group; data are presented as the mean ± SD, *$P < 0.05$

Conclusion

These results demonstrate the protective property of fluoxetine pretreatment on POCD through alleviating neuroinflammation, with putative involvement of repressing the TLR4/MyD88/NF-κB signaling pathway on activated microglia. Although the translatability of this study to humans and the safety of the novel fluoxetine application are unclear, the maturity of fluoxetine treatment in clinical practice provides reasonable enlightenment for further examination of its properties in preventing and alleviating POCD clinically.
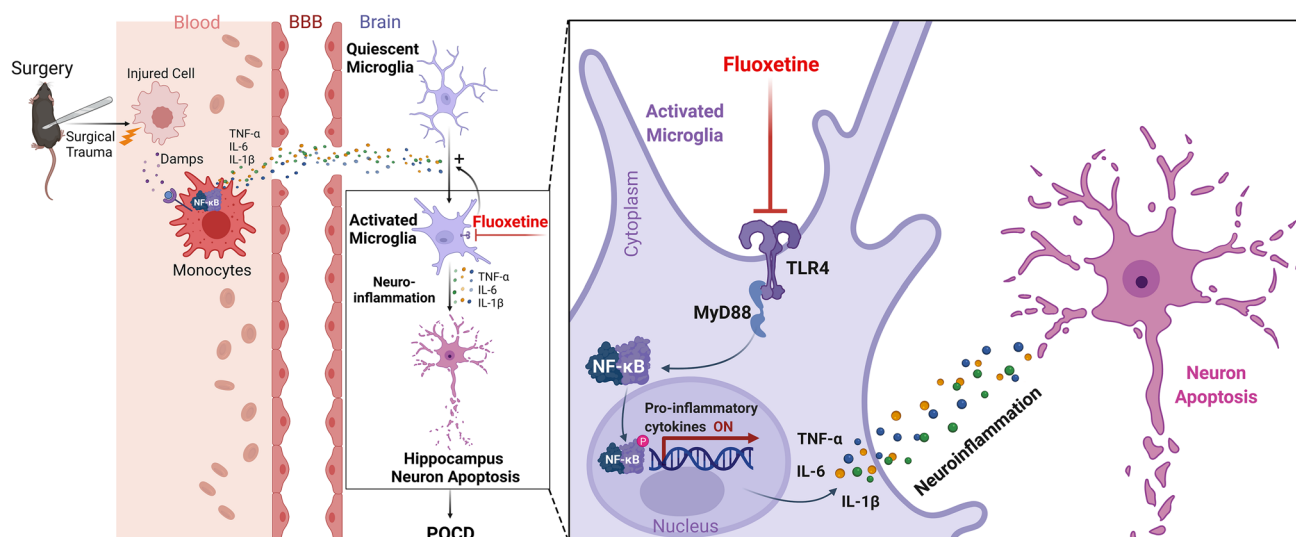
Fig. 9 Partial pathological mechanism of POCD and potential therapeutic effect of fluoxetine via inhibition of TLR4/MyD88/NF-κB pathway activation. Surgery triggers a peripheral inflammatory reaction extending to the central nervous system. Microglia are activated in the inflammatory setting, secrete proinflammatory factors, potentiate neural impairment, and eventually cause POCD. The magnified detailed graphic focuses on the anti-neuroinflammatory mechanism of fluoxetine explored in the current study

Author contributions YY, DL, and XZ: conceived and designed the experiments. DL, LL, and YW: performed the experiments. YC and YW: analyzed the data. DL and YC: wrote the draft. YY and XZ: revised the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that they have no competing interests.

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