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Original Article

Activation of NF-κB Signal Pathway and Downstream IL-1β Expression in Hippocampal Astrocytes of LPS-Induced Aged Rats

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ABSTRACT

Background: The activation of nuclear factor-kappa B (NF-κB) signal pathway and downstream expression of IL-1β may play a pivotal role in neuroinflammation and cognitive dysfunction. We previously reported that a single lipopolysaccharide (LPS) dose induces prolonged neuroinflammation which is associated with astrocytic NF-κB signal pathways in aged rats. Blockade of the pathway by pyrrolidine dithiocarbamate (PDTC) in astrocytes could markedly suppress them, which may provide innovative ideas for clinical improvement for postoperative cognitive dysfunction (POCD).

Methods: Rats were randomly assigned to vehicle, LPS, LPS + PDTC groups (n = 8 per group, per time point). 1) vehicle control (0.9% NaCl i.p.), 2) LPS (2 mg/kg i.p), 3) LPS + PDTC (LPS and PDTC50 mg/kg i.p.). After injection, whole brain tissues acquired at a series of time points (days 1, 3,7, 15 and 30) was prepared to carry out immunofluorescence, isolated hippocampal tissues were used for enzyme-linked immunosorbent assay (ELISA). NF-κB p65, p-IκBα and IL-1β were detected respectively by immunofluorescence and IL-1β protein levels were determined by ELISA. A separate cohort of rats (n=8-10/group) were tested in a Morris water maze (MWM) for spatial learning and memory.

Results: The PDTC treatment suppressed the LPS-induced canonical NF-κB signaling pathway- nuclear translocation of NF-κB p65 and IκBα phosphorylation, positive expression of GFAP and IL-1β in hippocampal astrocytes of aged rats by immunofluorescence and reduced cognitive dysfunction by MWM test; The LPS-induced IL-1β protein increase in hippocampus of aged rats was attenuated by PDTC with ELISA.

Conclusion: These findings suggest that NF-κB signaling pathway and downstream IL-1β in senescent astrocytes may play important roles in age-related neuroinflammation and cognitive dysfunction and provide potential discovery tool and therapeutic methods with PDTC for verifying the NF-κB signal pathway and treating cognitive impairment, especially POCD. (Funded by National Clinical Research for Geriatric Disorders of China, and Beijing Municipal Administration of Hospitals' Ascent Plan.)

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Cognitive dysfunction frequently occurs in the elderly, which is associated with multiple risk factors including surgery, trauma, infection and anesthesia. Among them, POCD has attracted much attention. Although the exact etiology and mechanism of POCD remain unclear and there are limited strategies that can effectively prevent its occurrence and provide treatment in clinical practice, studies have indicated that neuroinflammation could play a pivotal role in cognitive dysfunction.

Neuroinflammation impacts mainly glial cells, such as microglia and astrocytes, which coincidently become more activated during normal brain aging (1). Previous studies have shown that microglia activation is closely related to elderly cognitive dysfunction (2), and peripheral LPS challenges induce microglial hyperactivity and exaggerated expression of the pro-inflammatory IL-1 β, which is associated with persistent neuronal damage, changes in long-term potentiation, and cognitive dysfunction in aged mice (3, 4). However, systemic LPS treatment induces prolonged reactive astrocytes and neuroinflammation (5). In age- related neuroinflammation and cognitive impairment, chronic astrocyte reactivity is characteristic of the senescence-associated secretory phenotype (SASP) (6), which has been shown to exhibit increased glial fibrillary acidic protein (GFAP) expression, as well as related cytokines and protein accumulation of toxic matter. Therefore, there is an increased interest in the role of glial-produced pro-inflammatory cytokines regarding the modulation of learning and memory processes.

Inflammatory cytokines, such as TNF- α, IL-1β, IL-2, and IL-6, have been shown to play a role in disease, depression, fatigue, anxiety, apathy, and cognitive impairment (7, 8), as well as influence disease behavior (9). IL-1β overexpression in the hippocampus has been shown to correlate with memory impairment of hippocampal long-term potentiation (LTP) (10, 11). These cytokines are mediated by interactions between the brain and the immune system, impairing neurons and synaptic plasticity. The regulation of cytokine reactions can also lead to neurological complications, including cognitive damage (12, 13), anorexia, emotional disorders, and depression (14-18). Although many inflammatory factors are involved in cognitive functional damage, the role of inflammatory cytokines in the neuroinflammatory process remains unclear.

In our previous study, a single LPS dose induced prolonged neuroinflammation, which has been suggested to be associated with NF-κB in aged rats (14). Therefore, in the present study, we explored the impacts of IL-1β, which is secreted by astrocytes via the NF-κB signal pathway during prolonged hippocampal inflammation (14), on cognitive dysfunction in aged rats. We hypothesized that LPS-induced activation of NF-κB signal pathway may induce persistent IL-1β expression and then cognitive impairment.

PDTC, the NF-κB signal pathway inhibitor, was used to evaluate whether the cognitive function of aged rats was improved through the inhibition of neuroinflammation and IL-1β overexpression, which offer a new strategy for the prevention of cognitive impairment and alleviation of neuroinflammation.

MATERIALS and METHODS

Animals preparation

Male Wistar rats (20 months old) weighing 550–850g were used for all experiments. The rats were housed at 23°C on a 12-h light/dark cycle with ad libitum access to food and water. The rats were housed two to a cage (52 L × 30 W × 21 H in cm), in a standard animal colony shared by several experimenters. All experiments were conducted in accordance with protocols approved by the Capital Medical University Biomedical Ethics Committee Experimental Animal Ethics Branch (Approval No. LA2012-38). All efforts were made to minimize suffering and the number of animals used.

Rats were randomly assigned to Vehicle (n = 8), LPS, LPS + PDTC groups (n = 8 per group, per time point). 1) Vehicle control (0.9% NaCl i.p.), 2) LPS (2 mg/kg i.p., 055: B5, Sigma, St Louis, MO, USA), 3) LPS + PDTC (LPS and PDTC 50 mg/kg i.p., P8765, Sigma, St. Louis, MO, USA).

There was no death in rats of LPS group during the observation period based on previous work on LPS dosing (14). PDTC was administered 1 hr before LPS injection at a dose (50 mg/kg) shown to inhibit NF-κB activation (15). Vehi-

cle group received an identical volume of saline.

Sample preparation

Following isoflurane anesthesia (Forene, Abbott Laboratories, Queensborough, UK), the rats of LPS and LPS + PDTC groups were sacrificed on 1, 3, 7, 15, and 30 d, the rats of Vehicle on 1 d. After decapitation, the rat brains were rapidly excised and frozen in liquid nitrogen where they were maintained at −80°C for later use. All dissections were performed on an ice-cold, frosted, glass plate. Eight rats per group per time point were anesthetized for hippocampal harvesting. Four whole brains of each group were quickly mounted in OCT compound (Sakura Finetek USA, Inc., Torrance, CA, USA) and used for immunofluorescence. Brains from the remaining four rats from each group were used for ELISA.

Immunofluorescence

NF-κB p65 nuclear translocation and expression of p-IκBα and IL-1β were detected on adjacent tissue sections respectively by immunofluorescence. Brains were processed on a freezing microtome (CM1850, Leica Microsciences, Mannheim, Germany) and consecutive 20-μm thick hippocampal coronal sections were selected from Bregma −2.30 and Bregma −3.60 according to the atlas by Paxinos and Watson.

Tissue sections were fixed in ice-cold, 4% paraformaldehyde for 15 min and rinsed 4 times in PBS for 10 minutes each time. The sections were permeabilized using 0.3% Triton X-100 (Sigma-Aldrich, St Louis, MO, USA) for 1 h, blocked with 5% horse serum (8178102, Gibco) for 1 h at room temperature, and then incubated with the following primary antibodies: rabbit anti-NF-kB p65 IgG (1:100; catalog number Ab7970, Abcam, Cambridge, MA, UAS), mouse anti-p-IκBαIgG (1:100; catalog number Ab12135, Abcam, Cambridge, MA, UAS), goat anti-IL-1βIgG (1:100; catalog number AF-501-NA, R&D, Systems, Inc., Minneapolis, MN, USA), mouse anti-GFAP IgG (1:1000; catalog number MAB360 Millipore, Billerica, MA, USA) and rabbit anti-GFAP IgG (1:1000; catalog number Z0334; Dako) for 2 h at room temperature, and then overnight at 4°C.

The sections were washed three times with PBS and incubated for 2 h with a 1:500 dilution of the secondary antibodies: Alexa-488-coupled donkey anti-mouse IgG (1:500; catalog number: A21202, Invitrogen, Paisley, UK), Alexa 488-coupled donkey anti-rabbit IgG(1:500; catalog number: A21206, Invitrogen), Alexa-594-coupled donkey anti-rabbit IgG(1:500; catalog number: A21207, Invitrogen), Alexa-594-coupled donkey anti-mouse IgG(1:500; catalog number: A21203 Invitrogen), Alexa-594-coupled donkey anti-goat IgG (1:500; catalog number: A11058, Invitrogen).

Negative control sections, in which primary antibodies or secondary antibodies were replaced by PBS, revealed no labeled cells. Cell nuclei were counterstained with Hoechst 33342 (1:1000; Roche, Mannheim, Germany). Sections from all time points were stained simultaneously to provide uniform conditions for subsequent quantitative analysis by fluorescence staining. Samples were analyzed with a confocal microscope (Leica TCS SP5, Leica, Benshein, Germany) and the rate of NF-κB p65, p-IκBα and IL-1β positive expression in the GFAP-positive astrocytes of the hippocampal DG region were analyzed using Adobe Photoshop CS3V10.0.1.0. Ten visual fields were counted for each section, and 10 values of positive expression were counted and the mean value calculated by averaging the counts from the results of the three experiments at different time points (14).

Enzyme-linked immunosorbent assay

Isolated hippocampal tissues were homogenized in 100 mg tissue/ml RIPA Lysis Buffer (RIPA Lysis Buffer: 0.5 M Tris-HCL, pH 7.4, 1.5 M NaCl, 10% NP-40, 2.5% deoxycholic acid, 10 mM EDTA) (20-188, Millipore). Immediately prior to use, 1 μg/ml pepstatin (phosphatase inhibitor) and protease inhibitor cocktail tablets (Roche Diagnostics, Indianapolis, IN) were added to the RIPA Lysis Buffer. The resulting suspension was sonicated with an ultrasonic cell disrupter (Bandelin, OSTC) and centrifuged at 14,000 × g at 4°C for 15 min.

The supernatant was collected and stored at −80°C for ELISA. Protein quantification was performed using BCA Protein Assay Kit (23227, Thermo, USA) prior to ELISA assay. ELISAs were performed according to the manufacturer's instructions (IL-1β RLB00, R&D Systems.) and

optical density determined at 450 with a microplate reader (BioRad, Richmond, CA). IL- 1β protein levels were determined and normalized to total protein (pg/mg total protein). The ELISA assay was repeated twice.

The MWM test

One day after treatment, a separate cohort of rats (n = 8- 10/group) were tested in a Morris water maze for spatial learning and memory as previously described with some modification (16). The Morris water maze consisted of a large, circular, stainless steel pool (diameter 150 cm, height 60 cm) with a black-painted bottom. The maze was situated in a large room and was separated by a blue curtain from outside stimuli. It was filled with room temperature (25 ± 2°C) water to a depth of 20 cm, and a clear, plastic, circular platform (diameter: 10 cm) was located 1.5 cm below the water surface.

Various objects or geometric images, such as circles, squares, and triangles with different colors, were hung on the curtain as visual spatial cues. Swimming activity of each rat was tracked via a camera linked to a computer monitoring system. Escape latency to locate platform, total distance traveled, time and distance spent in each quadrant, and swimming speeds were calculated.

The classic Morris water maze test paradigm comprises two parts: the hidden platform test and the probe test.

Hidden platform test

The rats underwent four daily training trials for 5 consecutive days. The platform was located in quadrant II for all trials. During each trial, the rats were gently placed in the water facing the wall of the maze at one of the four equally spaced start positions (quadrant I, II, III, or IV). The rats could swim for 60 s to locate the hidden platform during each trial. When successful, the rat could stay for 5 s on the platform. If unsuccessful within 60 s, the rat was then physically placed on the platform for 20 s. A 3–5 min interval was allowed between each trial. The time to locate the platform and the distance to platform were recorded to compare between groups.

Spatial probe test

On day 7, a series of probe trials was conducted and the platform was removed. We choose quadrant IV to place the rats into the pool, and recorded the swimming activity of each rat in a 30-s period of time. Platform site crossovers and percentage of time spent in the previous platform quadrant during a 30-s period were determined.

Video tracking and analysis systems

All swimming behavior was recorded by software (version 2.4.50923) developed by Dr. David P. Wolfer (Institute of Anatomy, University of Zurich, Switzerland).

Statistical analysis

For statistical analyses Prism v5 software (GraphPad Software Inc., La Jolla, CA, UAS) was used. All values in the figures are presented as mean ± SEM. Hidden platform test was analyzed by repeated- measures two- way ANOVA followed by post hoc Bonferroni. Spatial probe test and other data was evaluated by one- way ANOVA followed by Tukey's multiple comparison test. P values < 0.05 were considered statistically significant.

RESULTS

LPS-induced expression of GFAP in hippocampal astrocytes of aged rats was suppressed by PDTC

Increased expression of GFAP is associated with the activation of astrocytes (17). We used immunofluorescence to detect GFAP expression levels in senescent astrocytes of the hippocampus by measuring the mean grey value between the three groups. Compared with the Vehicle group, there were a significant increase in GFAP in the hippocampus of LPS-induced rats from day 1 to day15 ($P < 0.001$) in the LPS group. Significant inhibition of GFAP in the hippocampus of L + P group rats was observed after PDTC treatment compared with the LPS group from day 3 to day 15 ($P < 0.001$) (Figure 1).

PDTC suppressed the LPS- induced canonical NF-κB signaling pathway in hippocampal astrocytes of aged rats

To determine whether the canonical NF-κB pathway was involved in the prolonged hippocampal inflammation and mediated IL- 1β elevation after i.p. injection of LPS, the nuclear localization

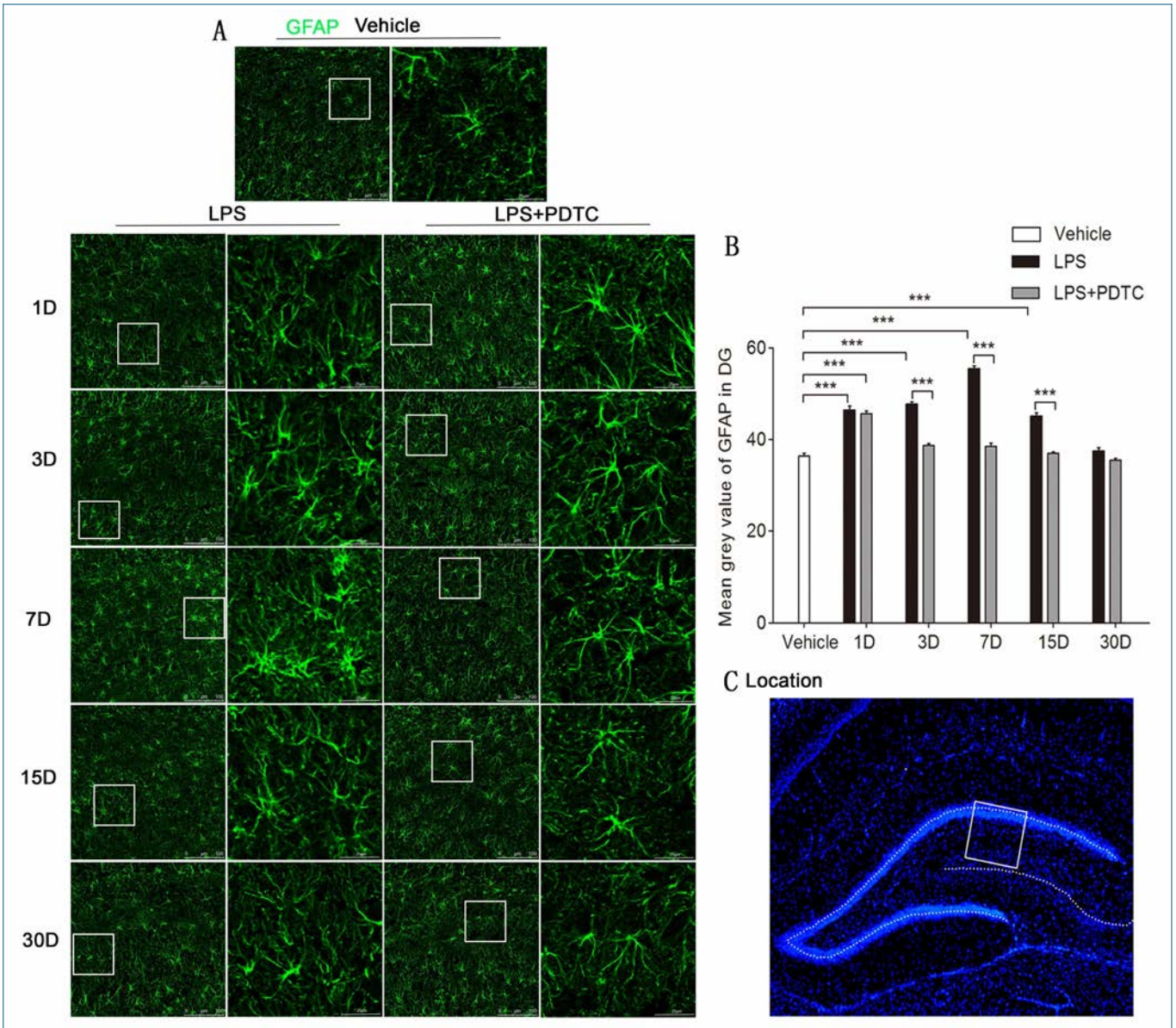

Figure 1. Astrocyte Activity was Measure with GFAP Immunofluorescence. PDTC Inhibited LPS-Induced Expression of GFAP.

Confocal immunofluorescence images showing labeling for GFAP (green) in the astrocytes of aged hippocampal DG regions in Vehicle, LPS, LPS+PDTC group at different time points from day1 to day30(A). Scale bars= 100μm, insets=25 mm. Compared with the Vehicle group, there were a significant increase in the mean grey value of GFAP in the hippocampus of LPS-induced rats from day1 to day15($P<0.001$) in the LPS group. Significant inhibition of GFAP was observed after PDTC treatment compared with the LPS group from day3 to day15 ($P<0.001$) (B). The location of hippocampus DG region in selection of confocal immunofluorescence images is shown in(C).

Data are expressed as mean ± SEM and compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

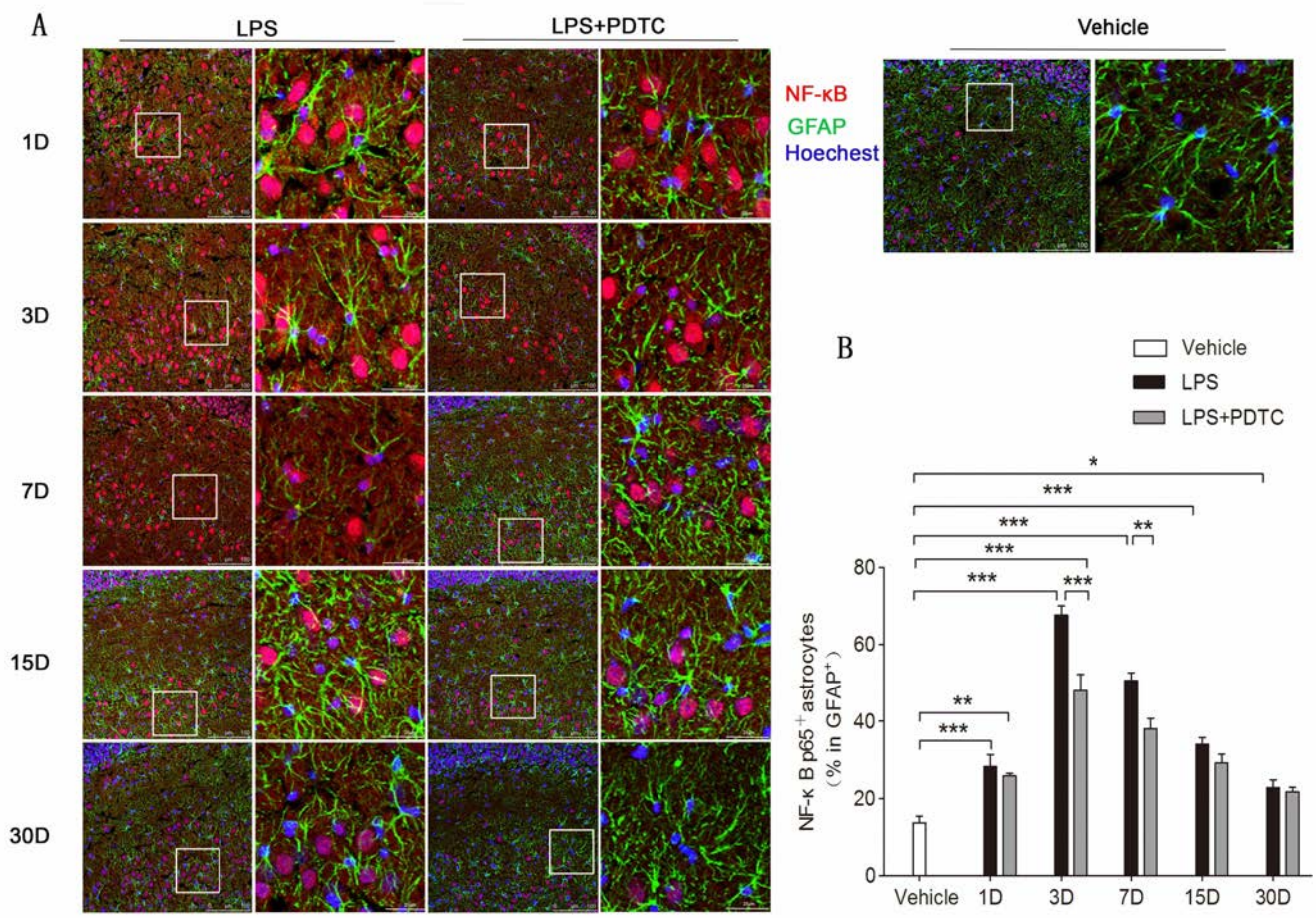

Figure 2. PDTC Diminished LPS Induced NF-κB p65 Activity in The Hippocampus.
Confocal immunofluorescence images of p65/glial fibrillary acidic protein (GFAP) in the hippocampus DG region (A). The ratio of NF-κB p65-positive astrocytes was significantly reduced in LPS+PDTC group compared to LPS (B).
Data are expressed as mean ± SEM (n=4) and compared by 1-way ANOVA with Tukey's Multiple Comparison Test. *P<0.05, **P<0.01, ***P<0.001 Scale bars=100 mm; insets=25 mm.

of p65 and the phosphorylation level of IκBα was detected and the inhibitory effects of PDTC on NF-kB activation were evaluated at a series of time points (days 1, 3,7, 15 and 30).

The result of nuclear translocation of p65 measured by NF-kB p65/GFAP double staining in astrocytes

To investigate the downstream nuclear translocation of NF-κB p65 (a subunit of NF-kB) in hippocampal astrocytes, immunofluorescence analyses at a series of time points were conducted. Double immunostaining for p65 and GFAP showed that nuclear translocation of p65 (red) occurred in the nuclei of GFAP-positive astrocytes (dark blue) in the hippocampal dentate gyrus region of the LPS group, compared with the Vehicle group. Nuclear translocation of NF-κB p65 in hippocampal astrocytes were significantly affected by LPS and PDTC treatment. From day 1 to 15 after LPS administration Nuclear translocation of NF-κB p65 were significantly in-

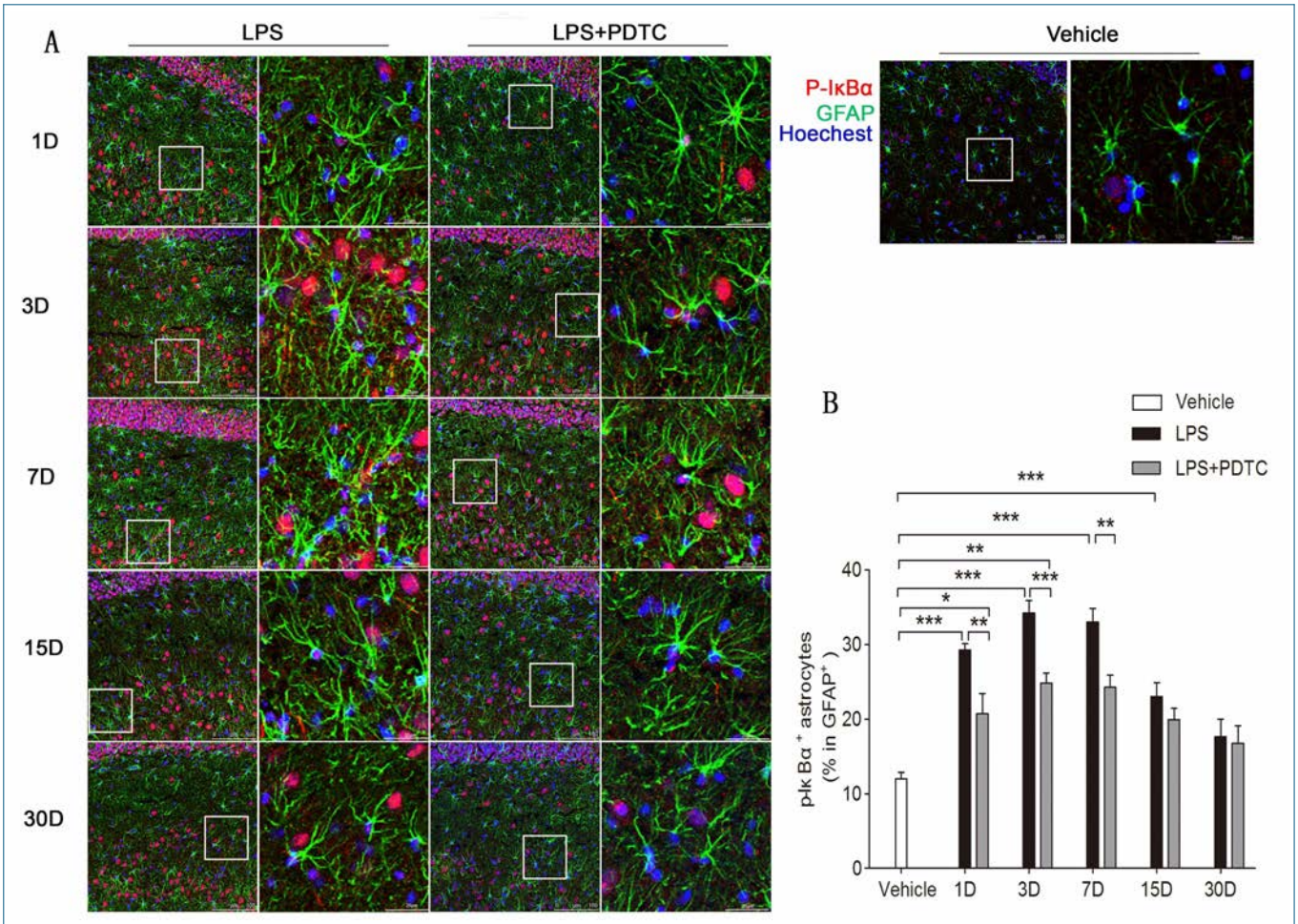

Figure 3. PDTC Inhibited LPS-Induced IκBα Phosphorylation in Aged Hippocampal Astrocytes.
Confocal immunofluorescence images of p- IκBα/glial fibrillary acidic protein(GFAP)in the hippocampus DG region (A). The ratio of p- IκBα-positive astrocytes was significantly reduced in LPS+PDTC group compared to LPS (B).
Data are expressed as mean ± SEM (n=4) and compared by 1-way ANOVA with Tukey's Multiple Comparison Test. *P<0.05, **P<0.01, ***P<0.001 Scale bars=100 mm; insets=25 mm.

creased compared to Vehicle (P<0.0001, respectively). These changes were significantly attenuated in the LPS+PDTC group compared to LPS group, especially on day 3 and 7 (P<0.001 and P<0.01, respectively) (Figure 2).

The result of p-IκBα contents measured by p-IκBα/GFAP double staining in senescence astrocytes

Because IκBα phosphorylation is an important part of the canonical NF-κB signaling pathway, we measured of p-IκBα to verify whether this signaling pathway played a pivotal role in LPS-induced prolonged inflammatory response in aged hippocampal astrocytes.

The obvious co-expression (purple) of p-IκBα (red) around cell nuclei (blue) of senescent astrocytes were also affected by different treatments. From day 1 to 15 after LPS administration, phosphorylated IκB-α was markedly increased in hippocampus compared to Vehicle (P<0.001, respectively), these increases were significantly attenuated by PDTC pre-treatment from day 1 to 7 (P<0.01, P<0.001, P<0.01, respectively) (Figure 3).

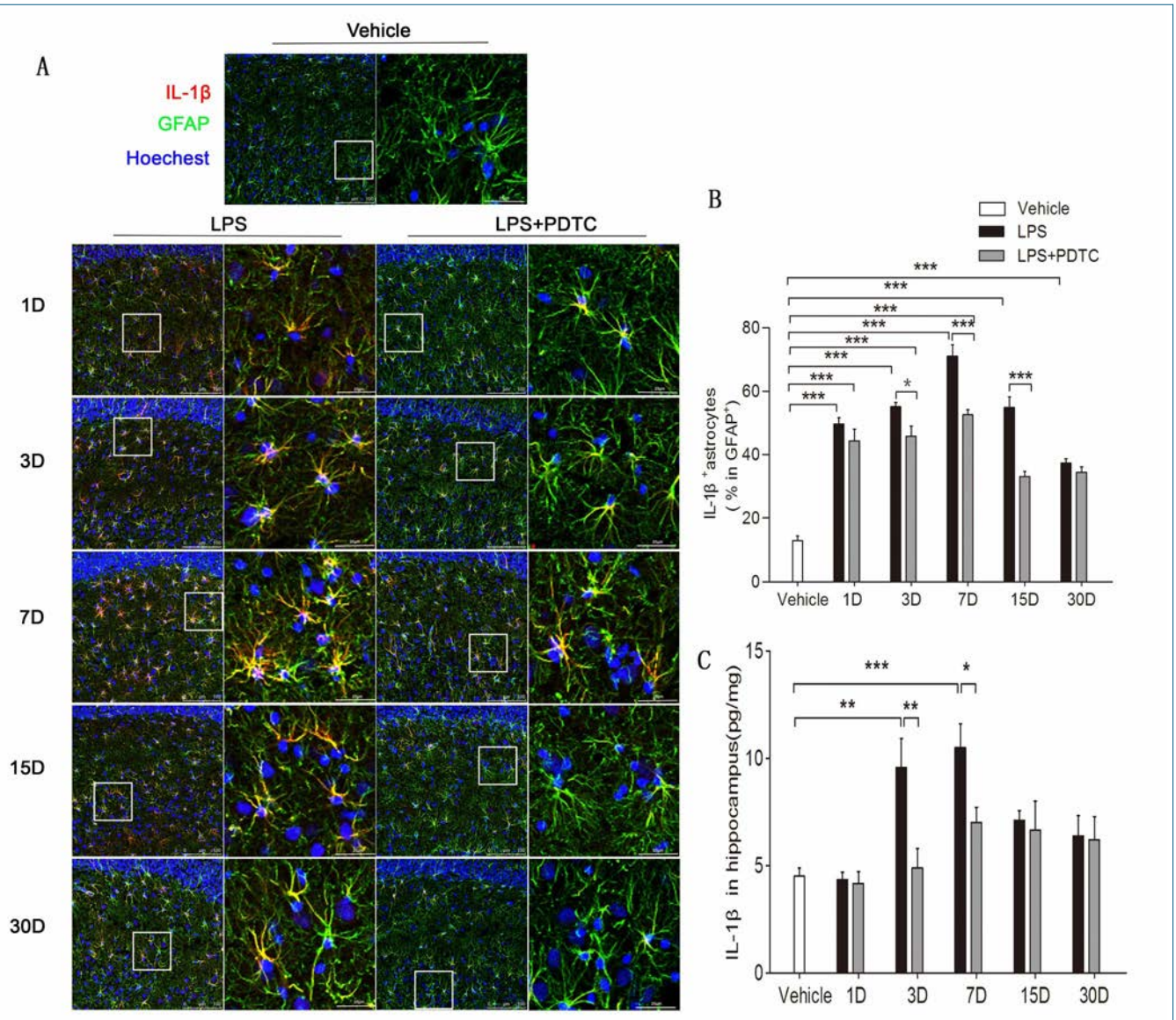

Figure 4. PDTC Attenuated the LPS Induced IL-1β Expression in The Hippocampus.
Confocal immunofluorescence images showed double labeling for IL-1β / GFAP in the astrocytes of hippocampal DG regions in the three groups (A). The ratio of IL-1β- positive astrocytes after PDTC treatment were induced significantly from day 3 to 15 compared to the LPS group (B). IL-1β protein levels showed a decreased trend after PDTC treatment compared with the LPS group on day 3 and especially on day 7 (C).
Data are expressed as mean ± SEM (n = 4) and compared by 1-wayANOVA with Tukey's Multiple Comparison Test. *P<0.05, **P<0.01, ***P<0.001 Scale bars = 100 mm; insets = 25 mm.

PDTC decreased LPS-induced IL-1β expression in hippocampal astrocytes of aged rats

Immunofluorescent detection of IL-1β

The IL-1β positive astrocytes in hippocampus were significantly increased from day 1 to 30 in both LPS and LPS + PDTC groups compared to the Vehicle (Figure 4). However, PDTC pretreatment significantly reduced LPS-induced IL-1β expression in astrocytes from day 3 to 15 ($P < 0.05$, $P < 0.001$, $P < 0.001$ vs LPS group, respectively).

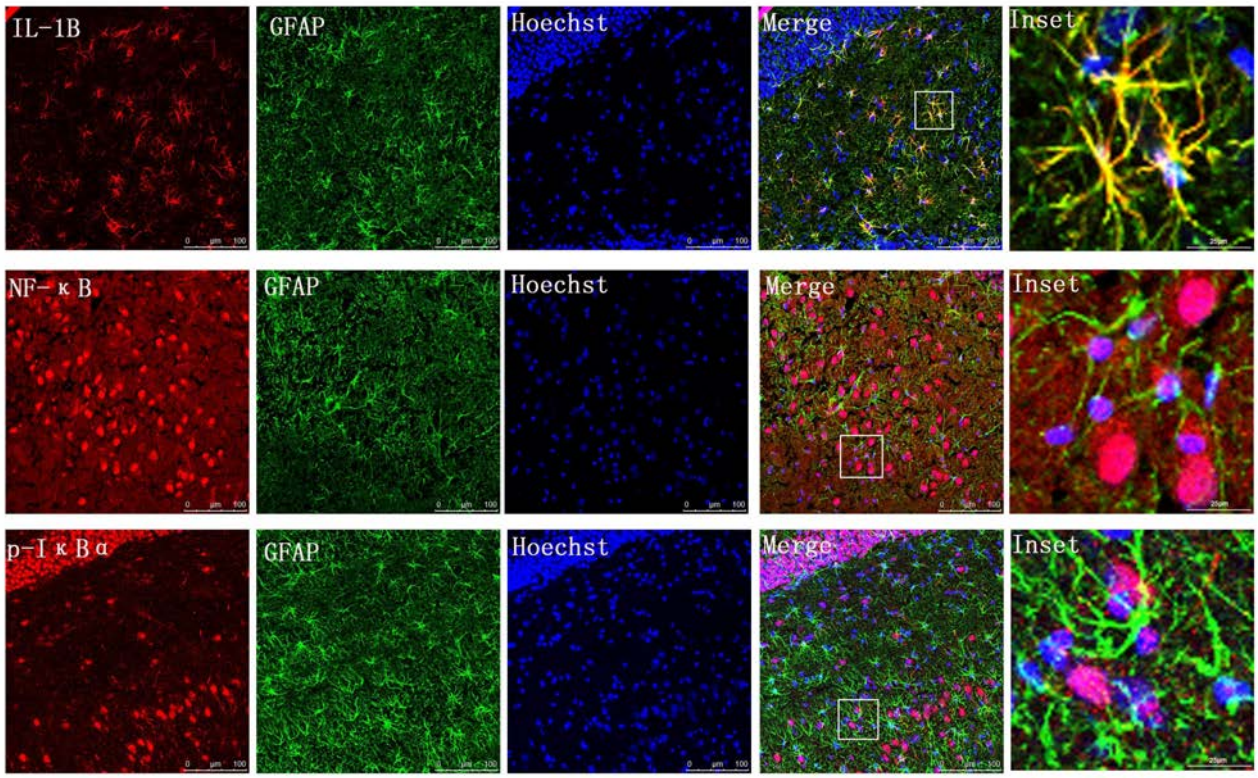

Figure 5. IL-1β/GFAP, NF-κB/GFAP and p-IκBα/GFAP Double Staining in Hippocampal Astrocytes of Aged Rats was Described by a Schematic Diagram.
Based on cell microstructural observations, cell positive expression changes after treatment by double-labeled immunofluorescence staining technique were described by a schematic diagram. IL-1β, NF-κB and p-IκBα were all stained red, the nuclei of tissues were blue counterstained with Hoechst 33342, and GFAP as an astroglial marker were stained green. Confocal immunofluorescence images showed double labeling for IL-1β, NF-κB P65 and p-IκBα with GFAP in aged hippocampal DG regions respectively. Yellow-orange labeling showed IL-1β/GFAP colocalization in cytoplasm of astrocytes; Purple labeling showed NF-κB/GFAP colocalization in neuclei of astrocytes; Purple labeling showed p-IκBα/GFAP colocalization in cytoplasm around nuclei of astrocytes. Scale bars = 100 mm; insets = 25 mm.

Detection of IL-1β protein expression by ELISA

The IL-1β protein level in hippocampus was further evaluated using ELISA. LPS significantly increased IL-1β level in hippocampus at day 3 and 7 compared to Vehicle ($P<0.01$, $P<0.001$, respectively), which was significantly attenuated by PDTC pretreatment ($P<0.01$, $P<0.05$ vs LPS group, respectively) (Figure 5).

PDTC attenuated LPS-induced learning and memory impairment

We used MWM to test learning and memory function in this inflammatory model. The part of orbits in testing 5 days showed obviously difference in those three groups (Figure 6). The escape latency was significantly affected by the testing days and drugs. All groups showed marked improvements in escape latencies over the 5 days of training ($F4, 228 = 27.91$, $P< 0.0001$, repeated measures ANOVA), indicating a memory in locating the escape platform. Repeated measures ANOVA revealed no interaction between training days and drugs ($F8, 228 = 2.49$, $P> 0.05$). This suggests that all the rats effectively learned the task. Post-hoc comparisons indicated that LPS significantly in-

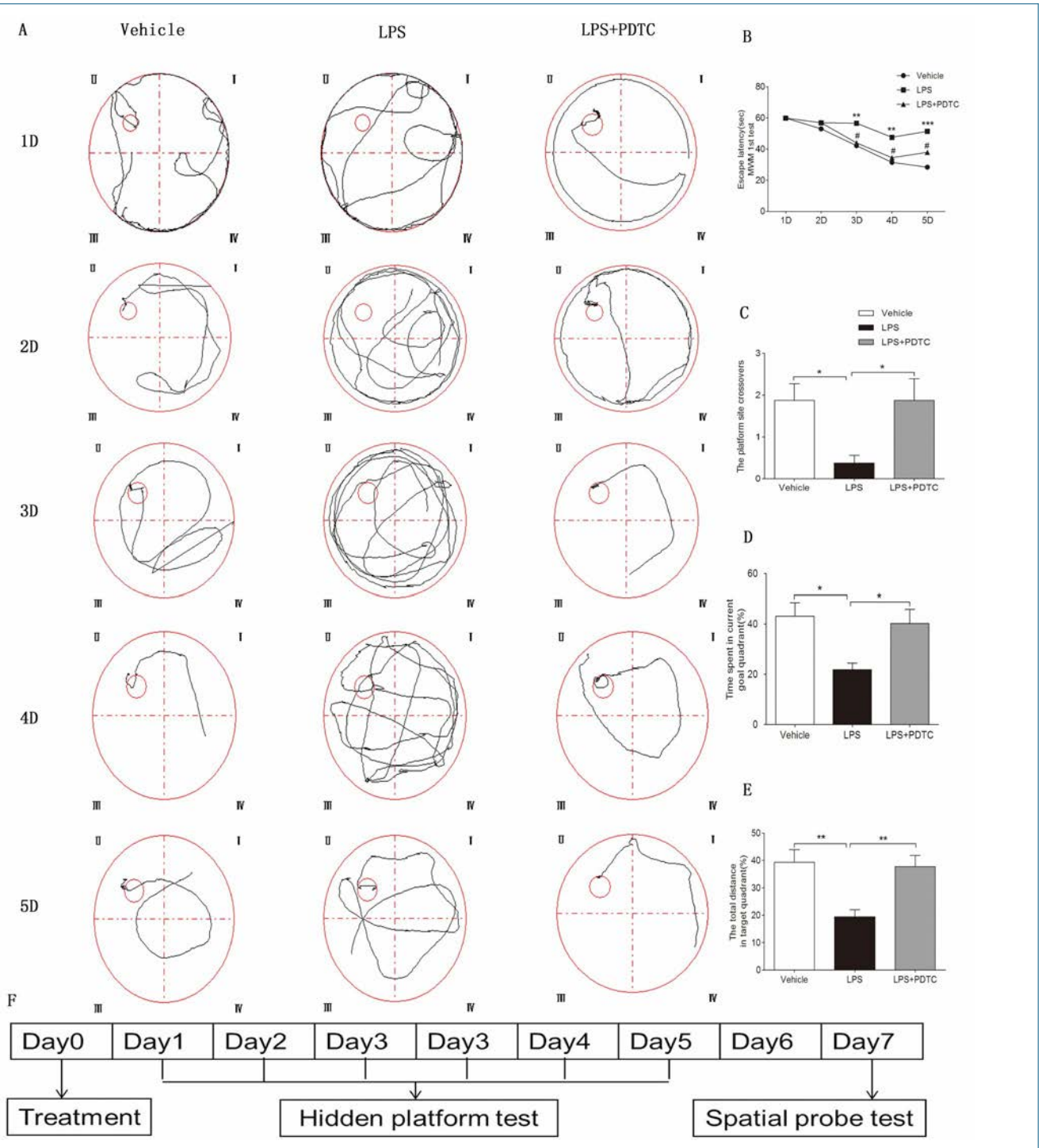

Figure 6. LPS Induced Cognitive Deficiency Was Ameliorated by PDTC.
The part of orbits in testing 5 days showed in (A). Escape latency in the MWM test (B). Platform site crossovers (C). Time spent in the target quadrant (%) (D). Distance spent in the target quadrant(%)(E). The schematic figure of the water maze protocol is shown in (F). Data are expressed as mean ± SEM (n=8-10). Hidden platform test was analyzed by repeated-measures two-way ANOVA followed by post hoc Bonferroni. Spatial probe test was evaluated by one-way ANOVA followed by Tukey's multiple comparison test. * P < 0.05, ** P < 0.01, *** P < 0.001 (C, D, E); * P< 0.05, ** P < 0.01, ***P < 0.001 vs. vehicle group, and # P<0.05 vs. LPS group (B).

creased the escape latency at day 3-5 in the hidden platform test compared to vehicle ($P<0.01$, $P<0.01$ and 0.001, respectively), PDTC significantly improved the LPS induced prolonged escape latency in the rats at day 3-5 ($P<0.05$ respectively, Figure 6). Furthermore, one-way ANOVA in the probe trial on day 7 revealed the effects of drug on the platform crossovers. Tukey's multiple comparison test indicated that rats with LPS challenge had significant less platform crossovers compared to vehicle or LPS + PDTC treated rats ($P<0.05$ and $P<0.05$, respectively) (Figure 6).

Similarly, one-way ANOVA revealed the effects of drug on the time in the target quadrant and the total distance in target quadrant. Tukey's multiple comparison test indicated that rats with LPS challenge had significant less time and distance spent in the target quadrant compared to vehicle or LPS+PDTC treated rats (Time: $P<0.05$ and $P<0.05$, respectively, Distance: $P<0.01$ and $P<0.01$, respectively) (Figure 6). All rats appeared to swim normally, with no significant differences in swimming velocities between the groups indicating that swimming speed did not influence escape latencies (not shown).

DISCUSSION

Inflammation plays a pivotal role in cognitive decline for patients undergoing surgery, infection, trauma, and anesthesia, especially for elderly patients. These insults trigger systemic inflammation, which affects the central nervous system (CNS) and consequently causes cognitive dysfunctions, including postoperative cognitive dysfunction (POCD) (18-21). In our previous study, we found that prolonged hippocampal astrocytic IL-1β expression was induced by a single LPS dose in aged rats and was associated with NF-κB p65 activation, an important part of the canonical inflammatory signaling pathway (14).

In this study, we used a single i.p. injection of LPS, a major component of the outer membrane of Gram-negative bacteria, to induce acute systemic inflammation for the purpose of examining the impact of neuroinflammation on cognitive function in aged rats. We also explored the role of IL-1β, which could be derived from astrocytic NF-κB signaling pathways, in establishing chronic neuroinflammation and its effects on cognitive function in aged rats.

NF-kB is a key and ubiquitous transcription factor for the turning on the transcription of many inflammatory mediators including iNOS, COX-2, TNF-α, IL-1β and IL-6 during inflammation. IκBα phosphorylation dependent on IKKβ activity is an important part of the activation of the canonical NF-κB pathway.

IL-1β is a pleiotropic pro-inflammatory cytokine involved in physiological and pathophysiological processes. The effect of IL-1β in the brain is complex; the lack of IL-1β expression or sustained, chronic IL-1β expression can induce hippocampal-dependent learning deficits (22, 23). Other studies have shown that IL-1β plays a pivotal role in hippocampal learning and memory functions (24, 25). Overexpression of IL-1β, however, interferes with long term potentiation (LTP) and impairs synaptic plasticity, which leads to cognitive decline (26). Prophylaxis with anti-TNF antibody can, in turn, reduce IL-1β expression, which prevents postoperative cognitive decline (21).

Additionally, IL-1β receptor knockout mice exhibit mitigated neuroinflammation and cognitive dysfunction (27). In the present study, astrocyte activation was detected in the hippocampal DG, and IL-1β expression was prolonged in hippocampal astrocytes up to day 30 by double-immunofluorescence, which was consistent with its ELISA results (Figure 4). Chronic IL-1β expression could be the primary source of prolonged neuroinflammation and cognitive dysfunction in the LPS group. PDTC successfully inhibited IL-1β expression in the L+P group, compared with the LPS group at different time points.

The present study utilized the MWM test to evaluate hippocampal cognitive function after LPS administration in aged rats. The Morris water maze is the classic method to test spatial learning and memory function (28). Results from the MWM test and probe test indicated that the aged rats LPS-induced exhibited significant spatial learning and memory impairment between day 3 and 7 of training. However, pretreatment with PDTC significantly ameliorated this cognitive function impairment (35). These results demonstrate that IL-1β overexpression, via the astrocyte-derived NF-κB signal pathway,

plays a primary role in the process of cognitive damage.

PDTC, which is a NF-κB pathway inhibitor that enters the brain through the blood–brain barrier (29), could block activation of the NF-κB signal pathway, inhibit IL-1β overexpression and alleviate further cognitive function damage in the aged rats. A previous literature showed that PDTC inhibited the increased IL-1β hippocampal expression and resulted in improved behavior (30), which was similar with results from the present MWM tests.

Hippocampal inflammation and increased IL-1β production in the brain is an important pathological basis of cognitive dysfunction (38, 4). The hippocampus is involved in learning and memory functions (31) and hippocampal damage can lead to learning and memory impairments in the MWM test (32). Results from the present study demonstrated the prolonged expression of astrocyte-derived IL-1β in the hippocampal DG region and cognitive impairment at different time points in aged rats from the LPS group. PDTC pretreatment inhibited nuclear translocation of NF-κB p65, IκBα phosphorylation, the prolonged IL-1β expression, and improved cognitive dysfunction in aged rats compared with the LPS group, which are also similar with our previous study (33).

Therefore, we suspected that LPS-induced acute systemic inflammation could activate the classical NF-κB signaling pathway of hippocampal astrocytes, leading to downstream increased IL-1β expression, which further induced hippocampal-dependent cognitive impairments in aging subjects whereas PDTC treatment inhibited these changes, which suggests that NF-κB signaling pathway and downstream IL-1β may play important roles in hippocampal neuronal damage and cognitive dysfunction in aged rats. Results from the present study may provide a better understanding about clinical POCD, thereby helping to develop novel prevention and treatment programs for susceptible-aged individuals.

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The authors declare no other conflicts of competing interest for this work.

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