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Chronic unpredictable mild stress accelerates lipopolysaccharide- induced microglia activation and damage of dopaminergic neurons in rats

Hua Kong[a,1], Li Yang[a,1], Can He[a], Jing-Wei Zhou[b], Wei-Zu Li[a], Wen-Ning Wu[a], Han-Qing Chen[c], Yan-Yan Yin[a,*]

[a] Department of Pharmacology, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, PR China
[b] First Clinical Medical College, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, PR China
[c] School of Food science and Engineering, Hefei University of Technology, 193 Tunxi Road, Hefei, Anhui 230009, PR China

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder, which is characterized by microglia activation and dopaminergic neurons affected by inflammatory processes. Inflammation has been recognized to be necessary for initiation and progress of PD. Emerging evidence indicates that NLRP3 inflammasome complex is involved in the recognition and execution of host inflammatory response. Stress is acknowledged to be a predisposing and precipitating factor in some neurodegenerative diseases. However, it is unknown whether chronic unpredictable mild stress (CUMS) sensitized microglia to pro-inflammatory stimuli. In this study, in vivo experiments are used to evaluate the effects of CUMS on lipopolysaccharide (LPS)-induced microglia activation and NLRP3 inflammasome activation. The results showed that CUMS pretreatment for 14 days significantly aggravated the behavioral dysfunction of PD rats, increased the activation of microglia. Pretreatment with CUMS for 14 days increased the levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-a (TNF-a) in the serum, and increased the expression of NLRP-3, ASC, Casepase-1 in the substantia nigra of PD rats. Our data showed that pretreatment with CUMS for 14 days increased the microglia activation and the DA neurons damage, and the mechanisms may be associated with the acceleration of the inflammatory response and activation of NLRP3 inflammasome.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, which is characterized by microglia activation and dopaminergic neurons affected by inflammatory processes. Bradykinesia, muscular rigidity, resting tremor, and sympathetic instability are primary symptoms of PD, whereas loss of dopaminergic neurons from the basal ganglia. Although the exact mechanisms of pathogenesis of PD remain unknown, chronic neuroinflammation has been found to be contributing factors in the progression of dopaminergic neuronal loss (Sharma and Nehru, 2015).

More and more evidences suggested that inflammation played a central role in the DA neurons loss in PD. Evidences had been accumulated since the presence of activated microglia in the substantia nigra (SN) of PD patients was firstly reported. In the different animal models of PD, the number of activated microglial cells and the expression of cytokines were increased. Lipopolysaccharide (LPS) is the active immunostimulant in the cell wall of Gram-negative bacteria, subtoxic doses of LPS induced the dopaminergic neuronal loss. Intracerebral injection of LPS induced the microglial cells activation and dopaminergic neuronal loss.

Stress is acknowledged to be a predisposing and precipitating factor in some neurodegenerative diseases. Some previous studies have suggested that chronic stress primes both the neuroinflammatory response to central proinflammatory challenges and the proinflammatory response of microglia to LPS (de Pablos et al., 2014a; Wohleb et al., 2012; Wohleb et al., 2011). It's reported that depression precedes PD development, indicating that it may be a risk factor for PD (Shen et al., 2013). The chronic unpredictable mild stress (CUMS)model is a well-known effective model imitating the pathogenesis of depression (Bhutani et al., 2009). Unpredictable chronic stress activated the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased corticosteroid (CORT) hormone levels, the marker of endocrine response to stress. Emerging literatures have demonstrated that chronic exposure to

* Corresponding author at: Department of Pharmacology, Anhui Medical University, Meishan Road, Hefei, Anhui 230032, PR China.
E-mail address: yinyanyan5678@126.com (Y.-Y. Yin).
[1] These authors contributed equally to this work.

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exogenous glucocorticoids (GCs) up-regulates the expression of the microglia activation antigens MHCII and Iba-1 (Hinwood et al., 2012a; Espinosa-Oliva et al., 2011a). However, it is unknown whether CUMS sensitizes microglia to pro-inflammatory stimuli.

Microglia are innate immune cells in the CNS, which survey the proximal environment through the Toll-like receptors and NOD-like receptors (NLRs). Activated microglia release a diverse array of toxic mediators including proinflammatory cytokines, nitric oxide and superoxide, which have been shown to play a critical role in DA neuronal degeneration.

IL-1β has been recognized to be necessary for initiation and progress of PD. Emerging evidence indicates that NLRP3 inflammasome complex is involved in the recognition and execution of host inflammatory response. The inflammasome is an intracellular multi-molecular complex for the activation of inflammatory caspases-1 which leads to the cleavage and secretion of interleukin (IL)-1β and IL-18. NLRP3 consists of nod-like receptor protein 3, adaptor protein ASC, and procaspase-1 precusor (Shao et al., 2015; Choulaki et al., 2015). Activated NLRP3 inflammasome maturates the caspase-1 and substrates IL-1β and IL-18. Excessive activation of NLRP3 is involved in the pathogenesis of diseases, such as Alzheimer's disease, rheumatoid arthritis, and depression (Lamkanfi and Dixit, 2012; Pan et al., 2014).

In the present study, we used the LPS intracerebral injection model explore whether the CUMS regulates the LPS-induced microglia activation and dopaminergic neurons degeneration via NLRP3 inflammasome activation.

2. Materials and methods

2.1. Reagents

Lipopolysaccharides from *Escherichia coli* O111:B4(LPS), dimethyl sulfoxide (DMSO) and apomorphine were purchased from Sigma–Aldrich (St. Louis, MO, USA). The antibodies (rabbit-anti-rat) of caspase-1, NLRP3, ASC, IL-1β, TNF-α, mineralocorticoid receptor (MR), primary antibody specific for neuronal Tyrosine hydroxylase (TH) were obtained from Abcam (Cambridge, MA, USA). The antibodies (rabbit-anti-rat) of glucocorticoid receptor (GR) were purchased from Cell signaling Technology (USA). Primary antibody against microglial marker ionized calcium binding adapter molecule 1 (Iba-1) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Streptavidin-biotin complex kit and diaminobenzidine (DAB) staining kit were purchased from Boster Biological Engineering Company (Wuhan, China). All other chemicals used were of the highest grade commercially available.

2.2. Animals and treatment

All the experimental procedures were performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by Animal Ethics Committee of Anhui Medical University (LLSC20160258). Male Sprague Dawley (SD) rats (220 to 250 g) were used for these studies. The rats were housed under standard conditions and kept on a 12 h light/dark cycle with ad libitum access to food and water. These animals were randomly divided into 6 groups (Fig. 1): the vehicle/nonstressed control group (sham group), which were received a single intranigral injection of 4 μL of vehicle into the left SN; the LPS/nonstressed group (LPS group), which were treated with a single intranigral injection of 5 μg LPS dissolved in 4 μL of vehicle into the left SN; the vehicle/stressed group (CUMS group), which were treated with a single intranigral injection of 4 μL of vehicle into the left SN and were stressed for 21 days; the LPS/stressed 7 d group (CUMS 7 d + LPS group): which were treated with a single intranigral injection of 5 μg LPS after being stressed for 7 days; the LPS/stressed 14 d group (CUMS 14 d + LPS group): which were treated with a single intranigral injection of 5 μg LPS after being stressed for 14 days; the LPS/stressed 21 d group (CUMS 21 d + LPS group): which were treated with a single intranigral injection of 5 μg LPS after being stressed for 21 days; the animals were housed isolated except for the sham and LPS group.

2.3. CUMS procedure

The rats were exposed to a chronic unpredictable stress procedure, which consisted of seven different stress situations. They involved tail pinch (2 cm apart from the end of the tail) for 1 min, foot shock, high-speed agitation for 1 min, water deprivation for 24 h, forced swimming in 4 °C cold water for 5 min, food deprivation for 24 h, day and night reversion. Unstressed control animals were housed undisturbed at room temperature of (23 ± 2) °C on a light-dark cycle of 12/12 h with free access to water and food. They had no contact with the stressed animals.

The schedule of stressors is given in Table 1. Application of stress started at different time from day to day (between 08:00 and 20:00) to minimize its predictability.

2.4. Neurosurgery

All surgical procedures were operated under the aseptic conditions and 10% chloral hydrate (3 mL/kg, i.p., Sigma) anesthesia. Rats were mounted in the stereotaxic apparatus (RWD Life Science Co. Ltd., Shenzhen, China) with the nose oriented 11° below the horizontal zero plane. The following coordinates were used: 5.2 mm posterior to bregma, 1.7 mm lateral to the midline, 7.8 mm ventral to the surface of the cortex. Left-unilateral lesions of the dopaminergic neurons located in the substantia nigra were induced by LPS (Zhou et al., 2005). LPS (5 μg), dissolved in 4 μL physiological saline solution, was administrated through a Hamilton syringe (Hamilton Co., Reno, NV) at a rate of 1 μL/min. After each injection, the needle was left in situ for 5 min before complete retraction. The sham operated rats were injected with saline solution.

2.5. Open-field test

Open-field test was proceeded on the 21 d after the LPS injection. The open field tests were performed in a black box (60 cm × 60 cm × 60 cm), which was located in an experimental room with a 50-dB background noise and a white light bulb (15 W) located 60 cm high above the center of the open field. The bottom of the black box is divided into 9 compartments (20 cm × 20 cm). The rat was put in the center of the open-field, locomotion frequency (the number of squares crossed) and rearing frequency (the number of times the animal stood on their hind paws) were recorded by ANY – maze Video tracking system (Stoelting Company, USA). The analysis of motor activity lasted for 3 min after a 2-minute pre-adaption.

2.6. Apomorphine-induced circling behavior

The animals were tested for rotational behavior by apomorphine after the open-field test. The rats were allowed to adapt to the testing environment for 10 min, then were injected hypodermically with 0.5 mg/kg apomorphine dissolved in physiological saline solution 5 min later. The measurement of rotational behavior lasted for 30 min under minimal external stimuli. The net number of turns performed during the entire 30 min testing period was counted.

2.7. Tumor necrosis factor (TNF)-α , interleukin (IL)-1β and interleukin (IL)-6 assays

After the apomorphine-induced circling behavior test, the rats were anesthetized and the blood was drawn through the abdominal aorta. Plasma was collected for measuring the levels of TNF-α, IL-1β and IL-6

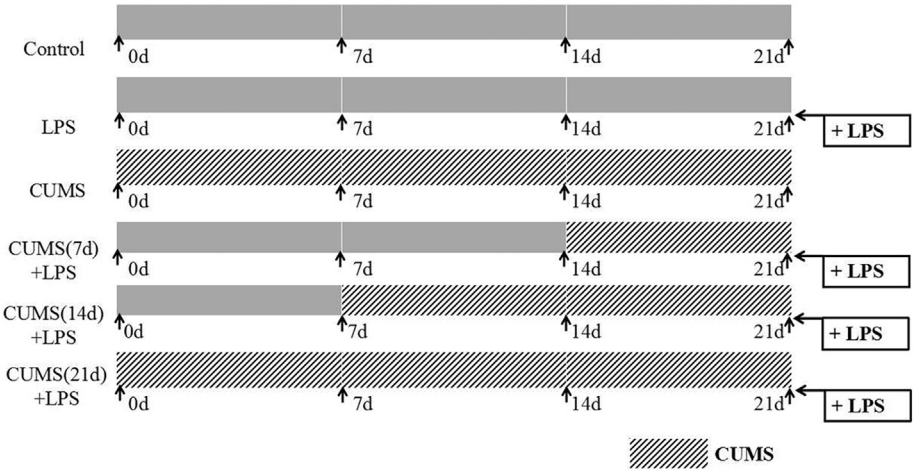

Fig. 1. Experimental groups and treatments. Intranigral injections of saline solution or LPS were given at day 21.

Table 1
Schedule of stressors used during the CUMS treatment +.

Day	Stressor	Time
1, 8, 20	Tail pinch	1 min
2, 13, 18	Foot shock	last 10 s/ time /min, total 30 times
3, 9, 15	High speed agitation	1 min
4, 12, 21	Water deprivation	24 h
5, 11,16	Forced swimming in 4 °C water	5 min
6, 14, 19	Food deprivation	24 h
7, 10, 17	Day and night inversion	24 h

with the enzyme-linked immunosorbent assay (ELISA) kits (Boster, Wuhan, China).

2.8. Immunohistochemistry

Following the rotational behavior test, eight rats in each group were randomly selected for histological assessment. At the end of behavioral experiments, the rats were anesthetized with chloral hydrate (350 mg/kg) and perfused through the ascending aorta with 50–100 mL of 0.9% saline solution followed by 4% paraformaldehyde. After perfusion, the brains were removed, and then fixed in a 4% paraformaldehyde solution and embedded in paraffin.

Paraffin sections were cut at 5 μm and affixed to slides to ensure adhesion. The sections of the SN (bregma −4.8 and −5.8 mm) were stained with primary antibody against neuronal TH (rabbit anti-rat,

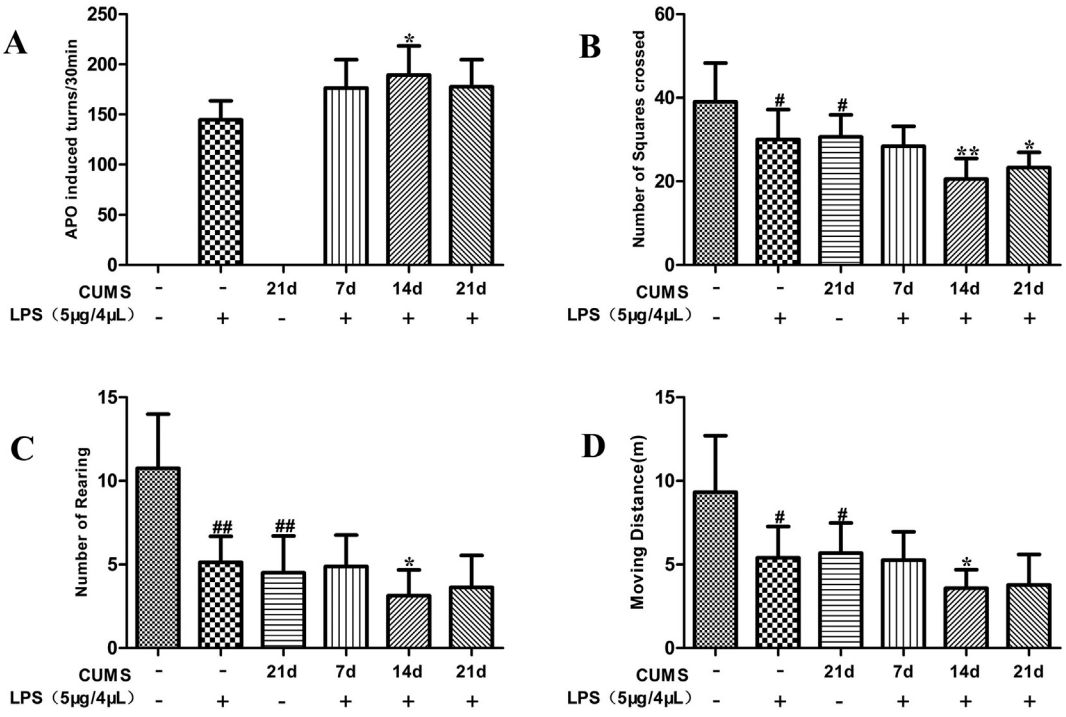

Fig. 2. Effect of CUMS on the behavioral dysfunction in LPS-induced PD model rats.
After LPS injection for 3 weeks, behavioral test was conducted to assess the motor dysfunction. The number of apomorphine-induced rotation in PD rats (A). The number of squares crossed in the open-field test (B). The number of rearing was recorded in the open-field test (C). Moving distance was recorded in the open-field test (D). Results are expressed as mean ± SD ($n = 8$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus sham group, $^{*}P < 0.05$, $^{**}P < 0.01$ versus LPS group.

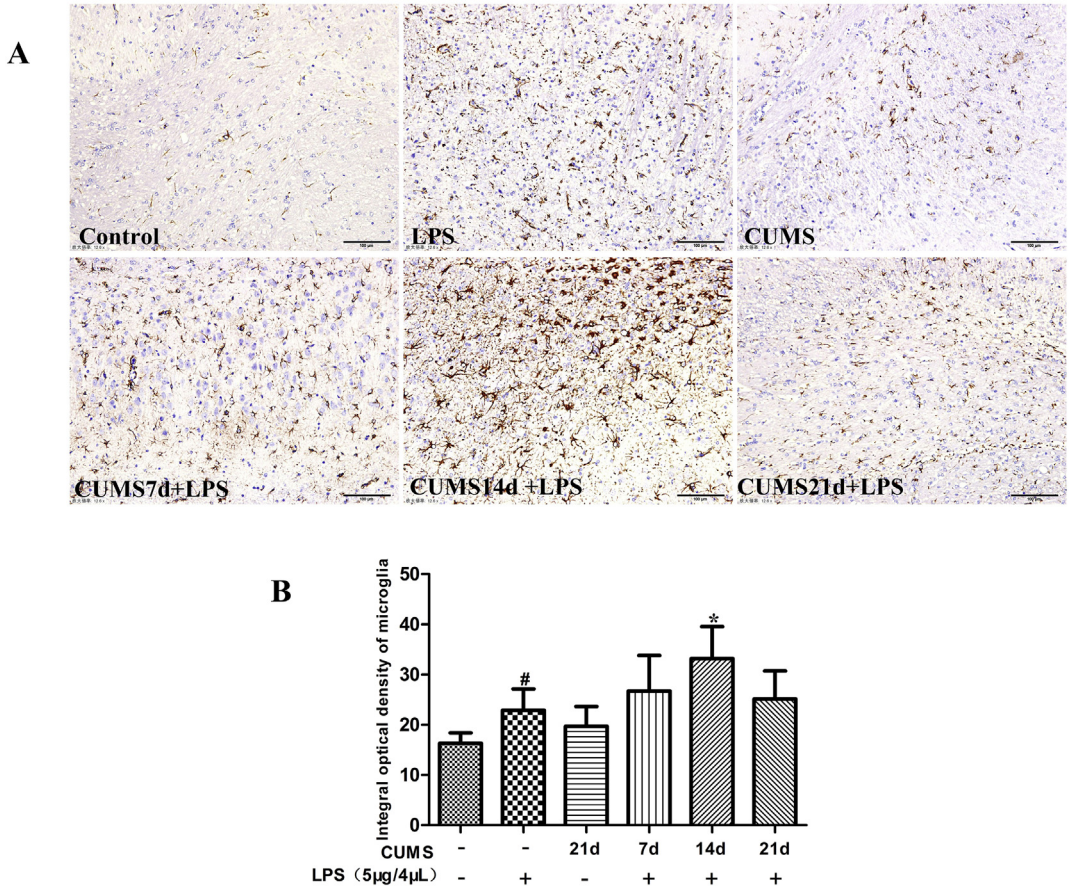

Fig. 3. Effect of CUMS on microglia activation in the substantia nigra of LPS-induced PD model rats (immunohistochemistry, ×200).
A. Effects of CUMS on microglia activation and morphological changes in SN induced by LPS in PD rats (immunohistochemistry). The bar is 100 μm.
B. Quantitative analysis of Iba1 positive expression in SN. Results are expressed as mean ± SD ($n = 8$). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

1:500). Adjacent sections were immunostained for the detection of the microglial marker Iba-1 (rabbit anti-rat, 1:500). The sections were incubated with diluted hydrogen peroxide (3%) for 10 min and blocked with non-immune goat serum for 30 min. In all cases, the primary antibody was left to react at 4 °C overnight. Then, the antibody was detected using the peroxidase method with a biotinylated secondary antibody (anti-rabbit, 1:500) for 30 min and DAB oxidation. The positive neurons were stained brown and taken pictures under the microscope.

2.9. Western blotting

The substantia nigra of rats were separated and prepared in an ice-cold lysis buffer. The lysates were centrifuged at 12000 rpm and 4 °C for 10 min, protein concentrations were measured by BCA Protein Assay Kit (Shanghai Sangon Bio-Tech). Protein extracts were separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Millipore Corp., Billerica, MA, USA), which blocked with 5% skim milk TBST. Membranes were further incubated sequentially with primary antibodies at 4 °C overnight. All primary antibodies were diluted at 1:500. After three washes, the blots were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:10000) for 1 h. After extensive washes, the protein bands were detected by chemiluminescence reagents (ECL kit; Amersham Biosciences, Little Chalfont, UK). The Chemi Q4800 mini-Imaging System (Shanghai Bioshine Technology) was used to visualize protein bands, and densitometry was performed with Image J software. The density of each immunoreactive band was normalized to the density of its corresponding band of β-actin.

2.10. Statistical analysis

Data were expressed as mean ± standard deviation. Results were analyzed by one way analysis of variance (ANOVA) and Student's *t*-test. Calculations were performed using the SPSS 13.0 statistical package (SPSS, Chicago, IL). A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of CUMS on LPS-induced behavioral dysfunction of rats

The apomorphine-induced rotation test is a classic method to evaluate behavioral dysfunction of PD rats, which is used to detect dopaminergic system damage. As shown in Fig. 2A, no rotation (0 turn per 30 min) was observed in the sham group and CUMS group rats, but rotation was observed in the LPS, CUMS(7 d) + LPS, CUMS (14 d) + LPS and CUMS(21 d) + LPS groups. Compared with the LPS group, CUMS(14 d) + LPS group increased the number of apomorphine-induced rotations of the rats ($P < 0.05$), CUMS(7 d) + LPS and CUMS(14 d) + LPS groups increased the rotation, but there were no significant differences between those two groups and the LPS group.

Open field test is also a behavioral test to evaluate the spontaneous behaviors of rats. Compared with the sham group, LPS and CUMS treatments decreased the number of square crossed, rearing and moving distance of rats during 3 min period in the open field. Compared with the LPS group, CUMS(14 d) + LPS group significantly decreased the number of square crossed, rearing and moving distance, CUMS (21 d) + LPS group decreased the number of square crossed (Fig. 2B,

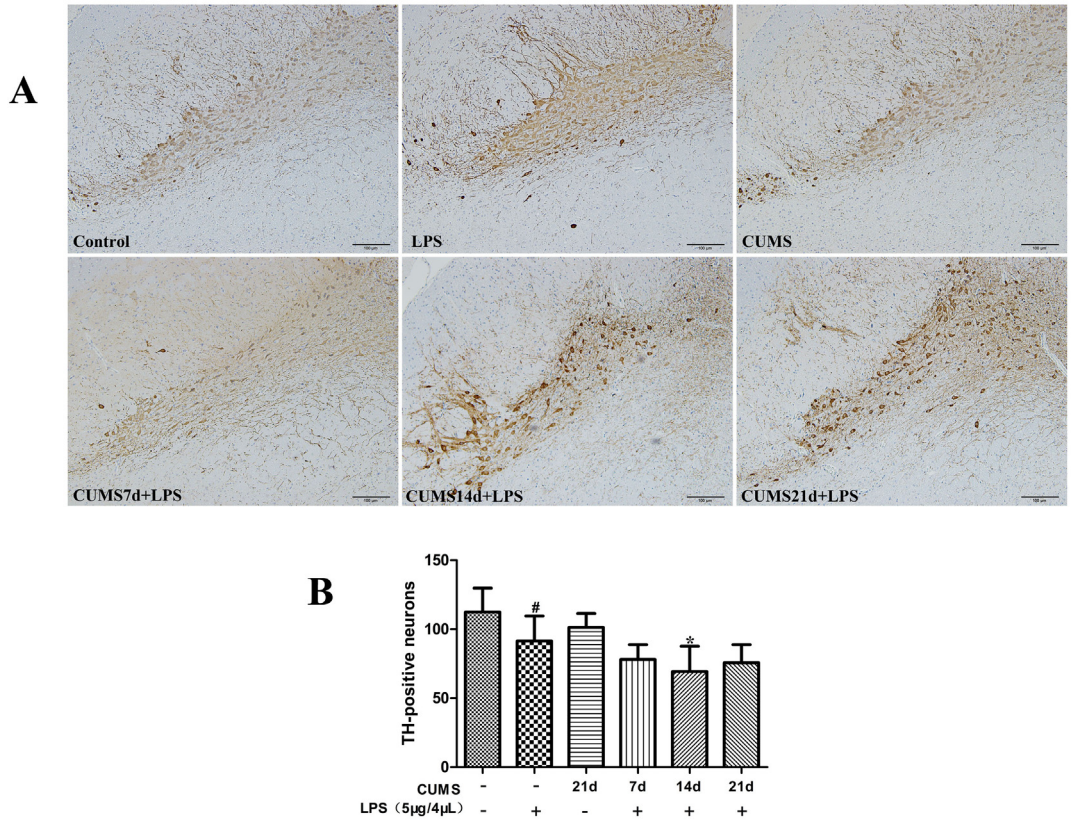

Fig. 4. Effect of CUMS on the number of TH-positive cells in the substantia nigra of LPS-induced PD model rats
A. Effects of CUMS on dopaminergic neurons (TH-immunoreactive cells) loss in SN induced by LPS in PD rats (immunohistochemistry). The bar is 100 μm.
B. TH-positive cells expression in SN. Results are expressed as mean ± SD (n = 8). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

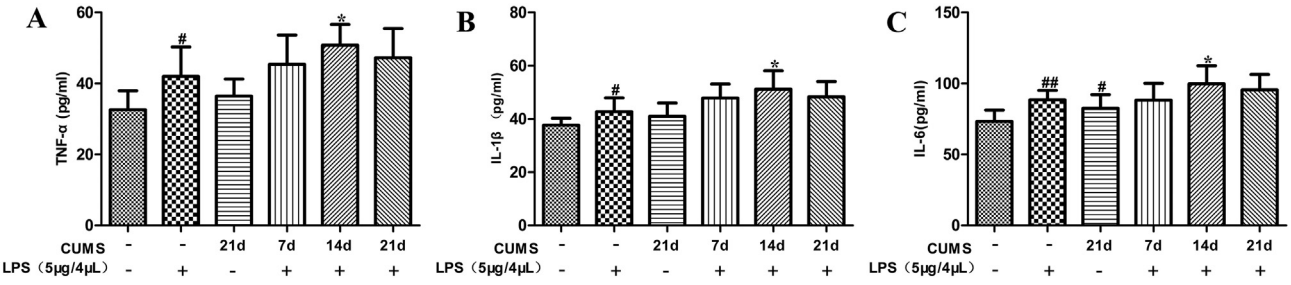

Fig. 5. Effects of CUMS on serum IL-1β, IL -6, and TNF-a in PD rats.
The content of IL-1β, IL -6, and TNF-a were detected using ELISA methods. Results are expressed as mean ± SD (n = 8). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

Fig. 2C and Fig. 2 D, $P < 0.05$ or $P < 0.01$). These results indicated that CUMS could aggravate the behavioral dysfunction of LPS-induced PD rats at different time.

3.2. Effects of CUMS on LPS-induced activation of microglia and the number of TH-positive cells in the substantia nigra of rats

Immunohistochemical staining of Iba-1 was used to evaluate microglial activation and morphological changes. Microglia transformed from resting cells to activated and large cells after LPS injection for three weeks. As shown in Fig. 3A, resting microglia in the substantia nigra of sham animals, while activated microglia was readily identified throughout the substantia nigra of LPS-treated rats by their thicker processes and more rounded cell bodies.

The integral optical density value was determined to represent the Iba-1 expression level in the substantia nigra. As shown in Fig. 3B, compared with the sham group, LPS treatment significantly increased the activation of microglia. Compared with the LPS group, CUMS (14 d) + LPS group significantly increased the Iba-1 expression level ($P < 0.05$), CUMS(7 d) + LPS and CUMS(21 d) + LPS groups increased the Iba-1 expression too, but the differences between those two groups and the LPS group were not statistically significant.

To further investigate the effect of CUMS on LPS-induced dopaminergic neurodegeneration, we observed the TH expression in the substantia nigra by immunohistochemical staining. As shown in Fig. 4, the bodies and fibers of dopaminergic cells (TH-immunoreactive neurons) in the substantia nigra of the sham group that showed intense staining could be easily detected. A considerable loss of TH-positive cells was observed in the LPS-treated group ($P < 0.01$) compared with the sham group. However, CUMS (14 d) aggravated the loss of TH-positive neurons in the LPS-injected substantia nigra significantly ($P < 0.05$).

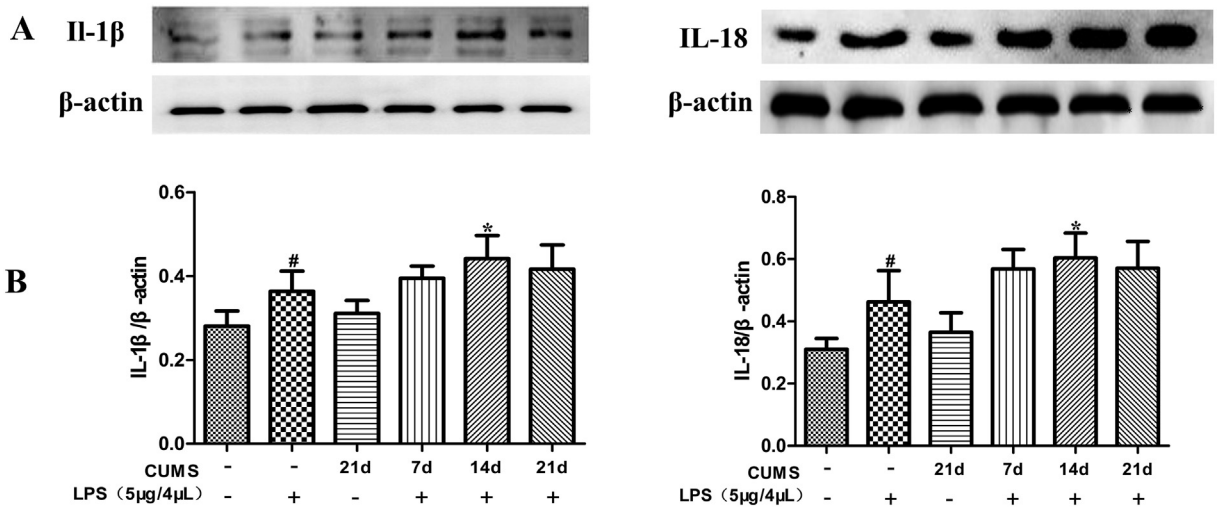

Fig. 6. Effects of CUMS on expression of IL-1βand IL -18 of brain in PD rats. Proteins were extracted from the SNs of rats. The IL-1βand IL-18 were determined by western blot assay. A. The expression of IL-1β and IL-18in brain. B. The relative density for expression of IL-1β and IL-18 relative to β-actin. Results are expressed as mean ± SD ($n = 5$). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

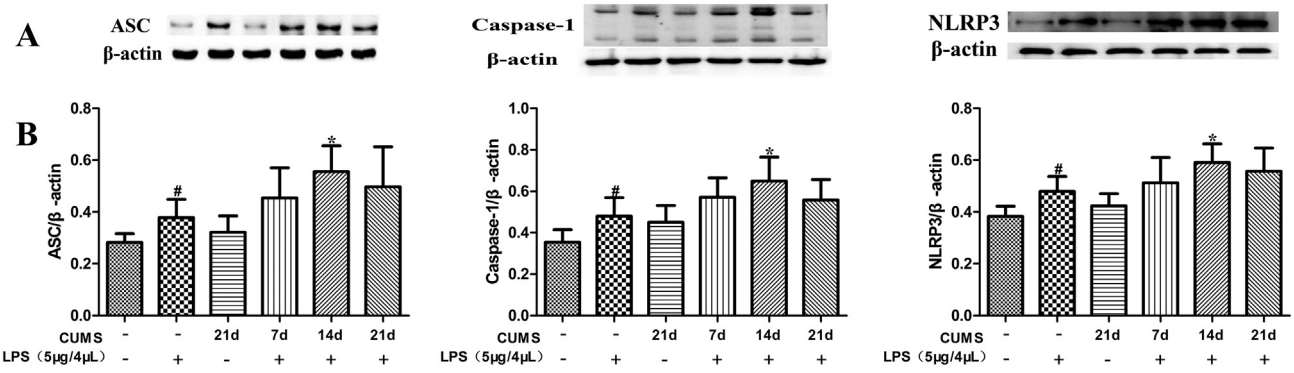

Fig. 7. Effects of CUMS on activation of NLRP3 inflammasome of LPS-induced PD model rats. Proteins were extracted from the SNs of rats. The ASC, Casepase-1, NLRP3 were determined by western blot assay. A. The expression of ASC, Casepase-1, NLRP3 in brain. B. The relative density for expression of ASC, Casepase-1, NLRP3 relative to β-actin. Results are expressed as mean ± SD (n = 5). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

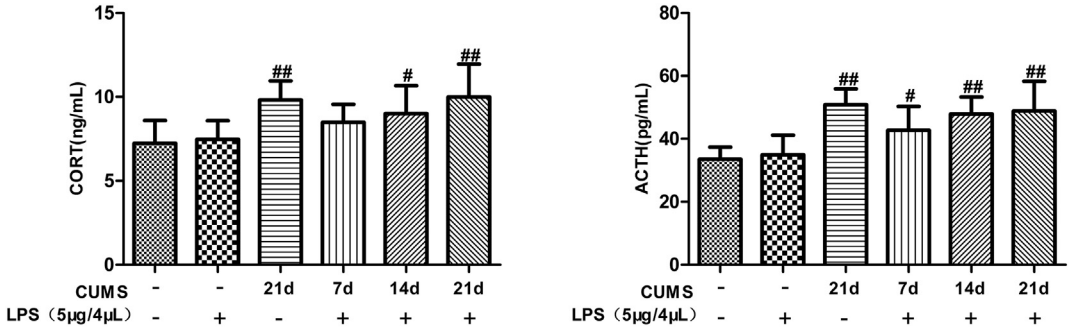

Fig. 8. Effects of CUMS on serum CORT and ACTH level in substantia nigra of LPS-induced PD model rats
The content of CORT and ACTH were detected using ELISA methods. Results are expressed as mean ± SD (n = 8). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$versus sham group,

3.3. Effect of CUMS on LPS-induced inflammatory responses in rats

We measured the levels of IL-1β, IL-6 and TNF-α in the serum of PD model rats by ELISA. As shown in Fig. 5, compared with the sham group, LPS injection caused the increase in IL-1β, IL-6 and TNF-α levels in the serum. After CUMS treatment for 14 days, the contents of IL-1β, IL-6 and TNF-α significantly increased.

We further detected the expression of IL-1β and IL-18 in the substantia nigra by western blot. As shown in Fig. 6, compared with the sham group, the expression of IL-1β and TNF-α were significantly increased in LPS treated rats. Compared with the LPS group, the expression of IL-1β and IL-18 were markedly increased in the CUMS (14 d) + LPS group. CUMS(7 d) + LPS and CUMS(21 d) + LPS groups increased the IL-1β and IL-18 expression, but there were no significant differences between those two groups and the LPS group.

3.4. Effect of CUMS on LPS-induced activation of NLRP3 inflammasome in rats

The effect of CUMS and LPS treatments on expression of NLRP-3,

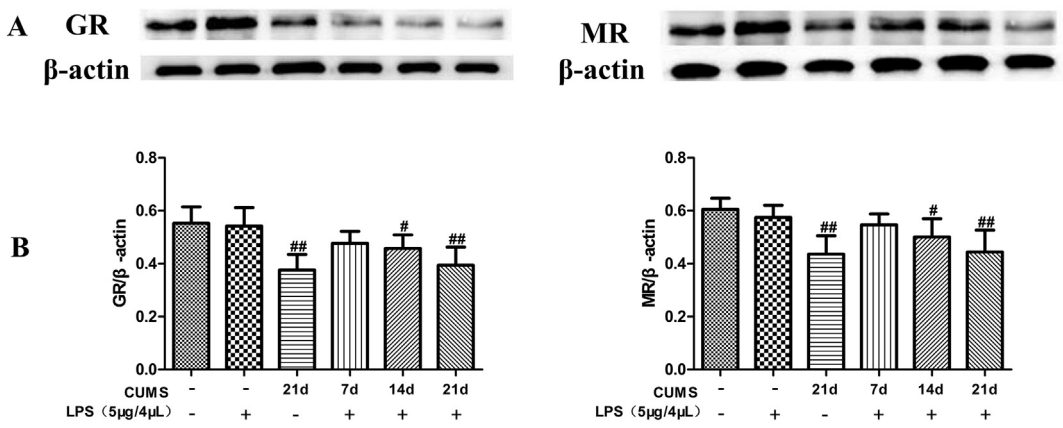

Fig. 9. Effects of CUMS on expression of GR and MR in substantia nigra of LPS-induced PD model rats
Proteins were extracted from the SNs of rats. The GR and MR were determined by western blot assay. A. The expression of GR and MR in brain. B. The relative density for expression of GR and MR relative to β-actin. Results are expressed as mean ± SD (n = 5). $^{\#}P < 0.05$ versus sham group, $^{*}P < 0.05$, versus LPS group.

ASC, and Casepase-1 in the substantia nigra brain tissues were examined by Western blot. As shown in Fig. 7, compared with the sham group, the expression of NLRP-3, ASC, and Casepase-1 in the LPS group were significantly increased. Compared with the LPS group, the expression of NLRP-3, ASC, and Casepase-1 in CUMS(14 d) + LPS group were significantly increased ($P < 0.05$).

3.5. Effects of CUMS and LPS on the levels of CORT and ACTH in serum and expression of GR and MR in substantia nigra

The levels of CORT and ACTH in the serum of PD model rats were measured by ELISA. As shown in Fig. 8, compared with the sham group, the level of CORT was increased in the CUMS, CUMS(14 d) + LPS and CUMS(21 d) + LPS groups, the level of ACTH was increased in the CUMS, CUMS(7 d) + LPS, CUMS(14 d) + LPS and CUMS(21 d) + LPS groups.

We further examined the influence of CUMS on expression of GR and MR in the substantia nigra brain tissues by immunoblot. As shown in Fig. 9, compared with the sham group, the expression of GR and MR were decreased in the CUMS, CUMS(14 d) + LPS and CUMS (21 d) + LPS groups. These results indicated that the CUMS treatment increased the levels of CORT and ACTH in serum, and decreased the expression of GR and MR in substantia nigra.

4. Discussion

In the study , we explored whether the CUMS regulates the LPS-induced microglia activation in rats. The CUMS activated the hypothalamic-pituitary-adrenal axis (HPA axis), increased the level of CORT in serum. The question is how CUMS sensitizes microglia activation and DA neurons to further damage. To answer this question, we used LPS-induced PD rats model (Liu and Bing, 2011), which is a model of dopaminergic neurons loss based on brain inflammation in the ventral mesencephalon induced by LPS. Our previous results showed that LPS (10 μg/4 μL) injection into substania nigra obviously induced the microglia activation. In the present study, in order to observe if CUMS accelerated LPS-induced microglia activation, we used LPS (5 μg/4 μL) injection to induce the microglia activation.

Recent studies have demonstrated that glucocorticoid modulates the immunophenotype of CNS microglia (Espinosa-Oliva et al., 2011b; Hinwood et al., 2012b; Frank et al., 2014). Several studies suggest that strss-induced sensitization of neuroinflammatory processes may be mediated by the glucocorticoids (GCs) response to stress. GCs play an important role in regulating the inflammatory and immune response and have been used since decades to treat various inflammatory and autoimmune disorders. Despite the primary anti-inflammatory actions of GCs, the literature contains a fairly sizable number of instances in which GCs have not inhibited inflammatory responses, and have even had pro-inflammatory effects (Duque Ede and Munhoz, 2016).

GCs can activate microglia, putting them in a primed state. The primed microglia can undergo changes, promoting a sensitized state without production of inflammatory or anti-inflammatory mediators, but, with further stimulus, can produce exaggerated levels of inflammatory mediators. It has been showed increased plasma IL-1β content shortly after the LPS challenge (Liberman et al., 2018; Frank et al., 2010). For example, CORT added to a macrophage culture 12 h before LPS potentiated the pro-inflammatory cytokine response to the LPS (Smyth et al., 2004).

The present results showed that CUMS strengthened the LPS-induced inflammatory, increased the espression of IL-1β, IL-6, TNF-α, NLRP-3, ASC and Casepase-1 in the substantia nigra brain tissues , upregulated the expression of the microglia activation antigens Iba-1, induced the NLRP3 inflammasome activation, led to an increase in the death of DA neurons in the SN. We found that CUMS treatment for 14 d significantly exacerbated the LPS-induced the inflammation, the CUMS 7 d and CUMS 21 d groups increased the dopaminergic neurons damage, too, but the differences between those two groups and the LPS group were not statistically significant. The reason may be related to the different levels of CORT. So we measured the CORT level in plasma, the CORT level of CUMS 14 d group was higher than that of CUMS 7 d group, but lower than that of CUMS 21 d group. The data suggest that the different CORT levels may have different effects on inflammation. Similar results (de Pablos et al., 2014b) were found when chronic stress strengthened the inflammatory stimulus such as LPS in the ventral mesencephalon, leading to an increase the death of dopaminergic neurons in the SN, and the effect was glucocorticoid-dependent.

Overall, our results show that CUMS, which chronically augment plasma CORT, and low-middle levels of CORT enhance the LPS-induced microglia activation as well as the expression of pro-inflammatory in the SN in LPS-induced rats. Further researches are warranted to investigate the particular associations, specific neuroinflammatory mechanisms of low-middle levels GCs exposure and parkinson's disease.

Ethics approval

Animal protocol was approved by the Institutional Animal Care and Use Committee of Anhui Medical University, Hefei, China. All animal procedures were performed to minimize pain or discomfort in accordance with current protocols.

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Competing interests

The authors declare that they have no competing interests.

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