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*Original Research*

# Environmental Enrichment Protects Against Cognition Deficits Caused by Sepsis-Associated Encephalopathy

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**Abstract**

**Background**: One of the most serious complications of sepsis is sepsis-associated encephalopathy (SAE), which impairs the cognition ability of survivors. Environmental enrichment (EE) has been demonstrated to alleviate cognition deficits under many kinds of brain injury conditions. However, EE's effects on SAE remain unknown. Therefore, this study aimed to determine EE's effect on cognition disorders under SAE conditions and the underlying mechanism. **Materials and Methods**: Adult male rats, subject to SAE or not, were housed under a standard environment (SE) or EE for 30 days. Subsequently, the rats were subjected to cognitive tests, such as the novel object recognition (NOR) test, the Morris water maze (MWM) test, an Open Field (OF) test, the elevated plus maze (EPM) test, and a sensory neglect (SN) test. Neuroinflammation, apoptosis, and oxidative stress changes in the brain were also detected. **Results**: The results revealed that SAE impaired somatesthesia, recognition memory, spatial learning and memory, and exploratory activity, which were significantly improved by EE housing. EE also prevented SAE-induced anxiety-like behavior. In addition, EE housing capable induced a decrease in pro-inflammatory cytokines, and an increase in anti-inflammatory cytokines and antioxidant properties in the brain. Moreover, EE housing exerted an anti-apoptosis function by upregulating the level of B-cell lymphoma/leukemia-2 (Bcl-2) level and downregulating the level of p53 level in the hippocampus. **Conclusions**: The results of the present study indicated that EE exerts a neuroprotective function on cognitive ability in SAE rats. The effect is achieved by increasing antioxidants, and anti-inflammatory and antiapoptotic capacities. EE can effectively rescue SAE-induced cognitive deficits.

**Keywords:** sepsis-associated encephalopathy; cecal ligation and perforation; environmental enrichment; cognition deficits; inflammatory cytokines; apoptosis; oxidative stress

## 1. Introduction

Systemic responses to infection commonly result in sepsis, the incidence of which is increasing gradually worldwide [1]. Sepsis is often accompanied by sepsis-associated encephalopathy (SAE) [2]. Over 50% of patients suffering from severe sepsis develop SAE, which is possibly one of the leading causes of mortality in intensive care units (ICUs) [3]. Acute SAE always manifests as a disturbance of consciousness and can ultimately lead to death [3–5]. In addition, survivors of SAE might carry a significant risk of long-term cognitive function disorder, such as changes in mental speed, learning and memory ability, spatial discrimination function, executive ability, and attention ability [6–8]. Long-term cognitive function disorder is a major cause of disability, leading to increased burdens on families and society, and poor life quality for patients. However, currently, we lack effective treatments for SAE-induced cognition disorder. Methods to improve SAE-induced cognition disorders have become a research hotspot.

Housing conditions comprising social interactions, novelty, and various forms of exercise are referred to as environmental enrichment (EE). Numerous studies have reported the positive effect of EE on cognitive function under various brain injuries [9]. For example, EE can improve the sensory and memory ability for traumatic brain injury (TBI) rats by changing the levels of proinflammatory cytokines and anti-inflammatory cytokines [10–12]. EE also inhibits strokeinduced cognition disorder by suppressing neuroinflammation and oxidative stress [13,14]. Moreover, in models of preeclampsia and Parkinson's disease, accumulating evidence has demonstrated the inhibitory effects of EE on cognition disorders by regulating inflammatory conditions and apoptosis levels [15–17]. These findings suggest that EE might be capable of rescuing cognitive ability impairment under SAE conditions.

Accordingly, in the present study, we investigated the effect of EE on SAE-induced cognitive impairment and the underlying mechanism. To this end, adult male rats were housed in EE or standard environment (SE) conditions for 30 days. The changes in cognitive function were then evaluated. Additionally, we detected the changes in neuroinflammation, apoptosis, and oxidative stress in the brain.

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**Fig. 1. Flow chart of the experimental process.**

## 2. Materials and Methods

### 2.1 Rats

The Animal Center of the China-Japan Friendship Hospital, Beijing, China provided the Sprague-Dawley (SD) rats (male; 60 days old; weighing 200–250 g). The *Guidelines for the use of animals in neuroscience research* (published in the Membership Directory of the Society, pp 27–28, 1992) were followed when performing all the animal experiments, and the committee of Animal Use for Research and Education of the China-Japan Friendship Hospital approved this study. The rats were reared in a temperature-controlled room (24 ± 1 °C) with a 12 h light/dark cycle and *ad libitum* access to water and food. The animals were allowed one-week of acclimation to the experimental room before the experiments.

### 2.2 SAE Induction

SAE was induced by cecal ligation and perforation (CLP) according to previous published methods [18,19]. In brief, the rats were subjected to sodium pentobarbital (50 mg/kg) intraperitoneal anesthesia. The cecum and adjoining intestine were exposed under aseptic conditions. A 3.0 silk suture was used to ligate the cecum at its base, beneath the ileocecal valve, followed by single perforation employing a 14-gauge needle (about 1 mm depth). A small amount of feces was extruded into the peritoneal cavity by gentle squeezing. The cecum was then returned to the peritoneal cavity, and 4.0 silk sutures were used to close the laparotomy incision. After surgery, the rats were given "basic support" (saline, 50 mL/kg) subcutaneously (s.c.) for recovery from anesthesia, immediately after and 12 h after CLP. All rats received antibiotic therapy by subcutaneous injection of ceftriaxone (30 mg/kg) and clindamycin (25 mg/kg) every 6 h for 3 days. Sham rats were given an identical cecum exposure but without ligation or puncture. At 7 days post-surgery, the model rats showed a 51% mortality rate, comparable to some previous studies [19,20] and indicative of severe sepsis. For the survivals, the neurobehavioral tests, including auricular reflex, corneal reflex, righting reflex, tail flick reflex and escape reflex were performed 3 days after the CLP as previous study [18]. 0 refers to no reflexes, 1 point refers to weakened reflexes (no reflexes within 10 s), 2 points refers to normal reflexes, and the highest score refers to 10 points. According to preliminary experiment, the SAE was determined when the scores was below 8.

### 2.3 Grouping and Housing Conditions

In the present study, SE and EE were used. For SE, the rats were reared in a standard-sized cage (25 × 40 × 20 cm) with two rats in each cage. SE allowed for moderate activity and exploration.

For EE, the rats were reared in a large cage (40 × 54 × 30 cm) with six rats in each cage. The cage included many kinds of objects, such as a running wheel, wooden blocks, and plastic tunnel [21,22]. In addition, ropes were provided to allow the rats to climb to higher levels. Twice a week, all the objects were replaced. At 30 days of EE housing, we removed the objects from the cages, with no further enrichment provided.

According to the results of preliminary experiments and previous studies [23], the sample size for each experiment was determined. The rats receiving CLP or sham operation were placed into four groups (n = 18 per group): Sham-SE group (sham injury + SE housing); Sham-EE group (sham injury + EE housing); SAESE group (CLP + SE housing); and SAE-EE group (CLP + EE housing).

After EE or SE housing, the mechanism of EE was investigated. We detected the level of inflammatory/anti-inflammatory mediators, oxidative/antioxidant activity, and apoptosis-related molecules and pathways. Thus, in each group, six rats were used to detect the level of apoptosisrelated molecules, six rats were used to detect the oxidative/antioxidant activity, and six rats were used to detect the levels of inflammatory/anti-inflammatory mediators.

### 2.4 Behavioral Testing

The experimental procedure is shown in Fig. 1. The rats were subjected to behavioral tests naively and were manipulated only when routine cleaning was performed. The tests were performed between 08:00 and 17:00 hours. To eliminate the confounding effect of testing time, we counterbalanced the testing order of the rats.

#### 2.4.1 Sensory Neglect

Somatesthesia was assessed using a sensory neglect (SN) test [24]. The SN test was performed 1 day after 30 days of SE or EE. In this test, we placed a 2 cm diameter sticker on the distal-radial area of both forelimbs. The rats were put into an empty cage and then we measured the time taken to remove the stickers (latency). Each rat was subjected to three trials at 5 min intervals and 2 min maximum trial time.

#### 2.4.2 Elevated Plus Maze

The elevated-plus maze (EPM) test was used to assess anxiety-like behavior. The EPM comprises two opposing open arms (10.2 × 10.2 cm), two opposing closed arms (10.2 × 10.2 cm; equipped with 30.5 cm high walls), and a common central square [25]. The EPM was elevated at 100 cm aboveground and lit indirectly using 85–90 Lux light intensity. Each rat was placed at the extreme end of a closed arm of the maze (facing opposite to the center) and given one 5 min trial. The arms were cleaned using 70% ethanol and dried using paper towels between each trial. The detection indices include time of occupancy of an open arm, number of entries into open or closed arms, latency of entry into an open arm, and time occupied by head dipping, rearing or grooming.

#### 2.4.3 Open Field Test

Locomotor and exploratory ability were evaluated using an open field (OF) test, as previously described [26]. The OF was 60 × 60 cm and was surrounded by 60 cm walls. The floor of the OF was divided into nine rectangles using black lines. The entire area of the OF was lit indirectly using 70 lux intensity in the center area. The rats were added to the central area with all paws on it and allowed free exploration for 5 min. The detection indices comprised: travel distance in the OF, rearing frequency (standing upright), number of times crossing the line (horizontal activity), duration in the center area or in the four corner squares. The field was cleaned using 70% ethanol between each trial.

#### 2.4.4 Morris Water Maze

Spatial learning and reference/working memory was assessed using a Morris water maze (MWM) [27–29]. The maze consisted of a dark circular tank with a diameter of 178 cm, which was filled with water to 37 cm deep and was separated into four quadrants (A, B, C, D). A plexiglass platform (diameter = 10.2 cm diameter) was placed at approximately 28 cm from the wall of the pool in quadrant C (the southeast quadrant) and was submerged at 2 cm below the waterline. Each animal was tested for 6 days. In this test, a 5-day testing block (four trials each day) was performed to detect the rat's spatial learning ability. In each trial, the rats were put into a random quadrant, facing the wall. In 120 s of given time, the latency to the reach the platform was recorded. If the rats could not reach the platform, we physically guided them to it. The rats were kept on the platform for 30 s after reaching it, followed by a 5-minute break before the next trial. To determine the role of non-spatial factors, on day 6, we raised the platform to 2 cm above the waterline such that was visible to the rats. On day 6, we also performed a single probe trial to determine memory retention. We removed the platform and the rats were allowed to explore the maze for 30 s. The detection indices were: proportion of time spent in the target quadrant (%) and the number times they crossed the platform location.

#### 2.4.5 Novel Object Recognition Test

The Novel Object Recognition (NOR) test was carried out to evaluate recognition memory (non-spatial working memory) [30,31]. The NOR test was performed according to a previously published method [32]. This test had two phases. In phase 1 (familiarization phase), the rats were allowed to freely explore two identical objects (placed in opposite corners of the field) for 5 min. Then, the rats were removed to their cages and rested for 1 h. During phase 2 (test phase), a novel object replaced one of the previous objects and the rats were allowed to explore them for 3 min. To reduce emotional instability-induced variation, rats were excluded from analysis when they exhibited excessive freezing behavior for more than 60% of the test phase (1 rat in Sham-SE group, 1 rat in Sham-EE group, 2 rats in SAE-SE group and 1 rat in SAE-EE group). When the nose of rats pointed at the objects and explored it at a distance less than 1 cm, the time taken to explore the object was measured. If the rats used the objects as props, sat on the objects or contacted but did not face the objects, these behaviors were not regarded as object exploration [33]. The total exploration and discrimination index (DI) was calculated as described in a previous study [34]. The total exploration = the sum of exploration time of the novel object (tn) + the sum of exploration time of the familiar object (tf). DI = (tn–tf)/(tn+tf) × 100.

After behavioral testing, rats were killed by decapitation. The brain was removed immediately and washed with ice-cold isotonic saline solution and placed in liquid nitrogen and stored at –80 °C.

### *2.5 Enzyme-Linked Immune-Absorbent Assay (ELISA)*

Inflammatory mediators and apoptosis-related molecules in the brain or blood were measured using ELISAs. The brain tissues were obtained according to our past study [35]. Briefly, the isolated brain was homogenized in normal saline, followed by centrifugation for 10 min at 4 °C and 2000 rpm. Interleukin (IL)-6, IL-10, IL-1$\beta$, tumor necrosis factor alpha (TNF-$\alpha$), B-cell lymphoma/leukemia-2 (Bcl-2), p53, Bcl-2 associated X (BAX), and caspase-3 levels were detected in the supernatant using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's instructions.

*2.6 Measurement of Oxidant Activity*

2.6.1 Lipid Peroxidation

The extent of lipid peroxidation was measured by determining the level of thiobarbituric acidreactive substances (TBARS), as previously described [35,36]. Isolated brain tissue was sonicated in 10% (w/v) radioimmunoprecipitation assay (RIPA) buffer added with a protease inhibitor cocktail (Sigma, St. Louis, MO, USA). Following incubation for 30 min on ice, the homogenates were centrifuged for 10 min at 4 °C and 600 × *g*. The supernatants were retained and placed at –80 °C for further analysis. Acid hydrolysis of tetraethoxypropane was used to produce a standard curve for malondialdehyde (MDA). The reaction between MDA and thiobarbituric acid proceeded at 95 °C for 10 min. We then measured the fluorescence of the reaction at an excitation wavelength of 515 nm and an emission wavelength of 548 nm.

2.6.2 Advanced Oxidation Protein Products (AOPPs)

To directly determine the amounts of oxidized proteins in biological samples, AOPPS were measured. Tissue was subjected sonication for homogenization by sonication in cold 50 mM $NaH_2PO_4$ and 1 mM EDTA buffer at pH 7.5. The, homogenates were then subjected to centrifugation for 10 min at 4 °C and 10,000 × *g*. According to Barsotti's method [35,37], a spectrophotometer was used to determine AOPP levels at 340 nm.

2.6.3 Nitric Oxide (NO)

*In vivo*, NO's final and stable end products are nitrates and nitrites, whose sum (NOx) represents total NO production. A colorimetric assay (Cayman Chemical Company, Ann Arbor, MI, USA) was used to determine NOx [35,38]. In sample homogenates, nitrates were converted to nitrites using nitrate reductase and NADPH. The Griess reaction was then used to monitor total nitrites (nmol/mg protein) at 540 nm.

*2.7 Determination of Hippocampal Antioxidant Activity*

2.7.1 Glutathione System

Reduced glutathione (GSH) and glutathione disulfide (GSSG; oxidized glutathione) concentrations were measured in brain extracts. Brain samples were subjected to homogenization in a 1:1 mixture of cold 10% metaphosphoric acid, 5 mM EDTA (pH 6.8) and 0.1 M potassium phosphate, followed by a 30 min incubation of ice. The homogenate was then centrifuged for 30 min at 10,000 × *g*. The supernatant was retained and used to determine GSSG and GSH using ophthalaldehyde (OPA), a fluorescent probe. For GSSG determination, aliquots were initially incubated with Nethylmaleimide to form complexes with GSH, thus avoiding interference. The sample was then incubated with OPA for 15 min, and the fluorescence was then determined at 420 nm (emission) and 350 nm (excitation). The Bradford protein assay was used to determine total brain protein levels. To determine glutathione peroxidase (GPx, EC 1.11.1.9) and glutathione reductase (GR, EC 1.8.1.7) activities, brain samples were sonicated in cold 50 mM $NaH_2PO_4$ and 1 mM EDTA buffer at pH 7.5, followed by centrifugation for 10 min at 4 °C and 10,000 × *g*. A kit (Nanjing Jiancheng Bioengineering Institute; Nanjing, Jiangsu, China) was used to measure GPx (lot number: A005-1-2) or GR (lot number: A104-1-1) activity in the supernatant, following to the suppliers guidelines.

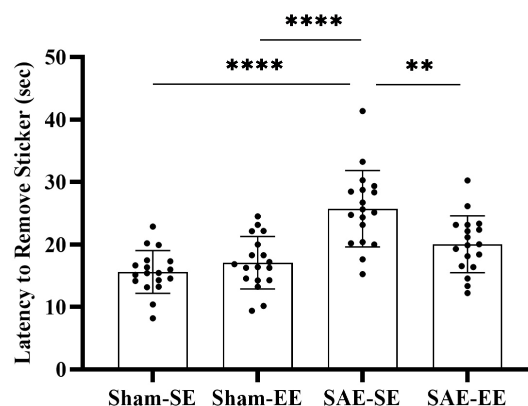


**Fig. 2. The rats in the SAE-SE group had longer latency to remove stickers than the rats in the Sham-SE or Sham-EE group.** However, the rats in the SAE-EE group had shorted latency to remove stickers than the rats in the SAE-SE group. $^{**}p < 0.01$, $^{****}p < 0.0001$. The assignment of order was counterbalanced across rats in this test. Each value represents means ± SEM, n = 18.

2.7.2 Superoxide Dismutase

Sonication was used to homogenize brain tissue as described in '*Lipid peroxidation*'. The homogenates were subjected to centrifugation for 10 min at 4 °C and 600 × *g*. A kit (lot number: A001-3-2; Nanjing Jiancheng Bioengineering Institute; Nanjing, Jiangsu, China) was used to determine the superoxide dismutase activity (SOD,EC 1.15.1.1.) in the supernatant.

*2.8 Statistical Analysis*

All data are shown as the mean ± the standard error of the mean (SEM). The Kolmogorov–Smirnov test and Brown–Forsythe test were performed to test normality and variance. When data complied with the normal distribution and variance equality, we used two-way analysis of variance (ANOVA) with a $p$ value $< 0.05$ being regarded as statistical significance, followed by Tukey's post-hoc analysis. GraphPad Prism 9.0 software (GraphPad Inc. La Jolla, CA, USA) was used to perform the statistical analysis.

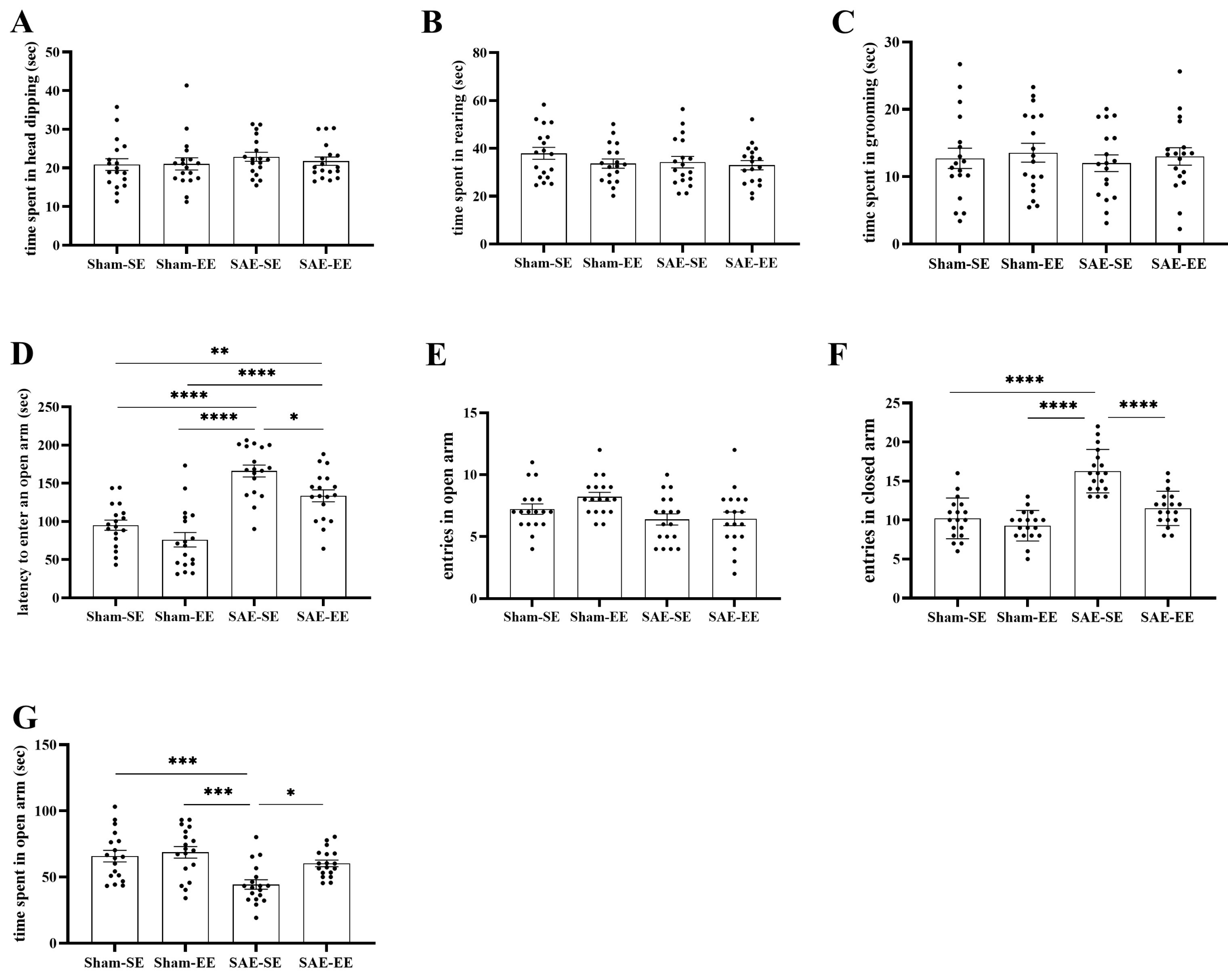


**Fig. 3. EE attenuated the SAE-induced anxiety-related behaviors.** (A–C) No statistical differences were found in dipping, rearing or grooming. (D) The rats in the SAE-SE or SAE-EE group spent more time before entering an open arm. In addition, the rats in the SAE-EE group spent less time before entering an open arm compared with the rats in the SAE-SE group. (E,F) There were no differences in entries in the open arms among four groups. The rats in the SAE-SE group had the increased entries in closed arms than the rats in the SAE-EE, Sham-SE or Sham-EE group. (G) The lower open arm time was found in the SAE-SE rats when compared with Sham-SE, Sham-EE or SAE-EE rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 18.

## 3. Results

### 3.1 EE Inhibited the SAE-Induced Sensory Neglect Deficits

First, we investigated somatosensory changes. The results of the two-way ANOVA showed an obvious interaction effect (SAE factor: F (1, 68) = 35.08, $p < 0.0001$; environmental factor: F (1, 68) = 3.643, $p = 0.0605$; interaction F (1, 68) = 10.56, $p = 0.0018$). Post-hoc analysis showed no obvious differences in latency to sticker removal were found between the Sham-SE and Sham-EE groups (Sham-SE *vs*. Sham-EE group: $p = 0.7788$; Fig. 2). After CLP, a significant increase in sticker removal latency was observed. Post-hoc analysis suggested that the SAE-SE rats had longer sticker removal latency than the Sham-SE or Sham-EE rats (SAE-SE *vs*. Sham-SE group: $p < 0.0001$, SAE-SE *vs*. Sham-EE group: $p < 0.0001$; Fig. 2), suggesting that SAE impaired the somatosensory ability. By contrast, when given EE housing, the SAE rats exhibited shorter sticker removal latency than the SAE rats housed in the SE condition (SAE-SE *vs*. SAE-EE group: $p = 0.0028$, Fig. 2). Thus, the above results suggested that EE inhibits sensory neglect deficits caused by SAE.

### 3.2 EE Attenuated the SAE-Induced Anxiety-Related Behaviors

The EPM test was used to detect changes in anxiety-related behaviors. The anxiety behavior indices included number of entries into the open/closed arms, latency of entry into an open arm, and time occupying open/closed arms, head dipping, rearing, and grooming. Results showed that SAE factor, environmental factor or interaction had no effect on the time spent in head dipping, rearing, and grooming among the four groups (head dipping: SAE factor: F (1, 68) = 0.9969, $p = 0.3216$; environmental factor: F (1,

68) = 0.1180, $p$ = 0.7323; interaction F (1, 68) = 0.2388, $p$ = 0.6266; rearing: SAE factor: F (1, 68) = 0.9700, $p$ = 0.3282; environmental factor: F (1, 68) = 1.598, $p$ = 0.2105; interaction F (1, 68) = 0.4592, $p$ = 0.5003; grooming: SAE factor: F (1, 68) = 0.2117, $p$ = 0.6469; environmental factor: F (1, 68) = 0.4602, $p$ = 0.4998; interaction F (1, 68) = 0.0035, $p$ = 0.9529; Fig. 3A–C). However, significant effects were found for open arm time (SAE factor: F (1, 68) = 15.72, $p$ = 0.0002; environmental factor: F (1, 68) = 6.229, $p$ = 0.0150; interaction F (1, 68) = 2.993, $p$ = 0.0881; Fig. 3G), latency of entry into an open arm (SAE factor: F (1, 68) = 64.47, $p < 0.0001$; environmental factor: F (1, 68) = 10.40, $p$ = 0.0019; interaction F (1, 68) = 0.7131, $p$ = 0.4014; Fig. 3D) and number of entries into a closed arm (SAE factor: F (1, 68) = 53.10, $p < 0.0001$; environmental factor: F (1, 68) = 25.38, $p < 0.0001$; interaction F (1, 68) = 11.39, $p$ = 0.0012; Fig. 3F). Post-hoc analysis revealed the SAE-SE rats displayed a lower open arm time when compared with that of the other three groups, (SAE-SE *vs*. Sham-SE group: $p$ = 0.0008; SAE-SE *vs*. Sham-EE group: $p$ = 0.0001; SAE-SE *vs*. SAE-EE group: $p$ = 0.0199; Fig. 3G). As expected, the SAE-SE rats showed increased escape latency to enter an open arm and increased entries in the closed arms compared with those in the other three groups (latency to enter an open arm: SAE-SE *vs*. ShamSE group: $p < 0.0001$; SAE-SE *vs*. Sham-EE group: $p < 0.0001$; SAE-SE *vs*. SAE-EE group: $p$ = 0.0268; entries into a closed arm: SAE-SE *vs*. Sham-SE group: $p < 0.0001$; SAE-SE *vs*. Sham-EE group: $p < 0.0001$; SAE-SE *vs*. SAE-EE group: $p < 0.0001$; Fig. 3D,F). Taken together, the results suggested that SAE induces anxiety-related behaviors, which can be inhibited by EE housing.

### *3.3 EE Inhibited SAE-Induced Exploratory Activity Decrease*

Exploratory activity was evaluated using the OF test. There were no effects of SAE factor, environmental factor or interaction in the number of line crossings, rearing, and total distance traveled among the four groups (number of line crossings: SAE factor: F (1, 68) = 3.956, $p$ = 0.0507; environmental factor: F (1, 68) = 2.593, $p$ = 0.1120; interaction F (1, 68) = 0.2714, $p$ = 0.6041; number of line rears: SAE factor: F (1, 68) = 0.04711, $p$ = 0.8288; environmental factor: F (1, 68) = 0.2944, $p$ = 0.5892; interaction F (1, 68) = 0.2385, $p$ = 0.6269; total distance traveled: SAE factor: F (1, 68) = 2.313, $p$ = 0.1329; environmental factor: F (1, 68) = 0.03263, $p$ = 0.8572; interaction F (1, 68) = 0.2541, $p$ = 0.6159). These results indicated that locomotor activity was not affected by sepsis or environment factors.

However, the exploratory behavior was impaired in the SAE-SE rats: they spent less time in the center square (SAE factor: F (1, 68) = 53.09, $p < 0.0001$; environmental factor: F (1, 68) = 10.10, $p$ = 0.0022; interaction F (1, 68) = 9.694, $p$ = 0.0027; Post-hoc analysis: SAE-SE *vs*. Sham-SE group: $p < 0.0001$; SAE-SE *vs*. Sham-EE group: $p < 0.0001$; Fig. 4E) and more time in the corner squares (SAE factor: F (1, 68) = 85.17, $p < 0.0001$; environmental factor: F (1, 68) = 11.06, $p$ = 0.0014; interaction F (1, 68) = 1.486, $p$ = 0.2270; Post-hoc analysis: SAE-SE *vs*. Sham-SE group: $p < 0.0001$; SAE-SE *vs*. Sham-EE group: $p < 0.0001$; Fig. 4D). The results also showed that SAE-EE rats spent less time in the corner squares (SAE-EE *vs*. SAE-SE group: $p$ = 0.0106; Fig. 4D), and more time in the center square (SAE-EE *vs*. SAE-SE group: $p$ = 0.0002; Fig. 4E) compared with the SAE-SE rats. Thus, EE is capable of suppressing the decreased exploratory activity caused by SAE.

### *3.4 EE Inhibited the SAE-Induced Spatial Learning and Memory Deficits*

The MWM was used to test the reference/working memory and spatial learning. The results for spatial learning revealed that the SAE-SE rats took more time to climb up the hidden platform compared with that of the Sham-EE or Sham-SE rats from day 3 or day 4 onward, suggesting that SAE impaired their spatial learning ability (SAE-SE *vs*. Sham-EE group; day 3: $p < 0.0001$; day 4: $p < 0.0001$, day 5: $p < 0.0001$; SAE-SE *vs*. Sham-SE group; day 4: $p < 0.0001$, day 5: $p < 0.0001$; Fig. 5A). From 3 day onward, the SAE-EE rats took less time to reach platform (improved latency to the platform) than did the SAE-SE rats (SAE-EE *vs*. SAE-SE group; day 3: $p$ = 0.0022, day 4: $p < 0.0001$, day 5: $p < 0.0001$; Fig. 5A). However, the four groups showed no differences in latency to the visible platform (SAE factor: F (1, 68) = 0.5112, $p$ = 0.4771; environmental factor: F (1, 68) = 2.907, $p$ = 0.0928; interaction F (1, 68) = 0.7743, $p$ = 0.3820; Fig. 5B), suggesting that the escape latency differences were not affected by vision. These findings suggested that EE ameliorated the SAE-induced impairment of spatial learning.

After removing the platform on day 6, the reference/working memory retention was determined by the number of times the rat crossed the platform area and duration of occupancy of quadrant C. The data showed that the Sham-SE, Sham-EE or SAE-EE rats exhibited a duration of occupancy of quadrant C than the SAE-SE rats, suggesting enhanced memory retention (SAE factor: F (1, 68) = 9.431, $p$ = 0.0031; environmental factor: F (1, 68) = 9.238, $p$ = 0.0034; interaction F (1, 68) = 6.270, $p$ = 0.0147; Post-hoc analysis: Sham-SE *vs*. SAE-SE group: $p$ = 0.0011; Sham-EE *vs*. SAE-SE group: $p$ = 0.0003; SAE-EE *vs*. SAE-SE group: $p$ = 0.0012; Fig. 5C). The number of times the rats crossed the platform area showed similar results. The Sham-SE, Sham-EE, or SAE-EE rats crossed the platform more frequently than did the rats in the SAE-SE group (SAE factor: F (1, 68) = 65.72, $p < 0.0001$; environmental factor: F (1, 68) = 9.722, $p$ = 0.0027; interaction F (1, 68) = 7.370, $p$ = 0.0113; Post-hoc analysis: Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; SAE-SE *vs*. SAE-EE group: $p$ = 0.0132; Fig. 5D).

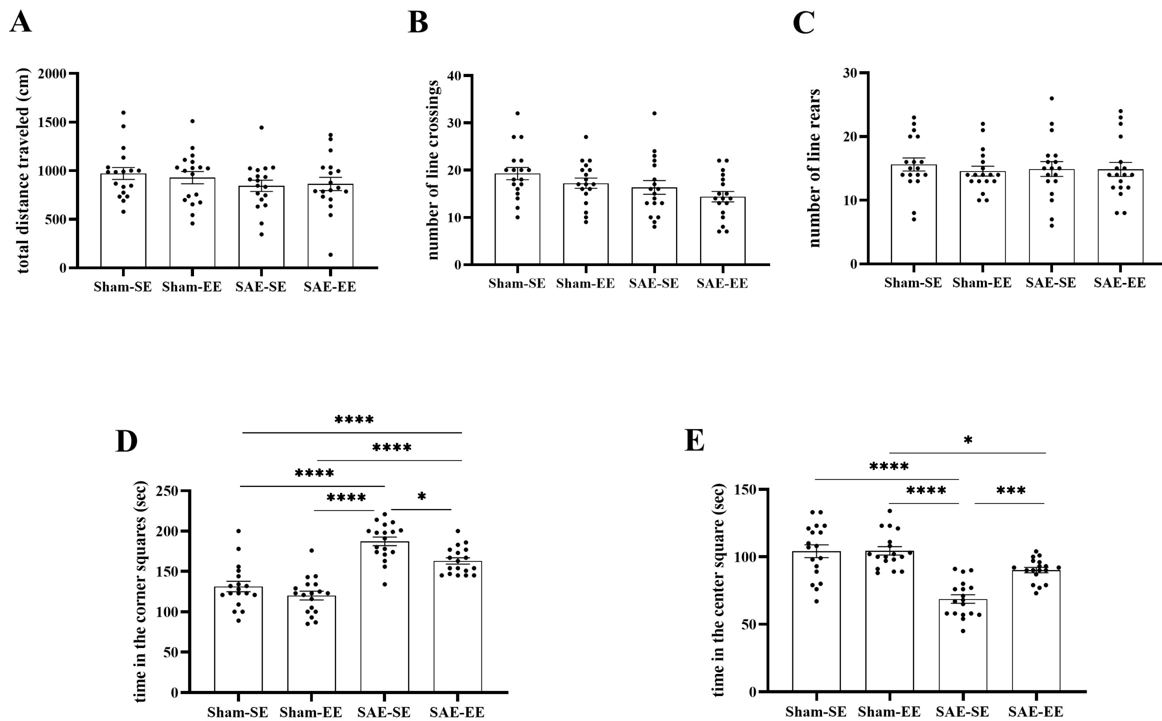


**Fig. 4. EE inhibited SAE-induced exploratory activity decrease.** (A–C) No differences were found in total traveled distance and times of line crossings and rears among the four groups. (D) The rats in the SAE-SE group had more time in the corner squares when compared with the rats in the Sham-SE, Sham-EE or SAE-EE group. (E) The rats in the SAE-SE group had less time in the center square when compared with the rats in the Sham-SE, Sham-EE or SAE-EE group. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 18.

There was no difference in swimming speed among the four groups (SAE factor: F (1, 68) = 1.20, $p$ = 0.2773; environmental factor: F (1, 68) = 0.0005, $p$ = 0.9807; interaction F (1, 68) = 0.9010, $p$ = 0.3459; Fig. 5E), suggesting that motor deficits did not contribute to the observed differences.

### *3.5 EE Ameliorated the Recognition Memory Impairment Induced by SAE*

The NOR test was adopted to detect the changes in non-spatial working memory (recognition memory). The results indicated that DI deteriorated after SAE (SAE factor: F (1, 63) = 9.896, $p$ = 0.0025; environmental factor: F (1, 63) = 14.93, $p$ = 0.0003; interaction F (1, 63) = 10.16, $p$ = 0.0022; Post-hoc analysis: Sham-SE *vs.* SAE-SE group: $p$ = 0.0002; Sham-EE *vs.* SAE-SE group: $p < 0.0001$; Fig. 6B), suggesting decreased recognition memory. However, when housed in EE, the recognition memory of the SAE rats improved in comparison with the SAE-SE rats (SAE-EE *vs.* SAE-SE group: $p < 0.0001$; Fig. 6B), suggested by the increased DI. However, the total exploration of objects showed no differences between the four groups (SAE factor: F (1, 63) = 1.151, $p$ = 0.2872; environmental factor: F (1, 63) = 0.0272, $p$ = 0.8694; interaction F (1, 63) = 0.0272, $p$ = 0.8694; Fig. 6A). These results indicated that EE could inhibit the recognition memory impairment induced by SAE.

### *3.6 EE Inhibited Inflammatory Cytokines, but Enhanced Anti-Inflammatory Cytokines in the Brains of SAE Rats*

Accumulating evidence demonstrates that inflammatory cytokines contribute to the impairment of cognitive function [39–41]. Therefore, we assessed the changes in brain inflammatory cytokine levels.

The results of the two-way ANOVA showed an obvious interaction effect on the levels of inflammatory cytokines (IL-1$\beta$: SAE factor: F (1, 68) = 9.04, $p$ = 0.0251; environmental factor: F (1, 68) = 32.13, $p < 0.0001$; interaction F (1, 68) = 14.22, $p$ = 0.0003; IL-6: SAE factor: F (1, 68) = 12.44, $p$ = 0.0008; environmental factor: F (1, 68) = 12.31, $p$ = 0.0008; interaction F (1, 68) = 13.08, $p$ = 0.0006; TNF-$\alpha$: SAE factor: F (1, 68) = 83.67, $p < 0.0001$; environmental factor: F (1, 68) = 8.918, $p$ = 0.0039; interaction F (1, 68) = 8.568, $p$ = 0.0047; IL10: SAE factor: F (1, 68) = 9.04, $p$ = 0.0251; environmental factor: F (1, 68) = 32.13, $p < 0.0001$; interaction F (1, 68) = 14.22, $p$ = 0.0003). Post-hoc analysis showed no differences in the levels of inflammatory cytokines (IL-1$\beta$, IL-6, TNF-$\alpha$, or IL10) between the Sham-SE and Sham-EE rats. However, there was an increase in brain IL-10, TNF-$\alpha$, IL-6 and IL-1$\beta$ levels in of the SAE-SE group when in comparison with those in the Sham-SE or Sham-EE rats (Sham-SE *vs.* SAE-SE group: IL-1$\beta$: $p$ = 0.0047, IL-6: $p < 0.0001$, TNF-$\alpha$: $p < 0.0001$, and IL-10: $p$ = 0.0005; Sham-EE *vs.* SAE-SE

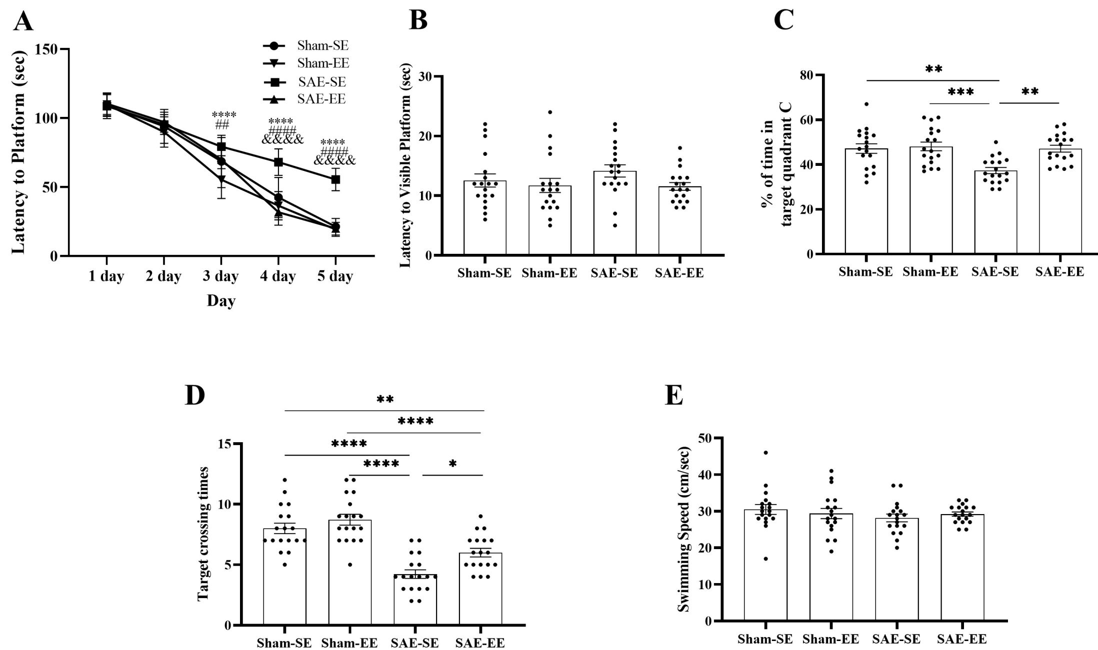


**Fig. 5. EE inhibited the SAE-induced spatial learning and memory deficits.** (A) At 3 day, 4 day or 5 day, the rats in the SAE-EE group exhibited shorter latency to platform when compared with the rats in the SAE-SE group. In addition, the rats in the Sham-EE or Sham-SE rats had the shorter latency to platform than the rats in the SAE-SE group from 3 day or 4 day on. SAE-SE *vs.* Sham-EE: ****$p < 0.0001$; SAE-SE *vs.* SAE-EE: ##$p < 0.01$, ####$p < 0.0001$; SAE-SE *vs.* Sham-SE: &&&&$p < 0.0001$. (B) There were no differences in latency to visible platform among four groups. (C) The rats in the SAE-SE group had less time spent in target quadrant (quadrant C) when compared with the rats in the Sham-SE, Sham-EE or SAE-EE group. (D) The rats in the SAE-EE rats, Sham-SE or Sham-EE group had more target crossing times than the rats in the SAE-SE group. (E) There were no differences in swimming speed among four groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 18.

group: IL-1$\beta$: $p < 0.0001$, IL-6: $p < 0.0001$, TNF-$\alpha$: $p < 0.0001$, and IL-10: $p = 0.0019$; Fig. 7A–D). In addition, the IL-1$\beta$, IL-6 or TNF-$\alpha$ level in the SAE-EE rats were lower than those in the SAE-SE rats (SAE-SE *vs.* SAE-EE group: IL-1$\beta$: $p < 0.0001$, IL-6: $p < 0.0001$; and TNF-$\alpha$: $p = 0.0005$; Fig. 7A–C). The IL-10 level increased further in comparison with that in the SAE-SE group (SAE-SE *vs.* SAE-EE group: IL-10: $p = 0.0036$; Fig. 7D), which suggested that in the whole brain, EE changed the SAEinduced pro-inflammatory profile to an anti-inflammatory profile.

Additionally, the inflammatory or anti-inflammatory cytokines in serum exhibited no differences among the four groups (IL-1$\beta$: SAE factor: F (1, 68) = 0.06095, $p = 0.8057$; environmental factor: F (1, 68) = 1.5724, $p = 0.2034$; interaction F (1, 68) = 0.0019, $p = 0.9653$; IL-6: SAE factor: F (1, 68) = 1.773, $p = 0.1874$; environmental factor: F (1, 68) = 0.1181, $p = 0.7322$; interaction F (1, 68) = 1.825, $p = 0.1022$; TNF-$\alpha$: SAE factor: F (1, 68) = 1.112, $p = 0.2953$; environmental factor: F (1, 68) = 2.763, $p = 0.1011$; interaction F (1, 68) = 0.0715, $p = 0.7900$; IL-10: SAE factor: F (1, 68) = 1.791, $p = 0.1810$; environmental factor: F (1, 68) = 0.0322, $p = 0.8581$; interaction F (1, 68) = 1.617, $p = 0.2079$; Fig. 8A–D), suggesting that the serum inflammatory or anti-inflammatory cytokines had returned to their normal levels at 30 days after SAE.

These results indicated that EE decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines in the whole brain of the SAE rats.

### *3.7 EE Regulated Apoptosis-Related Molecules in the Brain of SAE Rats*

SAE causes a significant increase in apoptosis, which also contributes to impaired cognitive function. p53 and Bcl-2 have been shown to regulate apoptosis in opposite manners [42,43].

SAE factor, environmental factor or interaction contributed to the difference of p53 or Bcl-2 level between four groups (p53: SAE factor: F (1, 68) = 56.97, $p < 0.0001$; environmental factor: F (1, 68) = 5.299, $p = 0.0244$; interaction F (1, 68) = 4.026, $p = 0.0488$; Bcl-2: SAE factor: F (1, 68) = 16.13, $p = 0.0001$; environmental factor: F (1, 68) = 4.577, $p = 0.0429$; interaction F (1, 68) = 14.88, $p = 0.0003$; Fig. 9A,B). The results also showed that the p53 level increased significantly in the SAE-SE rats in comparison with that in the Sham-EE or Sham-SE groups, whereas this increase was not observed in the SAE-EE rats (Sham-SE *vs.* SAE-SE group: $p < 0.0001$; Sham-EE *vs.* SAE-SE group: $p < 0.0001$; SAE-SE *vs.* SAE-EE group: $p = 0.0170$; Fig. 9A). The Bcl-2 level showed the reverse changes: After SAE, the brain Bcl-2 level was lower com-

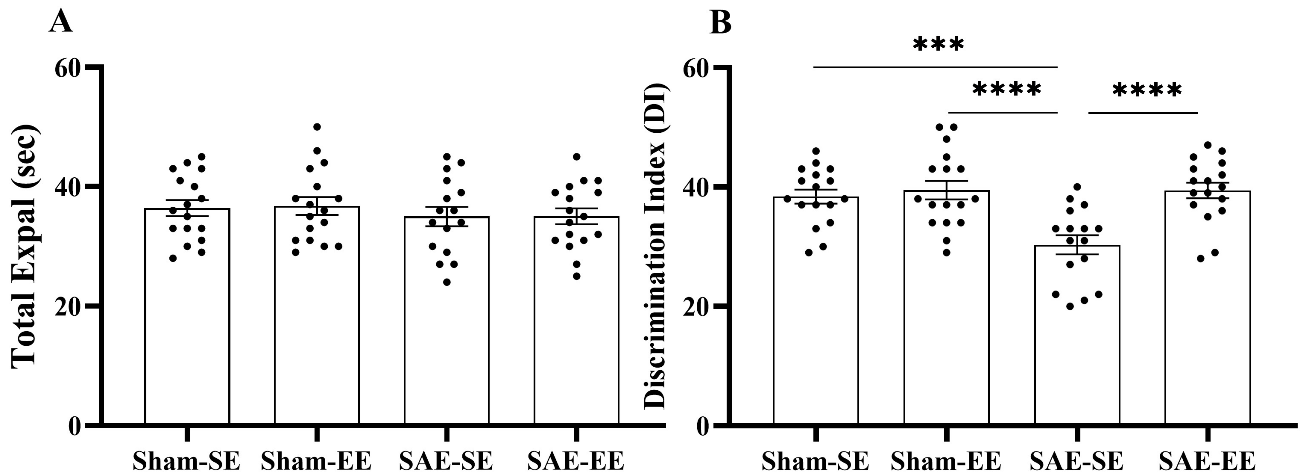


**Fig. 6. EE ameliorated the recognition memory impairment induced by SAE.** (A) total exploration. (B) DI. The total exploration exhibited no differences among four groups. The rats in the SAE-SE group had decreased DI when compared with the rats in the Sham-SE, Sham-EE or SAE-EE group. ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 16–17.

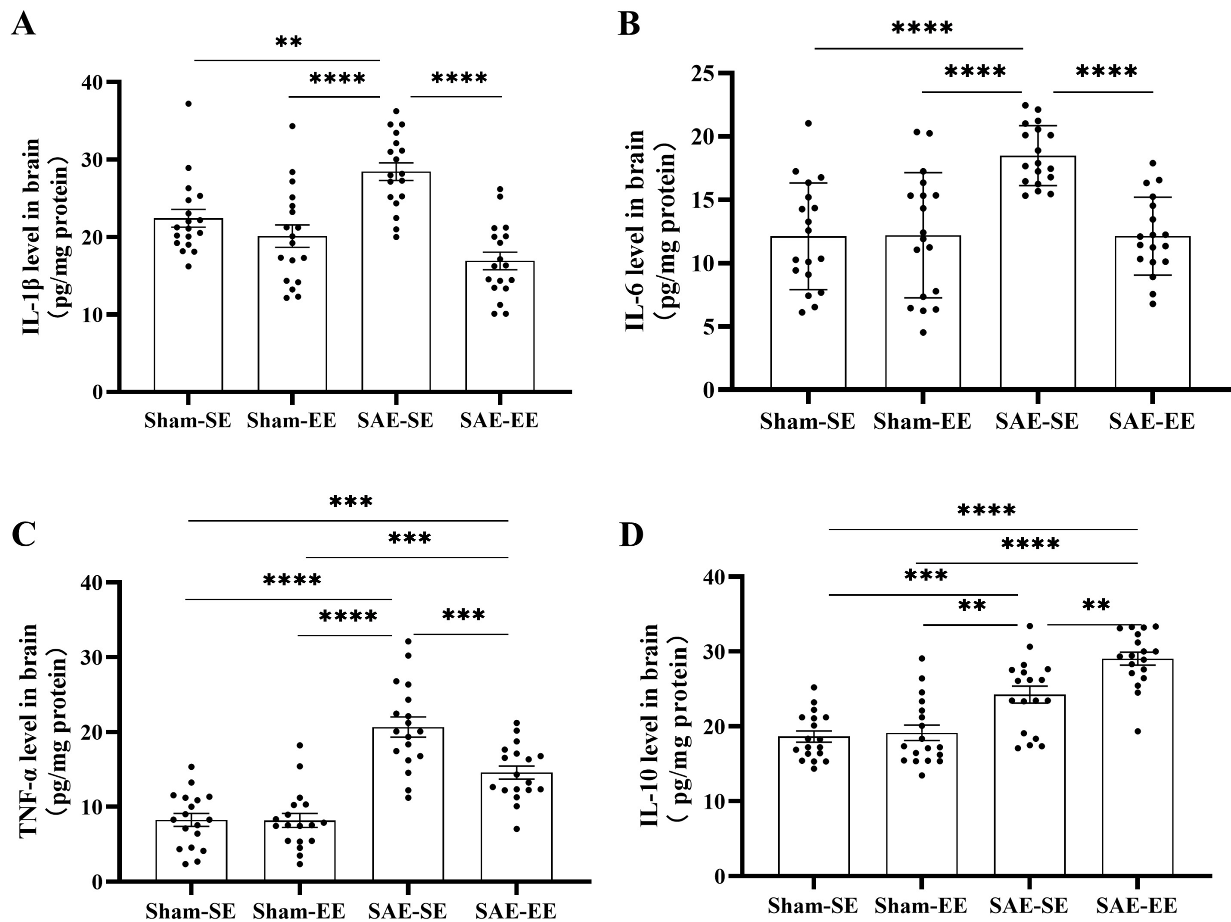


**Fig. 7. EE inhibited inflammatory cytokines, but enhanced anti-inflammatory cytokines in the brains of SAE rats.** (A) The whole brain of SAE-SE rats had higher IL-1$\beta$ level than that of the Sham-SE, Sham-EE or SAE-EE rats. (B) The whole brain of SAE-SE rats had higher IL-6 level than that of the Sham-SE, Sham-EE or SAE-EE rats. (C) The whole brain of SAE-SE rats had higher TNF-$\alpha$ level than that of the Sham-SE, Sham-EE or SAE-EE rats. (D) The whole brain of SAE-SE rats had higher IL-10 level than that of the Sham-SE or Sham-EE group. However, the IL-10 level in the brain of SAE-SE rats was lower than that of the SAE-EE rats. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 6.

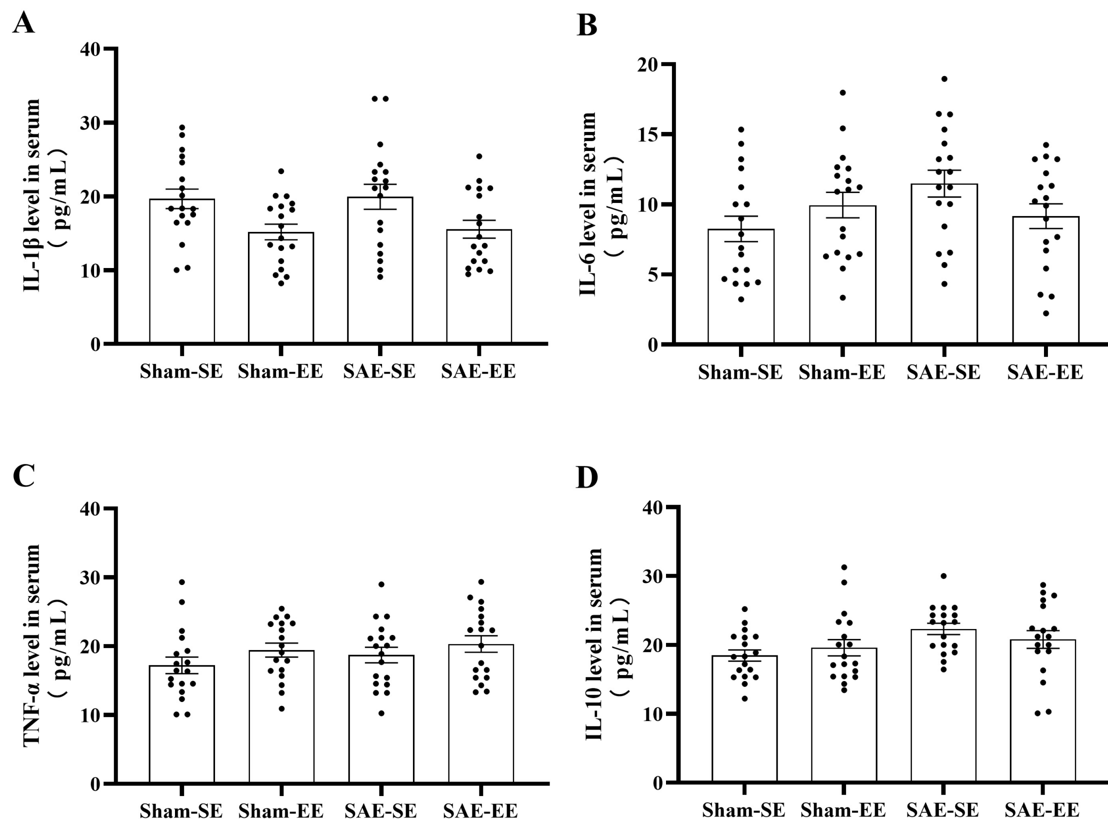


**Fig. 8. EE had no effect on the levels of inflammatory cytokines or anti-inflammatory cytokines in the serum.** (A) No differences were found in the IL-1$\beta$ in the serum among four groups. (B) No differences were found in the IL-6 in the serum among four groups. (C) No differences were found in the I TNF-$\alpha$ in the serum among four groups. (D) No differences were found in the I IL-10 in the serum among four groups. Each value represents means ± SEM, n = 6.

pared with that in the Sham-SE or Sham-EE groups (Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p = 0.0005$; Fig. 9B). As expected, the Bcl-2 level in the SAE-EE group was partially restored, being higher than that in the SAE-SE group (SAE-SE *vs*. SAE-EE group: $p = 0.0007$; Fig. 9B). These results suggested that EE could ameliorate the SAE-induced increase in the p53 level and the decrease in Bcl-2 level, ultimately countering the apoptosis induced by SAE.

Previous studies have shown that Bcl-2 reduced apoptosis by influencing BAX and caspase-3 [44]. Therefore, we also detected the changes in BAX and caspase-3 levels in the brain using ELISA. As expected, SAE increased the levels of BAX and caspase-3 (BAX: Sham-SE *vs*. SAE-SE group: $p < 0.0001$, Sham-EE *vs*. SAE-SE group: $p < 0.0001$; caspase-3: Sham-SE *vs*. SAE-SE group: $p = 0.0142$; Sham-EE *vs*. SAE-SE group: $p = 0.0067$; Fig. 9C,D) and EE could inhibit this increase in BAX and caspase-3 levels (BAX: SAE-SE *vs*. SAE-EE group: $p = 0.0014$; caspase-3: SAE-SE *vs*. SAE-EE group: $p < 0.0001$; Fig. 9C,D).

Thus, EE exhibited anti-apoptosis activity under SAE conditions by affecting the levels of p53 and the Bcl-2/BAX/caspase-3 signaling pathway.

### *3.8 EE Attenuated Oxidative Stress in Brain of SAE Rats*

It is widely reported that oxidative stress is responsible for the impaired cognitive function in various pathological conditions [45,46]. Consequently, we detected the changes in oxidant activity in the whole brain.

As shown in Fig. 10, there was a significant increase in TBARS level in the SAE-SE group, when compared with that in the Sham-SE or Sham-EE group (SAE factor: $F(1, 68) = 30.50$, $p < 0.0001$; environmental factor: $F(1, 68) = 8.096$, $p = 0.0059$; interaction $F(1, 68) = 12.06$, $p = 0.0009$; Post-hoc analysis: Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 10A), while the SAE-EE group showed a lower TBARS level than that in the SAE-SE group (SAE-SE *vs*. SAE-EE group: $p = 0.0002$; Fig. 10A). While in the Sham rats, EE or SE housing had no influence on the TBARS level (Sham-SE *vs*. Sham-EE group: $p = 0.9706$; Fig. 10A).

A similar response was found for AOPP (Fig. 10B). Under SAE conditions, the AOPP level in whole brain of the SAE-SE rats was markedly increased compared with that in the Sham-SE or Sham-EE groups (Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 10B). As expected, EE housing resulted

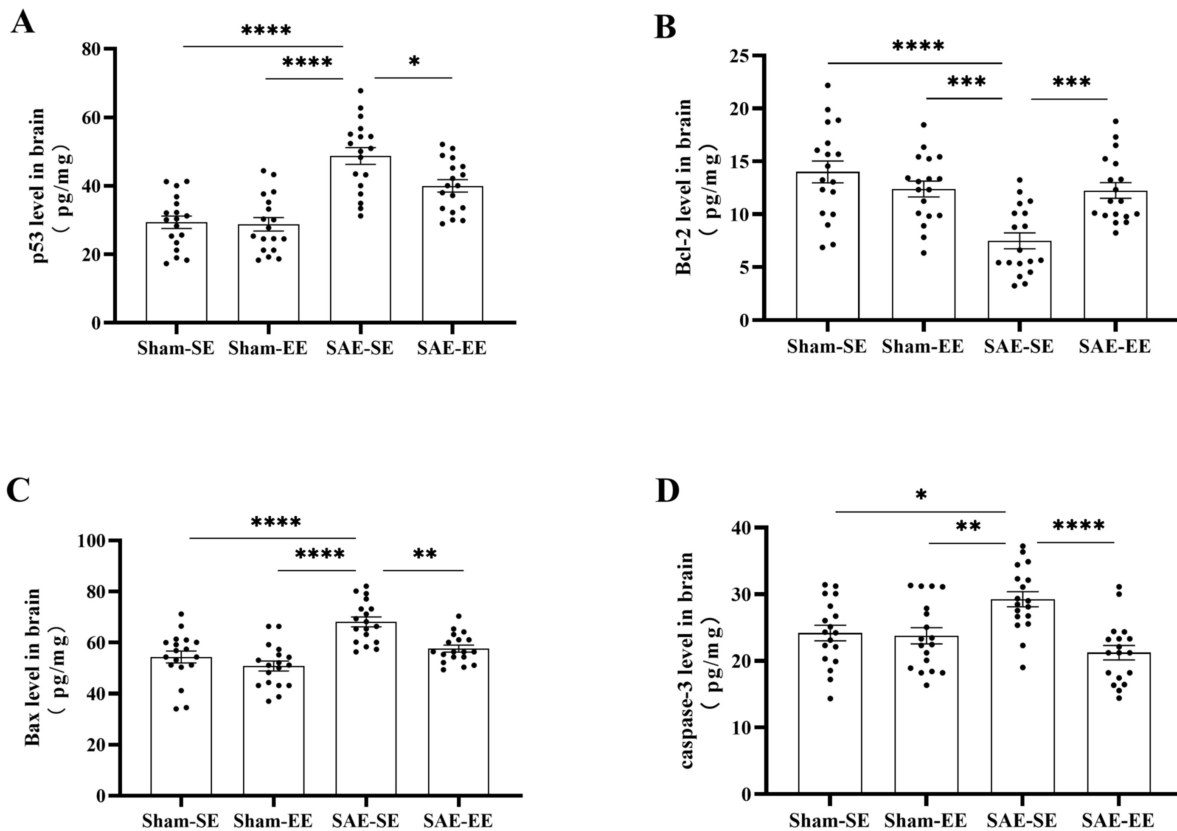


**Fig. 9. EE regulated apoptosis-related molecules in the brain of SAE rats.** (A) The p53 protein level in the whole brain of SAE-SE rats was obviously higher than that of the Sham-SE, Sham-EE or SAE-EE rats. (B) The Bcl-2 protein level in the whole brain of SAE-SE rats was less than that of the Sham-SE, Sham-EE or SAE-EE rats. (C) The Bax level in the whole brain of SAE-SE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. (D) The caspase-3 level in the whole brain of Sham-SE, Sham-EE or SAE-EE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represents means ± SEM, n = 6.

in a lower AOPP level (SAE-SE *vs*. SAE-EE group: $p$ = 0.0010; Fig. 10B).

We also detected the changes in NOx. As expected, SAE caused a significant enhancement in NOx levels relative to those in the Sham-SE or Sham-EE group (Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 10C). However, the NOx level in the SAE-EE group was lower compared with that in the SAE-SE group, which suggested that EE could reverse the increase in NOx induced by SAE (SAE-SE *vs*. SAE-EE group: $p < 0.0001$; Fig. 10C).

Analysis of the markers of oxidative stress showed that SAE caused significant oxidative stress, which could be inhibited by EE housing.

### *3.9 EE Enhanced the Antioxidant Activity in the Brain of SAE Rats*

An imbalance between oxidation and anti-oxidation leads to oxidative stress; therefore, the antioxidant activity in the brain was determined.

In the SAE-SE group, GSH was oxidized with a decreased level in whole brain relative to that in the Sham-SE or Sham-EE group (SAE factor: F (1, 68) = 26.32, $p < 0.0001$; environmental factor: F (1, 68) = 13.77, $p$ = 0.0004; interaction F (1, 68) = 4.771, $p$ = 0.0324; Post-hoc analysis: Sham-SE group *vs*. SAE-SE group: $p < 0.0001$; Sham-EE group *vs*. SAE-SE group: $p < 0.0001$; Fig. 11A). Correspondingly, an increase in GSSG was found in whole brain of the rats in the SAE-SE group (SAE factor: F (1, 68) = 76.32, $p < 0.0001$; environmental factor: F (1, 68) = 6.799, $p$ = 0.0112; interaction F (1, 68) = 5.481, $p$ = 0.0222; Post-hoc analysis: Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 11B), leading a decrease in the GSH/GSSG ratio in the SAE-SE group (Sham-SE *vs*. SAE-SE group: $p < 0.0001$; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 11C). However, after EE housing, the decrease in GSH and the increase in GSSG caused by SAE were inhibited (SAE-SE *vs*. SAE-EE group: GSH: $p$ = 0.0005, GSSG: $p$ = 0.0045; Fig. 11A,B), thereby increasing the GSH/GSSG ratio (SAE-SE *vs*. SAE-EE group: $p$ = 0.0460; Fig. 11C).

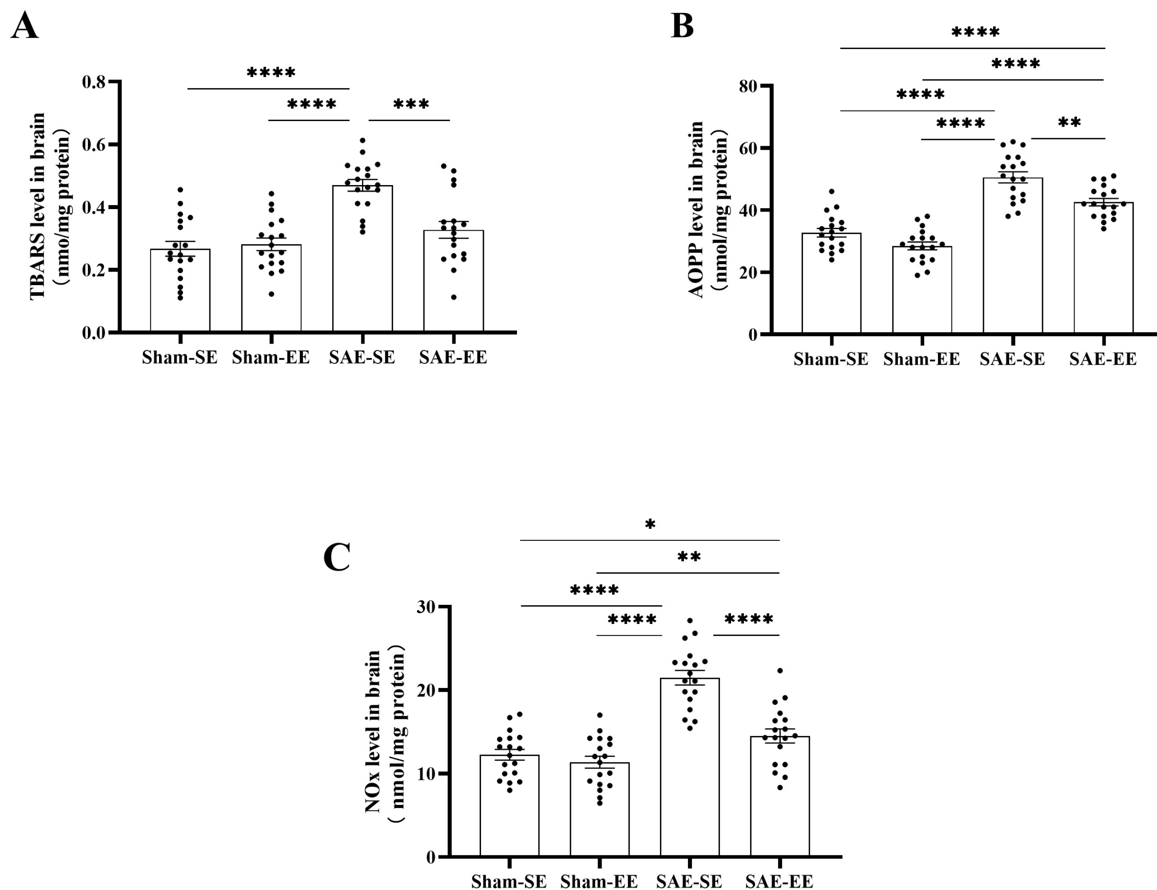


**Fig. 10. EE attenuated oxidative stress in brain of SAE rats.** (A) The TBARS level in the whole brain of SAE-SE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. (B) The AOPP level in the whole brain of SAE-SE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. (C) The NOx level in the whole brain of SAE-SE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represent means ± SEM, n = 6.

SOD is another important antioxidant enzyme, whose activity decreased significantly in the SAE-SE group (SAE factor: F (1, 68) = 44.25, $p < 0.0001$; environmental factor: F (1, 68) = 15.19, $p = 0.0002$; interaction F (1, 68) = 8.610, $p = 0.0046$; Post-hoc analysis: Sham-SE *vs*. SAE-SE group: p < 0.0001; Sham-EE *vs*. SAE-SE group: $p < 0.0001$; Fig. 5D). However, after EE housing, the SOD activity in whole brain was restored despite the presence of SAE (SAE-SE *vs*. SAE-EE group: $p < 0.0001$; Fig. 11D).

## 4. Discussion

Cognitive rehabilitation commonly uses EE as an effective and simple treatment. Our present results suggested that cognition impairment induced by SAE could be effectively alleviated using EE. Performances in behavioral tests (OF, EPM, MWM, and SN) indicated that after 30 days of enrichment, the negative effects of SAE on reference/working memory, spatial learning exploratory activity, anxiety-related behavior, and somatesthesia, were inhibited. Thus, rats with SAE reared under EE conditions had an improved outcome, which was similar to observations in other animal models of brain dysfunction [47–52]. In addition, EE also provides positive benefits to humans. For example, an enriched lifestyle is effective in slowing down the process of cognitive aging [53], ameliorates TBI-induced cognitive deterioration [50], and delays the onset of dementia [54].

The SN test is an effective examination to detect somatesthesia and reveals somatosensory changes resulting from injury to the prefrontal cortex [24]. The SN test showed that EE-housed SAE rats showed a marked enhancement in their response to somatosensory stimuli relative to SE-housed SAE rats. The EPM test is a good indicator of anxiety behavior [55]. The EPM test revealed that EE-housed SAE rats showed less anxiety-related behaviors compared with SE-housed SAE rats. This was consistent with previous well documented observations of EE's anxiolytic effect [25,56–58]. It should be noted that SAE commonly induces prefrontal damage that leads to an increased risk-taking behavior [59]. A previous study showed that open arm time could be increased by risk-taking behavior via a mechanism not associated with anxiety [60]. Therefore, in this test, we could not ignore the possible contribution made by risk-taking behavior to the increase in open

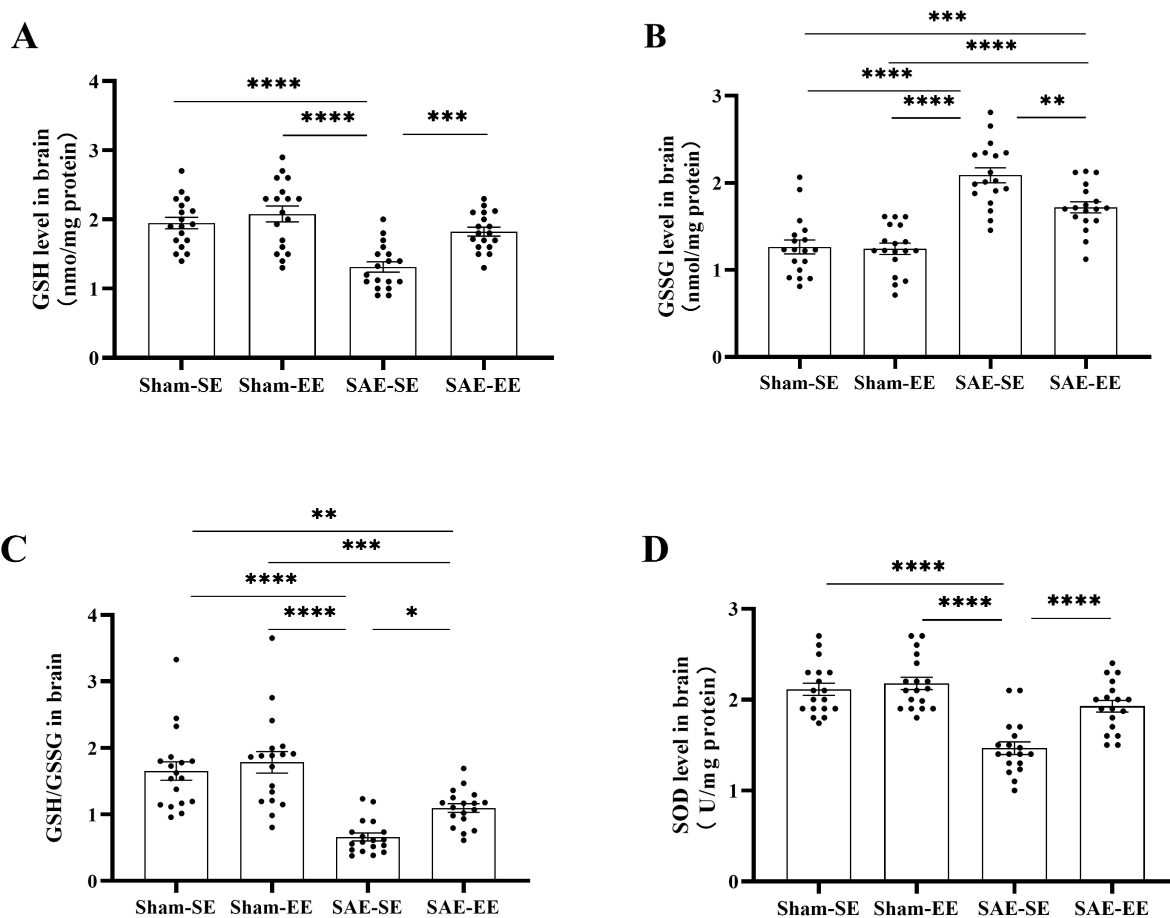


**Fig. 11. EE enhanced the antioxidant activity in the brain of SAE rats.** (A) The GSH level in the whole brain of SAE-SE rats was less than that of the Sham-SE, Sham-EE or SAE-EE rats. (B) The GSSG level in the whole brain of SAE-SE rats was higher than that of the Sham-SE, Sham-EE or SAE-EE rats. (C) The ratio of GSH/GSSG in the whole brain of SAE-SE rats was less than that of the Sham-SE, Sham-EE or SAE-EE rats. (D) The SOD level in the whole brain of SAE-SE rats was less than that of the Sham-SE, Sham-EE or SAE-EE rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Each value represent means ± SEM, n = 6.

arms time. MWM is a commonly used approach to assess hippocampus-dependent spatial learning and memory [61]. Herein, the MWM test revealed that EE could decreased the SAE-induced increase in latency to the platform. The rats find the invisible platform dependent only on memory and spatial cues; thus, decreased latency suggests enhanced memory and spatial learning. We also showed that the performance in the MWM test was not affected by locomotor factors. In addition, land-based locomotor impairment is not related to swimming speed, which also accounts for the independence from locomotor effects of learning and memory performance in the MWM test. There was no randomization of the target quadrant in this test, which might have allowed the rats to find the platform more easily, which is a limitation of this study. Herein, recognition memory was assessed using the NOR test, the results of which demonstrated that recognition memory impairment by SAE was effectively ameliorated by EE, and the preference for novelty was also increased following exposure to EE. The NOR test is used widely to detect recognition memory and mainly depends on innate recognition behavior, without externally applied rules or reinforcement. In addition to revealing the existence of familiar object in the rats' memory [62], the increased preference for novel objects also suggests a close relationship between EE and the desire to access novel stimuli [63]. In this study, we did not set a day of habituation to the testing arena prior to the familiarization phase as some previous studies [64] which may increase the novelty-induced anxiety and result in the excessive freezing behavior in the test phase.

The present study also showed that EE had no effect on Sham rats. In agree with this result, it has been shown that EE lacks effect on the cognitive function of control groups [65,66]. However, some studies also indicated a positive impact on the cognitive function of control groups [67]. This difference might be due to the duration of EE housing, the age or gender of experimental animal.

Sepsis-induced central nervous system (CNS) neuroinflammation is believed to be the mechanism of delayed cognitive dysfunction [68,69]. During sepsis, an im-

paired blood-brain barrier (BBB) allows brain transmission of systemic inflammation [70,71]. Increased levels of the pro-inflammatory cytokine, IL-1$\beta$, inhibit long term potentiation (LTP) in cornu ammonis 1, cornu ammonis 3, and the dentate gyrus of the hippocampus, representing the electrophysiological marker of learning and memory, by disrupting brain-derived neurotrophic factor signaling cascades, which impair F-actin formation in dendritic spines [72,73]. Overexpression of IL-6 also leads to cognitive dysfunction via disrupting neurotransmission in brain structures that regulate cognitive functions e. g. the prefrontal cortex and the hippocampus. IL-6 blockade prolonged the LTP and enhanced performance in a Y-maze task [74]. IL-6 induces glial cell expression of cyclooxygenase 2, leading to increased prostaglandin synthesis, especially prostaglandin E2, which activates the hypothalamus pituitary adrenal axis, resulting in behavioral alterations and fever [75]. In the present study, SAE induced significant increases in the levels of TNF-$\alpha$, IL-6, and IL-1$\beta$ in the brain, which further confirmed the role of neuroinflammation in SAE-induced cognitive dysfunction. Interestingly, our recent study showed that the hippocampal TNF-$\alpha$ content between Sham-SE, SAE-SE, and SAE-EE rats did not change [9]. We propose that neither an environmental factor nor a SAE factor influenced the hippocampal TNF-$\alpha$ level, suggesting that TNF-$\alpha$ is not involved in SAE-induced deficits in spatial learning and memory. Herein, SAE also induced an obvious increase in IL-10 levels, which is the most important anti-inflammatory cytokine. IL-10 increment might be the result of a self-protection mechanism against SAE. Moreover, EE further augmented the IL-10 level, suggesting that EE positive affects the anti-inflammatory ability of SAE rats. The present results also showed no difference was found in the pro-inflammatory cytokines level in serum between four groups after 30 days EE or SE housing. These results consist with some previous study that demonstrated that 10 days after sepsis induction, no sepsis signals could be observed in the plasma and the contents of inflammatory mediators in the plasma were similar to those of the control group [76]. We speculated that the sepsis enhances the self-anti-inflammatory capability that suppresses the increased inflammatory cytokines 10 days after sepsis induction.

The results of the present study showed that EE exposure could inhibit the SAE-induced increase in p53 and decrease in Bcl-2. P53 mainly regulates cell-cycle arrest, senescence, and apoptosis, while Bcl-2 inhibits the apoptotic pathway [77–79]. Thus, our study demonstrated an inverse relationship between Bcl-2 and p53 protein levels in the whole brain under SAE conditions. This finding is in line with previous studies concerning relationship between Bcl-2 and p53 protein levels [80]. Increased p53 and decreased Bcl-2 ultimately results in increased apoptosis of the essential brain cells under SAE conditions. Neuronal apoptosis can result in brain atrophy (especially in the cortex and hippocampus) and ventricle enlargement [81], finally leading to cognitive impairment. Of course, activation of mitogen activated protein kinase (MAPK) [82,83] and mechanistic target of rapamycin (mTOR) signaling pathways [84] produce apoptotic factors as a result of neuroinflammation.

Oxidative stress is also associated with long-term cognitive impairment [85,86]. Herein, oxidative stress marker levels were enhanced after SAE and EE could reduce these increases. Increased oxidative stress leads to mitochondrial damage, energy failure, overproduction of toxins and inflammatory factors, BBB breakdown, and ischemia, which all result in cognitive impairment. We also found that antioxidant activity was suppressed by SAE, and EE could alleviate this suppression, indicating that EE improved the antioxidant system. Increased oxidative stress produces reactive oxygen species in the brain, which play a positive role in modulating the production of pro-inflammatory mediators by preventing MAPK and nuclear factor kappa B (NF-$\kappa$B) activation in microglia cells [87]. Therefore, we concluded that EE alleviates the SAE-induced increase in oxidative stress and decreased antioxidant system, resulting in a decrease in pro-inflammatory cytokines.

This study had some limitations: Firstly, we assessed the cognition function, detected cytokine levels, oxidative stress, antioxidant activity and apoptosis-related molecules only at a single time point. In the future, the temporal effects of EE will be assessed by determining these indices at different exposure times. Secondly, we used young (60 days old), male rats; whereas, the majority of patients with SAE are middle-aged or older. EE positively affects rats of all ages; however, we consider that it might have more benefits in younger rats. Because female rats have estrus cycle that may disturb behavioral performance, we only used male rats in the present study. Thirdly, we believe that the SAE is a diffuse cerebral dysfunction and the SAE-induced cognition deficits attributes to the impairment of whole brain, not one brain region or several brain regions. Therefore, in the present study, we detected the inflammatory cytokines, apoptosis-related molecules, oxidative stress and antioxidant activity in the whole brain, not the discrete brain regions. Of course, hippocampus and perirhinal cortex were closely related with spatial orientation and object discrimination [88]. In the future, we will detect the changes of these discrete brain regions. Fourthly, the CLP rats showed a high mortality rate (51%) in the present study, which is similar with some previous studies [19,20], However, this high mortality rate may bias the research outcome. Therefore, a model of SAE by lipopolysaccharide (LPS) administration may be preferable.

## 5. Conclusions

The results of the present study indicated that EE could effectively counteract the cognitive deficits induced by SAE via a process related to reduced levels of pro-inflammatory cytokines, oxidative stress, and apoptosis,

and enhanced levels of anti-inflammatory cytokines and antioxidant activity. This study highlights the potential rehabilitation effect of EE to treat the consequences of SAE.

## Abbreviations

AOPP, Advanced oxidation protein products; BBB, blood-brain barrier; Bcl-2, B-cell lymphoma/leukemia-2; CNS, central nervous system; DI, discrimination index; EE, environmental enrichment; ELISA, Enzyme-linked immune-absorbent assay; EPM, Elevated Plus Maze test; GPx, glutathione peroxidase; GR, glutathione reductase; ICUs, intensive care units; MDA, malondialdehyde acid; MWM, Morris water maze test; NF-$\kappa$B, nuclear factor kappa B; NO, Nitric oxide; NOR, Novel Object Recognition test; OF, Open Field test; OPA, ophthalaldehyde; RIPA, radioimmunoprecipitation assay; SAE, sepsis-associated encephalopathy; SE, standard environment; SN, sensory neglect test; TBARS, thiobarbituric acid-reactive substances.

## Author Contributions

YJD and YFT—Collection of data and writing manuscript. SJ and JWG—Supervision and direction the project. BYG and SJ—Analysis and interpretation of data, and preparation of manuscript. RDG and XL—Collection of data. The authors read and approved the final manuscript.

## Ethics Approval and Consent to Participate

The study was approved by the committee of Animal Use for Research and Education in China-Japan Friendship Hospital (approval number: zryhyy61-21-03-24).


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## Conflict of Interest

The authors declare no conflict of interest.

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