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ARTICLE

Enriched environment alleviates post-stroke cognitive impairment through enhancing α7-nAChR expression in rats

Ambiente enriquecido alivia o comprometimento cognitivo pós-AVC em ratos por meio do aumento da expressão de α7-nAChR

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ABSTRACT

Background: Enriched environment (EE) is a simple and effective intervention to improve cognitive function in post-stroke cognitive impairment (PSCI), partly due to the rebalancing of the cholinergic signaling pathway in the hippocampus. α7-nicotinic acetylcholine receptor (α7-nAChR) is a cholinergic receptor whose activation inhibits inflammation and promotes the recovery of neurological function in PSCI patients. However, it is still unclear whether EE can regulate α7-nAChR and activate the cholinergic anti-inflammatory pathway (CAP) in PSCI. **Objective:** To investigate the effects of EE on cognitive impairment, and the role of α7-nAChR in PSCI. **Methods:** A PSCI rat model was induced by middle cerebral artery occlusion and reperfusion (MCAO/R) and were reared in standard environment (SE) or EE for 28d, control group with sham surgery. Cognitive function was determined by Morris water maze test. The long-term potentiation (LTP) was assessed by Electrophysiology. Histopathological methods were used to determine infarct volume, α7-nAChR expression and the cytokines and cholinergic proteins expression. **Results:** Compared with SE group, rats in EE group had better cognitive function, higher expression of α7-nAChR positive neurons in hippocampal CA1 region. In addition, EE attenuated unfavorable changes induced by MCAO/R in cytokines and cholinergic proteins, and also enhanced LTP promoted by nicotine and attenuated by α-BGT; but showed no significantly difference in infarct volume. **Conclusions:** EE markedly improves cognitive impairment and enhances neuroplasticity in PSCI rats, which may be closely related to enhancement of α7-nAChR expression.

Keywords: Cognitive Dysfunction; Stroke; Hippocampus.

RESUMO

Introdução: O ambiente enriquecido (AE) é uma intervenção simples e eficaz para melhorar a função cognitiva no comprometimento cognitivo pós-AVC, em parte devido ao reequilíbrio da via de sinalização colinérgica no hipocampo. O receptor nicotínico α7 de acetilcolina (α7-nAChR) é um receptor colinérgico cuja ativação inibe inflamação e promove a recuperação da função neurológica em pacientes com comprometimento cognitivo pós-AVC. No entanto, ainda não está claro se o AE pode regular α7-nAChR e ativar a via anti-inflamatória colinérgica (VAC) em comprometimento cognitivo pós-AVC. **Objetivo:** Investigar os efeitos do AE no comprometimento cognitivo e o papel do α7-nAChR no comprometimento cognitivo pós-AVC. **Métodos:** Modelo de comprometimento cognitivo pós-AVC foi induzido em ratos por oclusão e reperfusão da artéria cerebral média (MCAO/R), que foram criados em ambiente padrão (AP) ou em AE por 28d; grupo controle com cirurgia simulada. A função cognitiva foi determinada pelo teste do labirinto aquático de Morris. A potenciação de longo prazo (PLP) foi avaliada por eletrofisiologia. Métodos histopatológicos foram usados para determinar o volume do infarto, a expressão de α7-nAChR e a expressão de citocinas e proteínas colinérgicas. **Resultados:** Em comparação com o grupo AP, os ratos do grupo AE tiveram melhor função cognitiva, com maior expressão de neurônios positivos para α7-nAChR na região CA1 do hipocampo. Além disso, o AE atenuou alterações desfavoráveis induzidas por MCAO/R em citocinas e proteínas colinérgicas, e também aumentou a PLP promovida pela nicotina e atenuada por α-BGT, mas não mostrou nenhuma diferença significativa no volume do infarto. **Conclusão:** O AE melhora acentuadamente o comprometimento cognitivo e aumenta a neuroplasticidade em ratos com comprometimento cognitivo pós-AVC, o que pode estar intimamente relacionado ao aumento da expressão de α7-nAChR.

Palavras-chave: Disfunção Cognitiva; Acidente Vascular Cerebral; Hipocampo.

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INTRODUCTION

Post-stroke cognitive impairment (PSCI) is a common complication of ischemic stroke. About 25% of patients has severe PSCI three months after stroke, including memory, orientation, language and attention[1]. Cognitive impairment is a major contributor to longer duration of hospital stay, lower quality of life and difficulty returning to social life[2]. Prevention for PSCI can be implemented by lowering blood pressure, administration of statin, neuroprotective and anti-inflammatory drugs. However, there is no evidence of convincing efficacy. Recent studies also suggested that lifestyle interventions, physical activity and cognitive training may improve cognition of PSCI, but without sufficient controlled clinical trials[3].

Enriched environment (EE) is an effective rodent rehabilitation treatment method, in which several animals are accommodated in a large space equipped with various toys and receive more sensorial movements, perceptions and social stimulation than in standard conditions. EE has a neuroprotective effect on animal models of cerebral ischemia[4]. There is significant evidence that EE also benefits the clinical rehabilitation of post-stroke patients, including promoting greater exercise, social interaction and personal control[5]. Exploring the underlying mechanisms that EE improves cognitive function in PSCI is of great significance for providing more individualized cognitive rehabilitation programs in stroke patients.

α7-nicotinic acetylcholine receptor (α7-nAChR) is a cholinergic receptor that is abundantly-expressed in the hippocampus and the frontal cortex, and has been confirmed to play a critical role in improving cognitive function of learning and memory[6,7]. As the biological foundation of cognitive processes, synaptic transmission can be modulated by cholinergic pathway and evaluated by long-term potentiation (LTP). Activation of α7-nAChR contributes to a better induction of LTP[8], which is also essential for inhibiting cytokine synthesis, such as IL-1β, IL-6, by the cholinergic anti-inflammatory pathway (CAP)[9]. Activation of α7-nAChR inhibits inflammation in patients with stroke[10]. In the aging process of mice, EE not only increases the expression of choline acetyltransferase (ChAT) and α7-nAChR in the hippocampus but also improves spatial memory[11]. EE also significantly improves cognitive impairment in PSCI mice, induces hippocampal LTP, and enhances ChAT promoter acetylation[12].

These studies suggest that activation of cholinergic signals after stroke enhances neuroplasticity and cognitive function. However, it is still unclear whether EE can regulate α7-nAChR and the role of α7-nAChR in CAP and LTP of PSCI.

To explore the effects of EE on cognitive function, neuroplasticity and underlying mechanism in PSCI rats, a rat model of ischemic stroke was induced by middle cerebral artery occlusion and reperfusion (MCAO/R). We have characterized the effects of EE on cognitive function, expression of α7-nAChR protein, induction of LTP in hippocampus, and serum cytokine levels.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (200±20 g, 10 weeks) were purchased from Shanghai SLRC Laboratory Animal Co., Ltd. (Shanghai, China). Rats were housed under pathogen-free conditions and maintained at controlled temperature (20–24°C) and humidity (40–70%) on a 12h light/dark schedule, with food and water freely available. The experimental procedures were approved by the Animal Ethics Committee of Zhoupu Hospital.

Post-stroke cognitive impairment model

Ischemic stroke model was established through MCAO/R in rats with minor modifications[13]. Briefly, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg) and their body were maintained at 37°C of temperature via a thermal pad throughout the process. The midline neck incision was made for each rat to expose the right common carotid artery, internal carotid artery, and external carotid artery. The common carotid artery was ligated near the bifurcation of the carotid artery. A lysine-coated nylon monofilament (0.32±0.02 mm) (Beijing Sunbio Biotech Co., Ltd., Beijing, China) was inserted into right internal carotid artery via the common carotid artery, and was gently advanced to the origin of the middle cerebral artery. The filaments were pull out 1h later to restore blood flow (reperfusion). Twenty-four hours after MCAO/R, all surviving rats underwent neurobehavioral examination using a longa scoring system: score 0: no neurological deficit; score 1: completely unable to extend right forepaw; score 2: rotated right; score 3: toward right; score 4: can not walk spontaneously and consciousness level declines. Rats with scores of 1–3 were considered successful MCAO/R model and then divided into standard environment (SE) or EE housing condition according to the scores. Sham surgery received the same procedures except for the filament insertion.

Experimental design

Rats were divided into 3 groups according to surgery and housing condition: (1) Control group: sham surgery rats were housed in SE condition, n=16; (2) SE group: MCAO/R rats were housed in SE condition, n=16; (3) EE group: MCAO/R rats were housed in EE condition, n=16. In EE housing condition, 6–8 rats were housing together in a large cage (90 cm long × 75 cm wide × 50 cm high) with various objects, including climbing ladders, wood platform, toys or tunnels of different shape and color. These subjects changed 3 times a week to keep novelty. Four rats were housed together in each SE housing condition, which consisted of a standard cage (44 cm long × 32 cm wide × 20 cm high) with no objects. To further investigate the role of α7-nAChR in LTP induction by EE, nicotine (α7-nAChR activator, 0.5 mg/kg, i.p.) or α-BGT (α7-nAChR inhibitor, 1.0 μg/kg, i.v.) were administered daily for 28 days

according to a previously method[7], 4 rats for each administration. Rats were housed in EE or SE condition for 4 weeks.

Behavioral tests

Assessment of neurological function

Modified neurological severity scores (mNSS)[14] were evaluated on 7, 14, 28 days after MCAO/R to assess the degree of neurological deficits. mNSS is a comprehensive test including motor, sensory, reflex and balance tests and scaled from 0 to 18 score (score 0: normal; score 18: maximal deficit). The higher scores reflect more severe deficits. The scores were evaluated by two researchers who were blinded to the experiment grouping.

Morris water-maze test

Morris water-maze test was performed to measure the spatial learning and memory including two phases. The apparatus consisted of a circular tank (160 cm in diameter and 60 cm in height) that was filled with water at 25±1°C to a depth of 30 cm, and a circular hidden platform (12 cm diameter) was located in the middle of northeast quadrant of the pool and 2 cm below the water level. The first phase involved space learning trainings prior to experimental trail from 3 days after MCAO/R. Rats were randomly placed into pool facing the wall of tank from southeast and southwest directions, and allowed 90 s to reach the hidden platform. Each rat received 4 trainings prior to trail per day for 3 consecutive days. The second phase involved place navigation trial and probe trial at 7, 14 and 28 days after MCAO/R. The place navigation trial was carried out like space learning training and the duration reaching the platform was recorded as the escape latency (maximum 90 s). In the probe trail, platform was removed from the pool and the number of crossing over original platform position were recorded as platform crossing times.

Quantification of infarct volume

At 28 days after MCAO/R, rats were decapitated, and the brains were quickly frozen and sliced into coronal sections (2 mm thickness), and sections were stained with 1.2% 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma, St. Louis, Mucun, USA) at 37°C for 30 min, and then fixed in 4% paraformaldehyde overnight. Then, sections were observed and photographed. The infarcted tissue showed unstained (white) and normal tissue showed stained (red), and were analyzed using digital image analysis software (SigmaScan Pro, Jandel, San Rafael, CA, USA). The percentage of infarct volume was calculated using the following equation: percentage of infarct volume = (total infarct volume/whole brain section volume) × 100%. The volume was quantified by summing areas of sections multiplied by the section thickness (2 mm).

Expression of α7-nAChR in the hippocampus

Rats were decapitated at 28 days after MCAO/R, and the hippocampus was fixed overnight with 4% paraformaldehyde overnight and embedded with paraffin wax to acquire serial sections (5 μm thickness). After dewaxing, dehydration, incubation with 3% H_2O_2, and microwave antigen retrieval, the hippocampus serial sections were then incubated with rabbit anti-α7-nAChR primary antibody (1:100, ab10096, Abcam, UK) at 4°C overnight. After washing with phosphate buffered saline (PBS), sections were incubated with goat-anti-rabbit IgG labelled with HRP as secondary antibody (1:100, ab6721, Abcam, UK) at room temperature for 2h, and then were stained with diaminobenzidine (DAB) (Leica, Germany) and hematoxylin. The DAB stained brown cells are α7-nAChR positive neurons, which were counted from five random high magnification vision in CA1 region under a microscope (BX51, Olympus, Japan). The cell density was average number of α7-nAChR positive neurons per high magnification vision (cells/HP).

ELISA for cytokines and cholinergic proteins

Blood samples were obtained from caudal vein of rats at 7, 14 and 28 days after MCAO/R, and the ipsilateral hippocampal tissue at 28 days after MCAO/R. Both serum and hippocampal homogenates were separated by centrifugation at 5000 g for 10 min at 4°C to acquire serum and hippocampal homogenates. ELISA assay kits were used to measure serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), neuron-specific enolase (NSE) and brain-derived neurotrophic factor (BDNF) (R&D Systems, Minneapolis, MN, USA). Moreover, the ACh content (A105-1), ChAT (A079) and acetylcholinesterase (AChE) (A024) activities in the hippocampus were measured by commercial assay kits (Nanjing Jiancheng Biological Engineering Institute, Nanjing, China). The values of above proteins were determined by measuring the wavelength at 450 nm using a microplate reader (Ricso RK201, Shenzhen Ricso Technology Co., Ltd, China).

Electrophysiology

Rats were decapitated at 28 days after MCAO/R, brains were rapidly removed and the ipsilateral hippocampus region was cut into transverse slices (400 μm thickness). Slices were continuously perfused with ice-cold ACSF composed of the following: NaCl 124 mM, $CaCl_2$ 2.0 mM, KCl 4.5 mM, $MgCl_2$ 1.0 mM, $NaHCO_3$ 26 mM, NaH_2PO_4 1.2 mM, D-glucose 10 mM, and pH 7.4. A bipolar electrode was inserted into the ipsilateral Schaffer collaterals using microscope (Olympus BX50-wI, Olympus, Japan) to deliver the orthorhombic stimulus by a stimulator (SEN-3301, Nihon Kohden, Japan), and a recording electrode was inserted into the ipsilateral CA1 region to record the field excitatory postsynaptic potential (fEPSP). Baseline responses were recorded for 20 minutes prior to beginning the experiment with a constant current pulse (frequency 0.1 Hz, pulse duration 0.25 ms). Then, LTP was induced by high frequency stimulation (HFS, 100 Hz). LTP was recorded every 5 min for 120 min with the same stimulation intensity as pre-HFS. To further investigated the role of α7-nAChR in LTP induction by EE, α-BGT (1.0 μg/kg, i.v.) and nicotine (0.5 mg/kg, i.p.) were administered daily for 28 days.

Statistical analysis

All quantitative data were presented as mean±standard deviation (SD) and analyzed by SPSS 19.0 statistical software (SPSS, USA). The differences among groups in Morris water-maze test and serum cytokines were analyzed using two-way analysis of variance (ANOVA) with repeated measures, with following Bonferroni test for multiple comparisons. One-way ANOVA was applied to compare the differences in other data, followed by Bonferroni's post hoc. For statistically significance, $p<0.05$ was considered as criteria.

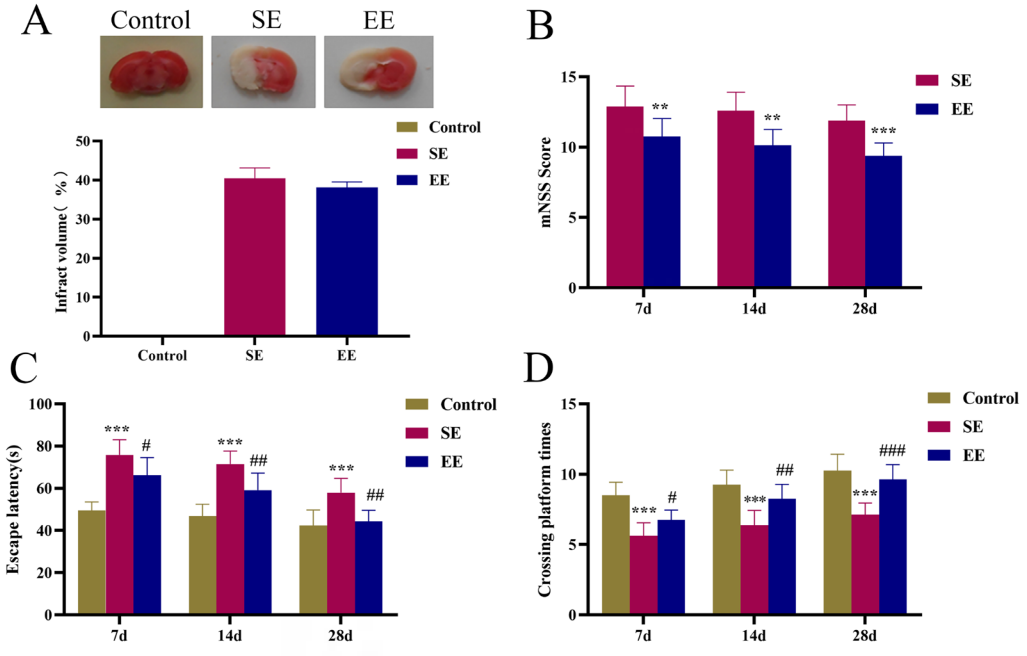
RESULTS

Enriched environment ameliorated cognitive deficits in ischemic stroke rats

At 28 days after MCAO/R, the infarct volume of EE group (38.13±1.44%; n=4) was slightly smaller than SE group (40.48±2.69%; n=4). However, with no significant difference ($p=0.189$) (Figure 1A). In terms of the functional recovery, mNSS scores were significantly decreased by 16.5, 19.6 and 21.1% at 7, 14 and 28 days after MCAO/R, respectively, compared with SE group ($p<0.001$; n=8 rats per group) (Figure 1B). In terms of learning and memory, data of Morris water-maze test suggested that, compared with control group, escape latency time was significantly increased and crossing platform times were significantly decreased in rats of SE group at different time points. Moreover, results were reversed significantly by EE treatment ($p<0.05$; n=8 per group) (Figures 1C and 1D). These results indicate that EE is capable of alleviating cognitive impairment induced by ischemic lesion.

Enriched environment enhanced α7-nAChR expression and cholinergic pathway of hippocampus

The density of α7-nAChR in the hippocampal CA1 region was detected by immunohistochemistry. Represent pictures were shown for the α7-nAChR positive neurons in control group (Figure 2A, photo 1), SE group (Figure 2A, photo 2) and EE group (Figure 2A, photo 3). Compared with the control group (46.75±4.88%), the cell density of α7-nAChR positive neurons was decreased in SE group (18.08±3.16%; $p<0.001$), and then restored by EE (36.43±2.73%; $p<0.001$ *vs.* SE group). It was then measured the ACh content, ChAT and AChE activities of hippocampal tissues with ELISA. MCAO/R significantly decreased the ACh content (43.93%; $p<0.001$) (Figure 2B) and ChAT activity (49.10%; $p<0.001$) (Figure 2C), but increased AChE activity (153.83%; $p<0.001$) (Figure 2D) in the hippocampal tissues. Moreover, these changes were significantly reversed by EE (ACh content, $p<0.001$; ChAT activity, $p=0.003$; AChE activity, $p<0.001$), n=4 per group. All these findings suggest that EE enhances α7-nAChR expression and cholinergic pathway of hippocampus.

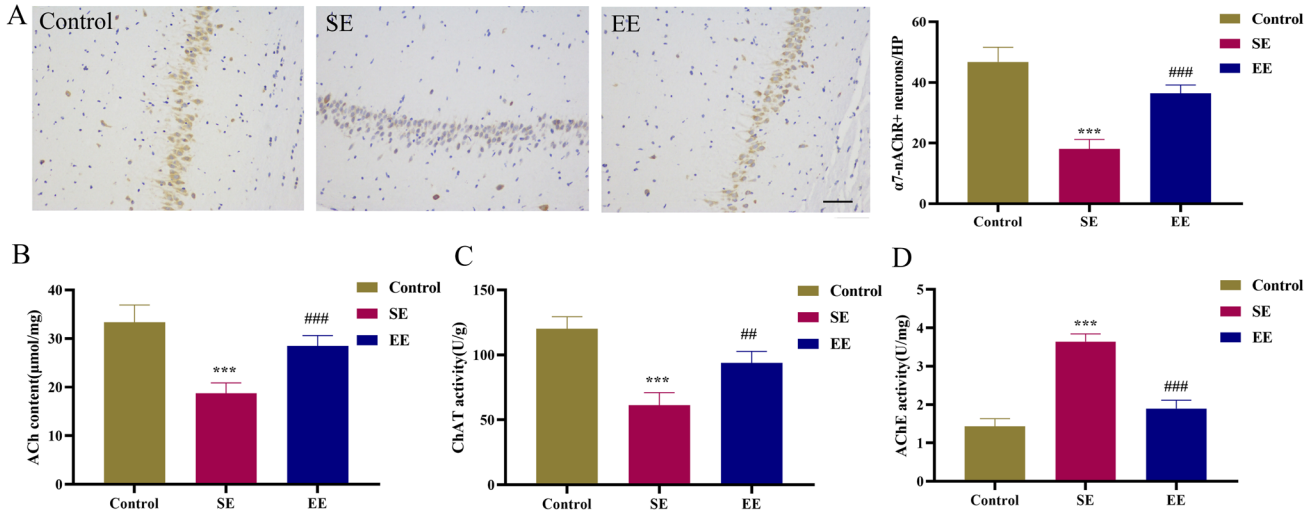


(A) Representative photographs of brain slices that are stained with TTC at 28 days after MCAO/R. The white areas are infracted tissue. EE slightly reduces infarct size, with no significant difference (n=4 rats per group). (B) EE significantly decreases mNSS score at 7, 14 and 28 days after MCAO/R. Date are expressed as mean±SD. **p<0.01, ***p<0.001 *vs.* SE group of the same time points (n=8 rats per group). (C-D) EE significantly decreases escape latency time and increases crossing platform times. Date are expressed as mean±SD. ***p<0.001 *vs.* control group of the same time points; #p<0.05, ##p<0.01, ###p<0.001 *vs.* SE group of the same time points (n=8 rats per group). SE: standard environment; EE: enriched environment, mNSS: Modified neurological severity scores.

Figure 1. Effects of enriched environment on infarct volume, neurological deficit scores and cognitive impairment.

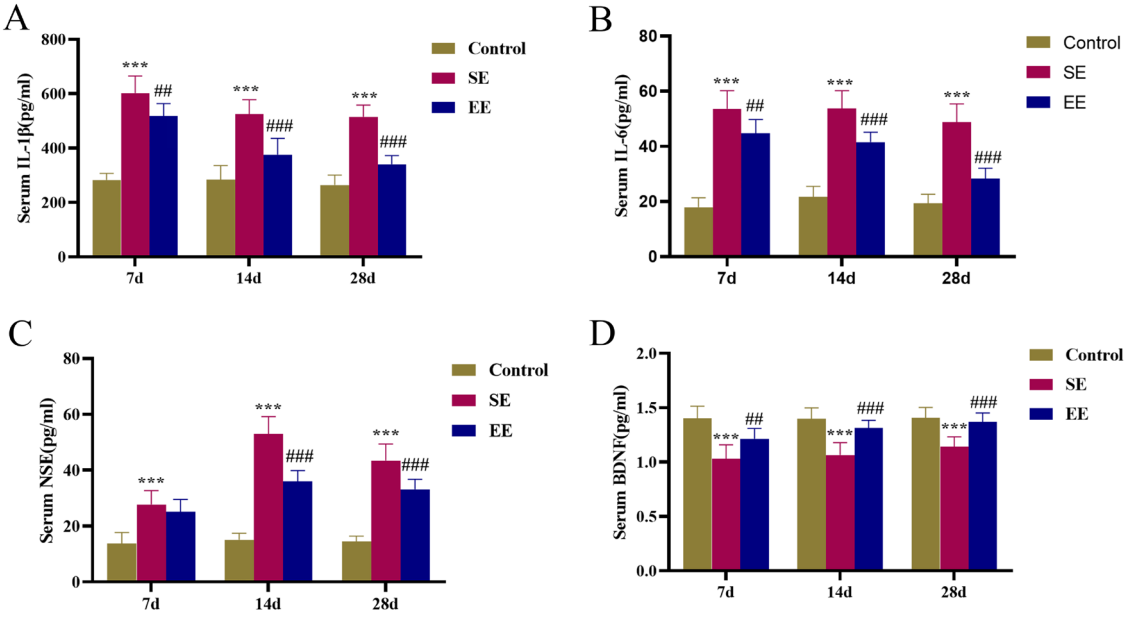
Enriched environment suppressed proinflammatory cytokines, brain damage and improved nerve regeneration

ELISA immunoassay were performed to detect the expression levels of serum proinflammatory cytokines (IL-1β and IL-6) (Figures 3A and 3B), NSE (Figure 3C) and BDNF (Figure 3D) at 7, 14 and 28 days after MCAO/R. Compared with control group, the level of proinflammatory cytokines and NSE were much higher ($p<0.001$) and BDNF was decreased significantly ($p<0.001$) at all time points after MCAO/R. EE significantly attenuated MCAO/R-induced change of proinflammatory cytokines, NSE and BDNF in



(A) Representative images of hippocampal α7-nAChR positive neurons in the hippocampal CA1 region are shown in rats of control group, SE group and EE group at 28 days after MCAO/R (×200). Scale bar=10 μm. EE increases the cell density of α7-nAChR positive neurons in the hippocampus ($p<0.05$). EE increases ACh content (B) and ChAT activity (C), and decreases AChE activity (D) in hippocampal tissue of MCAO/R rats. Date are expressed as mean±SD, ***$p<0.001$ *vs.* control group; ##$p<0.01$, ###$p<0.001$ *vs.* SE group. n=4 samples from 4 rats per group. ACh, acetylcholine; ChAT, choline acetyltransferase; AChE, acetylcholinesterase.

Figure 2. The effects of enriched environment on cholinergic pathway in the hippocampal tissue of middle cerebral artery occlusion and reperfusion rats.



EE significantly decreases serum IL-1β (A), IL-6 (B) and NSE (C) levels, and increases BDNF (D) level in rats at 7, 14 and 28 days after MCAO/R. Date are expressed as mean±SD, ***$p<0.001$ *vs.* control group of the same time points; ##$p<0.01$, ###$p<0.001$ vs. SE group of the same time points. n=8 samples from 8 rats per group. NSE, c.

Figure 3. Effects of enriched environment on serum cytokine levels in middle cerebral artery occlusion and reperfusion rats.

serum, n=8 per group. These results suggest that EE is capable to suppress proinflammatory cytokines, brain damage and improve nerve regeneration.

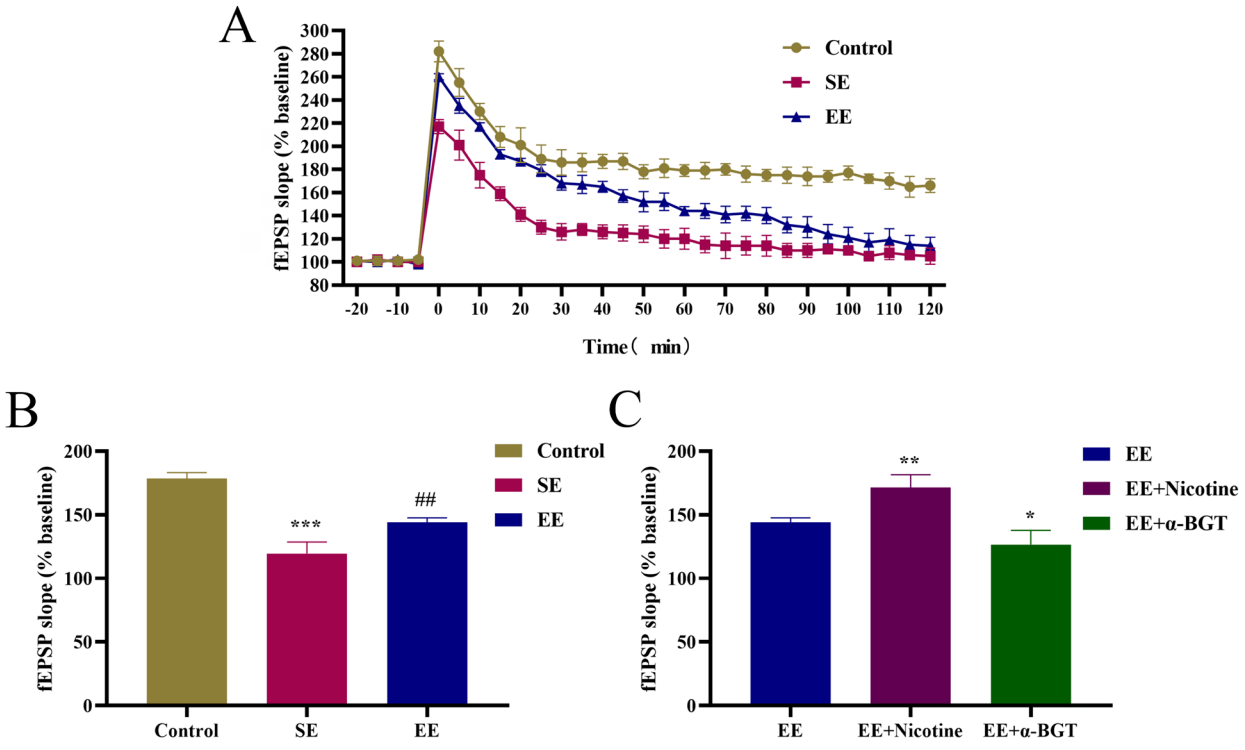
Enriched environment enhanced LTP in α7-nAChR dependent manner

To investigate the effect of EE on neural plasticity, hippocampal slices were stimulated by HFS to measure LTP. In the 5 min after HFS, the fEPSP slope of control group sharply increased compared baseline and then slowly decreased. EE could increase fEPSP slope in MCAO/R rats, especially before 90 min compared with SE group (Figure 4A). This indicates that ischemic stroke induces obvious damage on LTP, while EE enhances LTP after HFS. Average fEPSP amplitude at 60 min after HFS was significantly increased by 20.50% with EE, compared with SE group (p=0.002) (Figure 4B), and this increase was further enhanced by cotreatment with nicotine (19.10%; p=0.002 *vs.* EE group), and attenuated by cotreatment with α-BGT (12.15%; p=0.026 *vs.* EE group) (Figure 4C), n=4 per group. These results suggest that EE enhances synaptic plasticity and the effect is dependent on α7-nAChR.

DISCUSSION

The present study demonstrated effects and related mechanisms of EE in PSCI rats induced by MCAO/R. EE significantly improves neurological function and attenuates cognitive impairment in MCAO/R rats. In addition, α7-nAChR expression, hippocampal ACh content and ChAT activity was increased due to EE, and AChE activity was decreased due to EE, compared with SE group. EE also suppresses neuroinflammation, as evidenced by reduced serum levels of IL-1β and IL-6. NSE levels and BDNF level were reversed by EE after MCAO/R. Moreover, LTP was enhanced by EE in hippocampal slices, which was further enhanced by nicotine and attenuated by α-BGT. Together, these results provide evidence that EE has neuroprotective effects on PSCI rats. What's more, the mechanisms underlying this protection may be associated with enhanced expression of α7-nAChR, suppression of inflammation, and enhanced induction of LTP.

No significant difference in infraction volume was observed between EE and SE groups in our study, which is similar to recent studies in PSCI. For example, infarct volume on day 31 after MCAO in EE group is also smaller slightly, but no



(A) Time course and extent of LTP induction in rats 28 days after MCAO/R. fEPSP amplitude is normalized to average baseline. (B) EE increases the fEPSP slope. Date are expresses as mean±SD, ***p<0.001 *vs.* control group, ##p<0.01 *vs.* SE group. (C) The effects of EE on LTP are dependent on α7-nAChR. Average fEPSP amplitude at 60 min after HFS by EE are enhanced by cotreatment with nicotine, and attenuated by cotreatment with α-BGT. Date are expressed as mean±SD, *p<0.05, **p<0.01 *vs.* EE group. n=4 slices from 4 rats per group. α-BGT, α-bungarotoxin; LTP, long-term potentiation; HFS: high frequency stimulation; fEPSP: field excitatory postsynaptic potential.

Figure 4. The effects of enriched environment on long-term potentiation of middle cerebral artery occlusion and reperfusion rats.

statistically significant difference compared with SE group[15]. This result is also observed at 14 days after MCAO[16], possibly due to that the neuronal death is irreversible. Moreover, studies also found that pretreatment with EE for 2 or 5 weeks significantly decreases infract volume induced by ischemic stroke in rats, suggesting that pretreatment with EE protect neuronal death from stroke[17,18]. However, a previous systematic review found that EE increases infract volume of some degree without significantly statistic difference compared with SE group in two third of stroke-related studies, possibly due to the stress of new environment and cortical hyperexcitability[19]. Given that the small sample size in our study and no consensus on the effect of infarct volume by EE in PSCI, the effect of EE on infarct volume still need further research.

The anti-inflammatory effects of EE at different time point after stroke in this study is in accordance with some previous studies. For example, at four weeks after ischemic stroke, EE attenuates histopathological and oxidative damage to the brain, thereby improving cognitive function in chronic cerebral hypoperfusion (CCH) rats[20]. In an earlier phase of stroke, EE significantly decreases the expression of IL-1βat 5d after MCAO[21]. These show similar results with our study and indicate that suppressed neuroinflammation is an important mechanism of EE. The hippocampus is likely a good target of anti-inflammatory responses given that hippocampus is the main brain area responsible for cognitive function.

The present study shows that EE activates cholinergic pathway in MCAO/R rats. Agonists of α7-nAChR has an improvement effect on cognition, while reduced α7-nAChR expression has been found in various neurological disorders[22]. α7-nAChR is a major component of CAP and controls inflammation in central nervous system[23]. In ischemic stroke, α7-nAChR activates CAP and suppresses neuroinflammation, thus improving cognitive impairment[24]. These indicate that enhanced hippocampal α7-nAChR expression in our study may contribute to inhibition of neuroinflammation and improvement of cognitive function. α7-nAChR also might mediate the cognitive effect of EE. To our knowledge, our study is the first report on the up-regulation of α7-nAChR by EE in ischemic stroke. However, the intermediate links between EE and α7-nAChR remain largely unknown.

This study shows that EE increased ACh content and ChAT activity, and decreased AChE activity in the hippocampus of MCAO/R rats. ACh is a neurotransmitter and are associated with cognitive ability[25], who is regulated by two enzymes: ACh is synthesize by ChAT and degraded by AChE[26]. One report showed that acetylation of histones bound to the ChAT gene promoter and cholinergic circuits are enhanced by EE in PSCI mice, which supports our study. Increased AChE activity in the prefrontal cortex is associated with age-related cognitive decline[27], while decreased AChE activity and enhanced memory are found in physically enriched rats compared with rats reared in social environment[28]. These suggest that EE increases hippocampal ACh contents by increasing ChAT and decreasing AChE activities, thereby improving cognitive function.

EE enhances LTP in MCAO/R rats in this study. LTP manifests persistent strengthening of synapses and subsequent enhanced signaling between two neurons. Suppressed hippocampal LTP can result in cognitive impairment[29]. Hippocampal LTP is reduced in rats of CCH and reversed by EE[30]. Enhanced LTP indicates higher synaptic plasticity, and might be associated with increased BDNF by EE[31]. Furthermore, there is inter-regulation between BDNF and α7-nAChR. Choline could induce higher α7-nAChR activity and then lead to increased BDNF level[32]. Conversely, BDNF up-regulated α7-nAChR levels on subpopulations of hippocampal interneurons, which are important target cells that participate LTP[33,34]. The regulatory role of α7-nAChR in LTP was supported by our study, which is that LTP enhancement in hippocampal slices by EE was dependent on α7-nAChR.

In summary, EE demonstrates a marked preventive effect against cognitive impairment of MCAO/R rats. The mechanism underlying EE may be associated with enhanced expression of α7-nAChR, activation of CAP, and enhanced synaptic plasticity. The study reveals that EE may be a promising therapeutic method for PSCI patients, and provides potential possibility for the combination of EE with cholinergic activating agents.

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References

1. Jacquin A, Binquet C, Rouaud O, Graule-Petot A, Daubail B, Osseby GV, et al. Post-stroke cognitive impairment: high prevalence and determining factors in a cohort of mild stroke. J Alzheimers Dis. 2014;40(4):1029-38. https://doi.org/10.3233/jad-131580
2. Cumming TB, Brodtmann A, Darby D, Bernhardt J. The importance of cognition to quality of life after stroke. J Psychosom Res. 2014 Nov;77(5):374-9. https://doi.org/10.1016/j.jpsychores.2014.08.009
3. Mijajlović MD, Pavlović A, Brainin M, Heiss WD, Quinn TJ, Ihle-Hansen HB, et al. Post-stroke dementia - a comprehensive review. BMC Med. 2017 Jan;15(1):11. https://doi.org/10.1186/s12916-017-0779-7
4. Li YW, Li QY, Wang JH, Xu XL. Contribution of p38 MAPK to the ameliorating effect of enriched environment on the cognitive deficits induced by chronic cerebral hypoperfusion. Cell Physiol Biochem. 2016;40(3-4):549-57. https://doi.org/10.1159/000452568

5. White JH, Bartley E, Janssen H, Jordan LA, Spratt N. Exploring stroke survivor experience of participation in an enriched environment: a qualitative study. Disabil Rehabil. 2015;37(7):593-600. https://doi.org/10.3109/09638288.2014.935876

6. Wei XM, Yang W, Liu LX, Qi WX. Effects of L-arginine and N(omega)-nitro-L-arginine methylester on learning and memory and alpha7 nAChR expression in the prefrontal cortex and hippocampus of rats. Neurosci Bull. 2013 Jun;29(3):303-10. https://doi.org/10.1007/s12264-013-1331-1

7. Xu KL, Liu XQ, Yao YL, Ye MR, Han YG, Zhang T, et al. Effect of dexmedetomidine on rats with convulsive status epilepticus and association with activation of cholinergic anti-inflammatory pathway. Biochem Biophys Res Commun. 2018 Jan;495(1):421-6. https://doi.org/10.1016/j.bbrc.2017.10.124.

8. Udakis M, Wright VL, Wonnacott S, Bailey CP. Integration of inhibitory and excitatory effects of α7 nicotinic acetylcholine receptor activation in the prelimbic cortex regulates network activity and plasticity. Neuropharmacology. 2016 Jun;105:618-29. https://doi.org/10.1016/j.neuropharm.2016.02.028

9. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature. 2003 Jan;421(6921):384-8. https://doi.org/10.1038/nature01339

10. Neumann S, Shields NJ, Balle T, Chebib M, Clarkson AN. Innate immunity and inflammation post-stroke: an α7-nicotinic agonist perspective. Int J Mol Sci. 2015 Dec;16(12):29029-46. https://doi.org/10.3390/ijms161226141

11. Baraldi T, Schöwe NM, Balthazar J, Monteiro-Silva KC, Albuquerque MS, Buck HS, et al. Cognitive stimulation during lifetime and in the aged phase improved spatial memory, and altered neuroplasticity and cholinergic markers of mice. Exp Gerontol. 2013 Aug;48(8):831-8. https://doi.org/10.1016/j.exger.2013.05.055

12. Wang X, Chen A, Wu H, Ye M, Cheng H, Jiang X, et al. Enriched environment improves post-stroke cognitive impairment in mice by potential regulation of acetylation homeostasis in cholinergic circuits. Brain Res. 2016 Nov;1650:232-42. https://doi.org/10.1016/j.brainres.2016.09.018

13. Mao LL, Hao DL, Mao XW, Xu YF, Huang TT, Wu BN, et al. Neuroprotective effects of bisperoxovanadium on cerebral ischemia by inflammation inhibition. Neurosci Lett. 2015 Aug;602:120-5. https://doi.org/10.1016/j.neulet.2015.06.040

14. Chen SF, Hsu CW, Huang WH, Wang JY. Post-injury baicalein improves histological and functional outcomes and reduces inflammatory cytokines after experimental traumatic brain injury. Br J Pharmacol. 2008 Dec;155(8):1279-96. https://doi.org/10.1038/bjp.2008.345

15. Li MZ, Zhan Y, Yang L, Feng XF, Zou HY, Lei JF, et al. MRI Evaluation of axonal remodeling after combination treatment with xiaoshuan enteric-coated capsule and enriched environment in rats after ischemic stroke. Front Physiol. 2019;10:1528. https://doi.org/10.3389/fphys.2019.01528

16. Qian HZ, Zhang H, Yin LI, Zhang JJ. Postischemic housing environment on cerebral metabolism and neuron apoptosis after focal cerebral ischemia in rats. Curr Med Sci. 2018 Aug;38(4):656-65. https://doi.org/10.1007/s11596-018-1927-9

17. Karelina K, Norman GJ, Zhang N, DeVries AC. Social contact influences histological and behavioral outcomes following cerebral ischemia. Exp Neurol. 2009 Dec;220(2):276-82. https://doi.org/10.1016/j.expneurol.2009.08.022

18. Gonçalves LV, Herlinger AL, Alarcon Ferreira TA, Coitinho JB, Wanderley Pires RG, Martins-Silva C. Environmental enrichment cognitive neuroprotection in an experimental model of cerebral ischemia: biochemical and molecular aspects. Behav Brain Res. 2018 Aug;348:171-83. https://doi.org/10.1016/j.bbr.2018.04.023

19. Janssen H, Bernhardt J, Collier JM, Sena ES, McElduff P, Attia J, et al. An enriched environment improves sensorimotor function post-ischemic stroke. Neurorehabil Neural Repair. 2010 Nov-Dec;24(9):802-13. https://doi.org/10.1177/1545968310372092

20. Yang Y, Zhang J, Xiong L, Deng M, Wang J, Xin J, et al. Cognitive improvement induced by environment enrichment in chronic cerebral hypoperfusion rats: a result of upregulated endogenous neuroprotection? J Mol Neurosci. 2015 Jun;56(2):278-89. https://doi.org/10.1007/s12031-015-0529-2

21. Ruscher K, Johannesson E, Brugiere E, Erickson A, Rickhag M, Wieloch T. Enriched environment reduces apolipoprotein E (ApoE) in reactive astrocytes and attenuates inflammation of the peri-infarct tissue after experimental stroke. J Cereb Blood Flow Metab. 2009 Nov;29(11):1796-805. https://doi.org/10.1038/jcbfm.2009.96

22. Baranowska U, Wiśniewska RJ. The alpha7-nACh nicotinic receptor and its role in memory and selected diseases of the central nervous system. Postepy Hig Med Dosw (Online). 2017 Jul;71(0):633-48. https://doi.org/10.5604/01.3001.0010.3844

23. Duris K, Lipkova J, Jurajda M. Cholinergic anti-inflammatory pathway and stroke. Curr Drug Deliv. 2017;14(4):449-57. https://doi.org/10.2174/1567201814666170201150015

24. Han Z, Li L, Wang L, Degos V, Maze M, Su H. Alpha-7 nicotinic acetylcholine receptor agonist treatment reduces neuroinflammation, oxidative stress, and brain injury in mice with ischemic stroke and bone fracture. J Neurochem. 2014 Nov;131(4):498-508. https://doi.org/10.1111/jnc.12817

25. Micheau J, Marighetto A. Acetylcholine and memory: a long, complex and chaotic but still living relationship. Behav Brain Res. 2011 Aug;221(2):424-9. https://doi.org/10.1016/j.bbr.2010.11.052

26. Prado MA, Reis RA, Prado VF, de Mello MC, Gomez MV, de Mello FG. Regulation of acetylcholine synthesis and storage. Neurochem Int. 2002 Nov;41(5):291-9. https://doi.org/10.1016/s0197-0186(02)00044-x

27. Moreira EL, de Oliveira J, Nunes JC, Santos DB, Nunes FC, Vieira DS, et al. Age-related cognitive decline in hypercholesterolemic LDL receptor knockout mice (LDLr-/-): evidence of antioxidant imbalance and increased acetylcholinesterase activity in the prefrontal cortex. J Alzheimers Dis. 2012;32(2):495-511. https://doi.org/10.3233/JAD-2012-120541

28. Nawaz A, Batool Z, Shazad S, Rafiq S, Afzal A, Haider S. Physical enrichment enhances memory function by regulating stress hormone and brain acetylcholinesterase activity in rats exposed to restraint stress. Life Sci. 2018 Aug;207:42-9. https://doi.org/10.1016/j.lfs.2018.05.049

29. Grasselli G, Hansel C. Cerebellar long-term potentiation: cellular mechanisms and role in learning. Int Rev Neurobiol. 2014;117:39-51. https://doi.org/10.1016/B978-0-12-420247-4.00003-8

30. Bayat M, Sharifi MD, Haghani M, Shabani M. Enriched environment improves synaptic plasticity and cognitive deficiency in chronic cerebral hypoperfused rats. Brain Res Bull. 2015 Oct;119(Pt A):34-40. https://doi.org/10.1016/j.brainresbull.2015.10.001

31. Leal G, Bramham CR, Duarte CB. BDNF and hippocampal synaptic plasticity. Vitam Horm. 2017;104:153-95. https://doi.org/10.1016/bs.vh.2016.10.004

32. Johansson J, Formaggio E, Fumagalli G, Chiamulera C. Choline up-regulates BDNF and down-regulates TrkB neurotrophin receptor in rat cortical cell culture. Neuroreport. 2009 Jun;20(9):828-32. https://doi.org/10.1097/WNR.0b013e32832b7324

33. Massey KA, Zago WM, Berg DK. BDNF up-regulates alpha7 nicotinic acetylcholine receptor levels on subpopulations of hippocampal interneurons. Mol Cell Neurosci. 2006 Dec;33(4):381-8. https://doi.org/10.1016/j.mcn.2006.08.011

34. Lau PY, Katona L, Saghy P, Newton K, Somogyi P, Lamsa KP. Long-term plasticity in identified hippocampal GABAergic interneurons in the CA1 area in vivo. Brain Struct Funct. 2017;222(4):1809-27. https://doi.org/10.1007/s00429-016-1309-7