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Research report

Enriched environment improves post-stroke cognitive impairment in mice by potential regulation of acetylation homeostasis in cholinergic circuits

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ABSTRACT

Post-stroke cognitive impairment (PSCI), commonly seen in the clinical practice, is a major factor impeding patient rehabilitation. Enriched environment (EE) intervention is a simple and effective way to improve cognitive impairment, partially due to the rebalancing of the basal forebrain-hippocampus cholinergic signaling pathway. Epigenetic changes have been identified in many cognitive disorders. However, studies on the effects of EE on epigenetic regulation of cholinergic circuits in PSCI animal models have not yet been reported. In this study, we established a photothrombotic mouse PSCI model and showed that after EE intervention, mice with PSCI had significantly improved water maze performance, better induction of hippocampal long-term potentiation (LTP), enhanced function of the basal forebrain-hippocampus cholinergic circuits of contralateral side of stroke and relatively balanced acetylation homeostasis compared to those of PSCI mice in standard environments (SE). In addition, PSCI mice in EE expressed much higher levels of p-CREB and CBP than in SE, and the chromatins bound to M-type promoter of ChAT gene were more acetylated. These results demonstrate that EE plays an important role in the improvement of PSCI and the underlying mechanism may involve in the acetylation of histones bound to the ChAT gene promoter in cholinergic circuits.

1. Introduction

Post-stroke cognitive impairment (PSCI), which is commonly seen in clinical practice, is an important factor impeding patient rehabilitation. As reported in the 2014 Chinese Stroke Conference, there are 2.5 million new cases of stroke in China every year and one of the most common manifestations of stroke is cognitive impairment. The incidence rate of cognitive impairment within one week after acute ischemic stroke is 61% while 37% of the patients still suffer from cognitive impairment after 6 months (Dong et al., 2012). In addition to physical impairment, the long-term impact of PSCI can lead to difficulties in patients' perception and ability to adapt to the external environment (Pustokhanova and

Abbreviations: Ach, , acetylcholine; CBP, , CREB binding protein; ChAT, , choline acetyltransferase; EE, , enriched environment; HDACs, , histone deacetylases; LTP, , long-term potentiation; p-CREB, , phosphorylated cAMP response element binding protein; PSCI, , post-stroke cognitive impairment; TSA, , Trichostatin A; HFS, , High-frequency stimulation; fEPSP, , field excitatory postsynaptic potential; mACSF, , modified artificial cerebrospinal fluid

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Morozova, 2013). As a result, patients encounter obstacles in accommodating themselves to social life and lack the abilities to live and work independently. This significantly impedes the overall rehabilitation of patients (Brainin et al., 2015; Pasi et al., 2012). Therefore, effective treatment is urgently needed to improve not only the cognitive function but also the life quality of stroke patients.

The neurotransmitter acetylcholine (ACh) and all the cholinergic circuits in the brain, especially in the basal forebrain-hippocampus (BF-HIP), are greatly involved in cognitive functions (Mitsushima et al., 2013; Lim et al., 2012; Robinson et al., 2011). In both animal models and patients with Alzheimer's disease (AD) or other cognitive disorders, ACh levels within the cholinergic circuits in certain brain regions such as the hippocampus are dramatically reduced and the function of cholinergic circuits is impaired (Lombardo and Maskos, 2015; Haider et al., 2014). In recent years, increasing attention has been paid to the relationship between epigenetic modifications and cognitive functions. It has been shown that in AD patients, altering the DNA methylation of mononuclear cells in peripheral blood is negatively correlated with cognitive function (Di Francesco et al., 2015). Age-related cognitive impairment is associated with the disordered chromosome modification in related brain regions including the hippocampus (Spiegel et al., 2014). Our previous study also revealed chromatin modification in the hippocampus and frontal lobes of PSCI mice (Wang et al., 2013). However, there are very few studies that have been reported to examine the relationship between the effects of cholinergic circuits and epigenetic modifications in cognitive functions.

Enriched environment (EE) intervention is a new, simple and effective treatment widely used in the medical practice, including in cognitive impairment rehabilitation (Young et al., 2015; Yu et al., 2014). Compared to a standard environment (SE), EE maintains much more novelty due to the larger activity space, more participating members, greater numbers of items provided to meet the activity requirements, as well as periodic changes of the combination of multiple stimulants (e.g. sound, light and color) (Yu et al., 2014). EE provides not only sufficient multi-sensory and motor stimuli, but also opportunities for training and learning including social interaction, space exploration and spontaneous physical activities (Fan et al., 2016; Yu et al., 2014). Through EE, the chances for humans and animals to receive physical and social stimuli from the environment are greatly increased. Thus, EE may play an important role in brain plasticity and behavioral improvements. Previous studies have confirmed that EE could help to improve the cognitive function in aged mice and AD mice (Rodriguez et al., 2013; Lores-Arnaiz et al., 2006). However, the underlying mechanism has not been fully elucidated. Studies have suggested that the improvement of cognitive impairment in EE is related to the restoration of hippocampal neurogenesis or increased length of hippocampal myelinated nerve fibers (Speisman et al., 2013; Qiu et al., 2012). It is also possible that EE induces change in mouse hippocampal histone modifications (Lopez-Atalaya et al., 2011; Fischer et al., 2007).

Nevertheless, EE still fails to improve certain types of cognitive impairments. For example, EE cannot improve the cognitive impairment in mice with CaMKII autophosphorylation site mutations (Need and Giese, 2003). This suggests that the mechanism of EE intervention is not fully understood.

In this study, we evaluated the effects of EE in a mouse PSCI model, and examined whether the effects of EE were correlated with the epigenetic regulation of cholinergic circuits in the brain.

2. Results

2.1. Photothrombotic stroke induces infarcts in mouse brains

As shown from magnetic resonance image (MRI) scanning (Fig. 1A), a fairly large lesion was clearly seen in the right cortex and subcortex (including hippocampus) 24 h after surgery, indicating the successful establishment of the focal thrombotic ischemic stroke model using the Rose Bengal photothrombotic method. The average size of the largest slices of the lesions in all mice was $(4.99 \pm 0.23) \times (2.35 \pm 0.11)\ mm^2$ (Fig. 1A, PSCI 4, light arrow).

To examine the location and degree of damages in PSCI brains, five randomly selected PSCI mice were sacrificed. Their location and degree of damages were similar. Large area of damages and scar tissues were found in the right cortex and subcortex of the PSCI mice brains. The average size of the scar tissues was approximately $(5.50 \pm 0.18) \times (2.48 \pm 0.09)\ mm^2$ large (Fig. 1B).

2.2. EE improves spatial learning ability and memory in mice with PSCI

The Morris water maze test is widely used in spatial learning ability and memory research. After three days' training (Fig. 2A), the escape latency (Fig. 2B) and searching distance (Fig. 2C) of the mice in the PSCI+SE (PSCI mice in Standard Environment) group remarkably increased compared with those of the mice in the SHAM+SE (sham-operated mice in Standard Environment) group ($p < 0.01$) on the fourth day, and the percentage of distance in the target quadrant (Fig. 2D) and times of pass over platform (Fig. 2E) of the mice in the PSCI+SE group remarkably decreased compared with those of the mice in the SHAM+SE group ($p < 0.01$) on the fifth day. After being housed in EE, mice in the PSCI+EE (PSCI mice in Enriched Environment) group exhibited significantly reduced escape latency (Fig. 2B) and searching distance (Fig. 2C) compared with those of the mice in the PSCI+SE group ($p < 0.05$), whereas the percentage of distance in the target quadrant and times of pass over platform (Fig. 2D, E) of the mice in the PSCI+EE groups were significantly increased compared with those of the mice in the PSCI+SE group ($p < 0.05$). However, these indexes of Morris water maze test of the mice in the PSCI+EE groups were still significantly different from those of the mice in the SHAM+SE group ($p < 0.05$). The indexes of Morris water maze test of the mice in the SHAM+EE (sham-operated mice in Enriched Environment) group (Fig. 2B, C, D, E) showed a slight but not significant change compared with those of the mice in the SHAM+SE group ($p > 0.05$).

2.3. EE induces long-term potentiation (LTP) in mice

The effect of EE on the basal transmission of CA1 synapse of contralateral side of stroke was examined. Slopes of field excitatory postsynaptic potential (fEPSP) were plotted against stimulus intensity ranging from 0.1 to 0.9 mA. Input/output (I/O) curve (Fig. 3A) showed that the slope of fEPSP in slices obtained from PSCI+SE mice was significantly lower compared to that from SHAM+SE mice ($p < 0.01$), indicating the impaired synaptic transmission. After being housed in EE, PSCI mice exhibited markedly rescued synaptic dysfunction, and the slope of fEPSP in slices obtained from PSCI+EE mice significantly increased compared to that from PSCI+SE mice ($p < 0.05$, Fig. 3A). The slope of fEPSP in slices obtained from SHAM+EE mice also significantly increased compared to that from SHAM+SE mice ($p < 0.05$), indicating increased synaptic transmission.

High-frequency stimulation (HFS) was found to evoke a stable potentiation of fEPSP slope in SHAM+SE mouse slices ($150.29 \pm 8.81\%$ at 60 min post-HFS, n =15 slices/five mice), but