

# Electroacupuncture Enhances Cognition by Promoting Brain Glucose Metabolism and Inhibiting Inflammation in the APP/PS1 Mouse Model of Alzheimer's Disease: A Pilot Study

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## Abstract.

**Background:** Alzheimer's disease (AD) is a neurodegenerative disease, yet there is no effective treatment. Electroacupuncture (EA) is a complementary alternative medicine approach. In clinical and animal studies, EA promotes cognition in AD and vascular dementia. It has been previously reported that cognitive decline in AD might be closely related to reduced glucose intake in the brain. It is worth mentioning that the regions of glucose hypometabolism are usually found to be associated with neuroinflammation.

**Objective:** This study is to explore whether the protective mechanism of EA on cognition is related to the regulation of glucose metabolism and neuroinflammation.

**Methods:** APP/PS1 mice were randomly divided into AD group and the treatment (AD + EA) group. In the AD + EA group, EA was applied on Baihui (GV20) and Yintang (GV29) for 20 min and then pricked at Shuigou (GV26), once every alternate day for 4 weeks. Morris water maze (MWM) tests were performed to evaluate the effects of EA treatment on cognitive functions. <sup>18</sup>F-FDG PET, immunofluorescence, and western blot were used to examine the mechanisms underlying EA effects.

**Results:** From MWM tests, EA treatment significantly improved cognition of APP/PS1 mice. From the <sup>18</sup>F-FDG PET, the levels of uptake rate of glucose in frontal lobe were higher than the AD group after EA. From immunofluorescence and western blot, amyloid-β (Aβ) and neuroinflammation were reduced after EA.

**Conclusion:** These results suggest that EA may prevent cognitive decline in AD mouse models by enhancing glucose metabolism and inhibiting inflammation-mediated Aβ deposition in the frontal lobe.

Keywords: Alzheimer's disease, cognition, electroacupuncture, <sup>18</sup>F-FDG PET, frontal lobe, glucose metabolism, neuroinflammation

## INTRODUCTION

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Alzheimer's disease (AD) is a neurodegenerative disease that is associated with old age and has become

a major burden on health care systems worldwide [1, 2]. It is still one of the urgent problems in the medical field to explore the pathogenesis of AD and find effective ways to prevent it. Neuroimaging has significant advantages in the diagnostic accuracy of AD, as well as the evaluation of therapeutic effects [3]. <sup>18</sup>F-FDG PET is a well-established imaging technique for assessing cognitive decline in AD that can quantify neuropathology in the brain by using glucose analogues. Studies have suggested that cognitive impairment to a certain extent results from decreased cerebral glucose metabolism in AD patients [4]. It is especially interesting that the regions of glucose hypometabolism are usually found to be associated with neuroinflammation [5], and that is a key part of the etiopathogenesis of AD [6]. Microglia, the resident phagocytes of the innate immune system in the central nervous system, can be activated by the pathological process of AD, such as A $\beta$ . Activated microglial cells play a role in the recognition and phagocytosis of A $\beta$  [7]. While the initial inflammatory response is conducive to ameliorating neuronal injury, prolonged abnormal microglial activation potentially exacerbates neurodegeneration and has a series of harmful effects.

Despite new advances in understanding the neurobiology and pathophysiology of AD, only a handful of drugs are currently available for patients. Based on this consideration, many non-drug therapies have been proposed for prevention. Acupuncture is a therapeutic approach that has curative effects and few side effects. EA is widely used due to its adjustable strength and easy quantification in China compared to manual acupuncture [8]. In recent years, a functional MRI study confirmed that acupuncture can activate certain cognitive-related regions in AD patients, and acupuncture improved learning-memory ability in AD mice [9, 10]. In addition, we have previously found that the expressions of A $\beta$  in the hippocampus of APP/PS1 mice were reduced after EA therapy, as well as cognitive impairment was improved,

suggesting that EA intervention is helpful to the cognitive decline of AD [11, 12].

The aim of the current study was to investigate the mechanism of EA in enhancing cognition. We hypothesized that EA at Governor Vessel (GV) acupoints would alter cerebral glucose metabolism in a manner associated with neuroinflammation to enhance cognition. In this study, 6-month-old APP/PS1 mice were used, which was a well-established transgenic AD mouse model. First, we assessed the learning and memory abilities of the AD animals after EA treatment using the MWM test. Next, we evaluated changes in glucose metabolism in the frontal lobe using <sup>18</sup>F-FDG PET. Finally, the changes of A $\beta$  and inflammatory response in the frontal lobe were tested using immunofluorescence and western blot. The schematic diagram of this study is detailed in Fig. 1.

## MATERIALS AND METHODS

### Experimental animals

6-month-old male APP/PS1 mice and age- and gender-matched C57BL/6N mice were purchased from Beijing HFK Bioscience Company, Ltd. (experimental animal license number: SCXK (Jing) 2014-0004). The mice weighed  $28.0 \pm 2.0$  g and were housed separately in standard mouse cages at the Experimental Animal Center of Beijing University of Chinese Medicine. The room temperature was maintained at  $24 \pm 2^\circ\text{C}$ , and the humidity was 40–60%. The mice were maintained on a standard 12 h light-dark cycle (dark cycle 8 : 00 PM–8 : 00 AM). All mice had *ad libitum* access to food and water. The bedding material was replaced daily to keep the cages clean and dry. The experiment began 8 days after the animals entered the room so that the mice could acclimate to the housing conditions. Every effort was made to minimize the suffering of the mice during the experimental procedure.

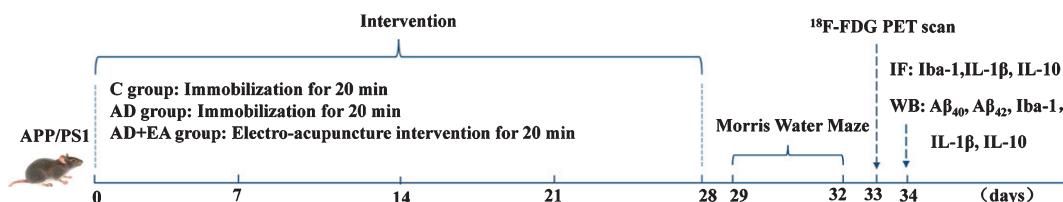


Fig. 1. Schematic diagram of this study to detect the effect of EA on AD.

Table 1  
The primary apparatus used in this study

Apparatus	Source	Details
Disposable sterile acupuncture needle	Beijing Zhongyan Taihe Medical Instrument, Co., Ltd	Model: ZYTH2013030504; specification: 0.25 × 13 mm
Electroacupuncture (EA) apparatus	Peking University Institute of Science Nerve and Beijing Hua Wei Industrial Development Company	Model: HANS-LH202
Morris water maze	Shanghai Xinruan Information Technology, Co., Ltd	Model: XR-XM101
Software of image acquisition and analyzing system	China Daheng Group, Inc.	China Daheng Group, Inc., Beijing Image Vision Technology Branch
Micro-PET scanner	the Chinese Medicine Research Institute PET Room	–
PET imaging system	Siemens INVEON PET/CT imaging system	–

### Apparatuses

The primary apparatuses are listed in Table 1.

### Animal grouping and intervention

A total of twenty APP/PS1 mice were randomly and equally divided into AD model (AD) and EA treatment (AD + EA) groups ( $n=10$  per group). Ten C57BL/6N mice composed the control group (C).

In this experiment, GV20, GV29, and GV26 were selected based on traditional Chinese medicine (TCM) theory and our previous studies. First of all, acupoints have a near-therapeutic effect. The three acupoints are all located at the head, so they have the effect of regulating mental activities. In addition, according to the theory of TCM, GV and brain are closely related. GV20, GV29, and GV26 are the important points in the GV, so stimulation at the three acupoints can improve brain function. The acupuncture points GV20, GV29, and GV26 on the mice were located based on the Acupoint Standard for Experimental Animals.

In the AD + EA group, EA was applied once every alternate day for 4 weeks. Mice in the C and AD groups were immobilized for 20 min when EA was performed. To immobilize the mice, we made special bags based on the size of the mice. After the mice crawled inside the bags, two clips were put in place to close the open backs of the bags, which absolutely controlled and immobilized the mice well. As the front third of each bag was made of mesh, the experimental mice could breathe unimpeded. The acupoint locations and operations of EA in this study are shown in Table 2 and Fig. 2 [12].

Table 2  
Acupoints location and operation of EA in this study

Acupoint	Location	Operation of EA
GV20	On the top of the head, at the midpoint of the line connecting the ears.	First, insert needle obliquely in an upward direction to a depth of 5 mm. Then, connect the needle to the EA apparatus set to a frequency of 2 Hz and a current of 1 mA. Continue the intervention for 20 min.
GV26	On the face, in the upper one-third of the philtrum.	Deliver a fast prick after the end of EA treatment.
GV29	On the forehead, in the depression between the two eyebrows medial end.	Insert needle obliquely in a downward direction to a depth of 5 mm. The other operations are the same as for GV20.

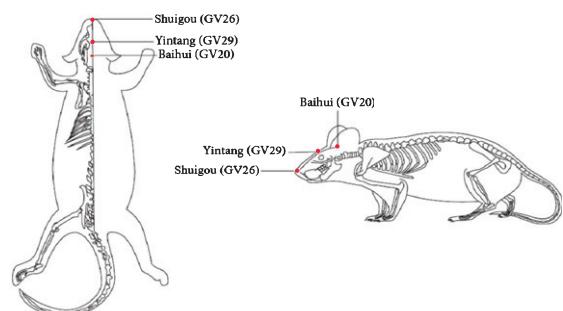


Fig. 2. The location of GV acupoints applied in this study. Red points indicate the locations of GV20, GV26, and GV29 on the head of mice (left: top view; right: side view).

### MWM test

The MWM test system consists of a water maze device, water maze image automatic acquisition, and software analysis system. The water maze device is

the main site of the experiment, which consists of a circular pool (diameter: 90 cm; height: 50 cm; divided into four quadrants: I, II, III, and IV) and a removable circular platform. The camera is placed 2 m above the center of the pool to automatically collect the swimming images of the animal. These signals are directly input into the computer for automatic analysis and processing by the automatic image acquisition and analysis system (Daheng Group, China). The experimental surroundings are required to maintain sound insulation, and the objects in the room were unchanged for the duration of the test. During the formal experiment, the pool was filled with water up to 30 cm depth, and the addition of milk powder rendered the water opaque. A thermometer was used to monitor the water temperature that was maintained at  $20 \pm 1^{\circ}\text{C}$ , keeping the indoor light intensity consistent during the experiment.

In training trials, the platform was fixed in the center of quadrant III (the target quadrant), located 2 cm below the water surface. The animals were first placed on the platform for 10 s in each training trial. Then, the mice were gently placed into the water from quadrants I, II, and IV. The entry point is the equal distance to the center of the pool in quadrants I, II, and IV. The time of finding the platform was recorded. When the mouse stayed on the platform for 5 s, the collection stopped automatically, which was deemed successful, and time was recorded. If the animal failed to climb onto the platform within 60 s, the escape latency was recorded as 60 s. The training trial lasted 4 days.

A probe trials were performed 1 day after the last training trial. In the probe trials, the platform was removed, and the animals were gently placed into the water from the entry points of quadrants I, II, and IV. The swimming trajectory and the duration spent in quadrant III were recorded. This spatial probe trial was used to evaluate the spatial memory ability of the mice.

#### *Micro-PET imaging*

Four mice from each group were randomly selected for micro-PET imaging. Before the micro-PET scan, we first monitored the blood glucose of the mice. When the levels of blood glucose were in the normal range (7.0–10.0 mmol/L), we could start experiments. Before the micro-PET scan, the mice were deprived of water for 6 h. After the mice were completely anesthetized with isoflurane inhalation (2% in 100% oxygen, 1 L/min).  $^{18}\text{F}$ -FDG tracer at a dose of 14.8–16.5 MBq was injected via the tail vein.

After the injection for 60 min, the mouse was placed on the scan bed in the prone position, the mouse and scanner long axis were parallel, and the head of the mouse was located within the scanner field of view. Then, the micro-PET began to collect the image. The scan lasted 10 min, and mice wore breath mask with isoflurane continuously carried to ensure entirely unconscious during this progress.

Micro-PET captured a single frame image, and then filtered back projection and CT photon attenuation correction were used for image reconstruction (pixel size  $0.2 \times 0.2 \times 0.8$  mm, 30 s/frame). Next, the three-dimensional region of interest selection (ROIs) of PET/CT images in the frontal lobe were selected manually by the experimenter, which were in transverse, coronal, and sagittal planes. Finally, we calculated the uptake rate per gram with ROIs.

#### *Immunofluorescence*

Twenty-four hours after micro-PET scanning, four mice in each group were randomly anesthetized. After heart perfusion, brains were fixed in 4% phosphate-buffered paraformaldehyde for 24 h, followed by ethanol dehydration, xylene treatment for transparency, paraffin embedding, and coronal sectioning. Slices were incubated at  $4^{\circ}\text{C}$  with anti-Iba-1 (1 : 2000, ab178847, Abcam, England), anti-IL-1 $\beta$  (1 : 100, ab9722, Abcam, England), and anti-IL-10 (1 : 100, ab9969, Abcam, England) antibodies. After permeabilization and blocking overnight, appropriate secondary antibodies (GB21303, Servicebio, China) were used at a dilution of 1 : 300. After the sections were washed 3 times with PBS, they were incubated with DAPI (G1012, Servicebio, China) for 10 min, followed by live imaging. We captured and scanned the frontal lobe images with a confocal laser microscope (FV1000, Olympus, Japan) at  $200\times$  and  $400\times$  magnification.

#### *Western blot*

Twenty-four hours after micro-PET scanning, six mice in each group were randomly anesthetized, and the frontal lobe was collected. Tissues were lysed with RIPA lysis buffer (Sainobo, China). The protein concentration was determined using a bicinchoninic acid assay kit (Sainobo, China) according to the manufacturer's instructions. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto PVDF membranes at  $4^{\circ}\text{C}$  at 300 mA for 2 h.

The levels of A $\beta$ <sub>40</sub>, A $\beta$ <sub>42</sub>, Iba-1, IL-1 $\beta$ , and IL-10 were measured by incubating with the primary antibodies against A $\beta$ <sub>40</sub> (1 : 1000, ab17295, Abcam, England), A $\beta$ <sub>42</sub> (1 : 2000, ab201060, Abcam, England), Iba-1 (1 : 2000, ab178847, Abcam, England), IL-1 $\beta$  (1 : 2000, ab9722, Abcam, England), and IL-10 (1 : 2000, ab9969, Abcam, England) at 4°C overnight. GAPDH (1 : 20000, YM3029, Immunoway, China) was used as an internal control. Subsequently, the membranes were washed with TBST and incubated with the corresponding secondary antibody for 1 h at room temperature. The immunoreactive bands were detected using a chemiluminescence gel imaging system (C600, Azure Biosystems, USA). Quantitative results are expressed as a ratio of target proteins to GAPDH and then compared to all groups to measure relative changes.

#### Statistical analysis

All data are expressed as the means  $\pm$  SD. Statistical analysis was performed using IBM SPSS Statistics 20 software. Comparisons between groups were analyzed using repeated measures two-way ANOVA in the training trial. One-way ANOVA followed by the least significant difference (LSD) multiple-range test was used to analyze group differences in the space probe trial and the uptake rate of <sup>18</sup>F-FDG and WB. Statistical significance was set at  $p < 0.05$ , and high statistical significance was set to  $p < 0.01$ .

## RESULTS

#### *EA treatment alleviated cognitive impairments in APP/PS1 mice*

To assess the spatial learning ability of the mice, a training trial was conducted from day 1 to day 4 (Fig. 3A, B). We recorded the time spent by the mice in searching to climb onto the platform to escape the water (escape latency). The mice in the AD group showed a longer escape latency than the C group. The results suggested that the ability of spatial learning in the AD group had obvious deficits. However, the escape latency of the AD + EA group gradually shortened with the training time. Compared to day 1, the mice from the AD + EA group showed significantly lower escape latency on days 3 and 4 (Fig. 3C).

After assessing the spatial learning ability, the maintenance of memory was tested by a probe trial on day 5. The longer duration that the mice stayed

in quadrant III (platform quadrant), the better their memory retention [13]. Compared with the C group, the AD group showed a significantly lower platform crossing number and duration percentage in quadrant III. After EA treatment, the AD + EA group showed a significantly longer duration in quadrant III compared with the AD group (Fig. 3D). Representative probe traces of each group intuitively reflected the search strategy of mice (Fig. 3E). The above results demonstrated that 6-month-old APP/PS1 mice had significant cognitive impairment, and EA treatment ameliorated cognitive decline by enhancing spatial learning and memory.

#### *EA treatment enhanced glucose metabolic activity in the frontal lobe of APP/PS1 mice*

After observing that EA alleviated cognitive impairments, we investigated the mechanism responsible for enhancing cognition. The dysregulation of energy metabolism in the brain is a significant causative factor in the development of AD. A reduction in glucose metabolism could result in neuronal dysfunction. <sup>18</sup>F-FDG PET displays the rate of cerebral glucose metabolism, and the ROIs for PET data analysis are a well-established evaluation of the metabolic rate of glucose [14]. From the image, the same color standard and color code from top high to bottom low were used to display the metabolic rate of the glucose. ROIs of PET images in the frontal lobe of APP/PS1 mice were shown in Fig. 4A. Obviously, the metabolic rates of glucose from micro-PET scanning in the C and AD + EA groups were higher than that in the AD group (Fig. 4B). The ROI data confirmed that the uptake rates of <sup>18</sup>F-FDG in the frontal lobe of the AD group were significantly lower than that in the C group, which recovered after EA stimulation in the AD + EA group (Fig. 4C).

#### *EA treatment attenuated A $\beta$ deposition in the frontal lobe of APP/PS1 mice*

As A $\beta$  deposition contributed to AD pathogenesis, we hypothesized that the improvement of glucose metabolism could be attribute to the reduction of A $\beta$  load. To test this hypothesis, we measured expressions of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> by western blot. The results showed that the levels of both A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> in the frontal lobe of AD + EA group were significantly markedly reduced following EA treatment as compared with levels in the AD group (Fig. 5).

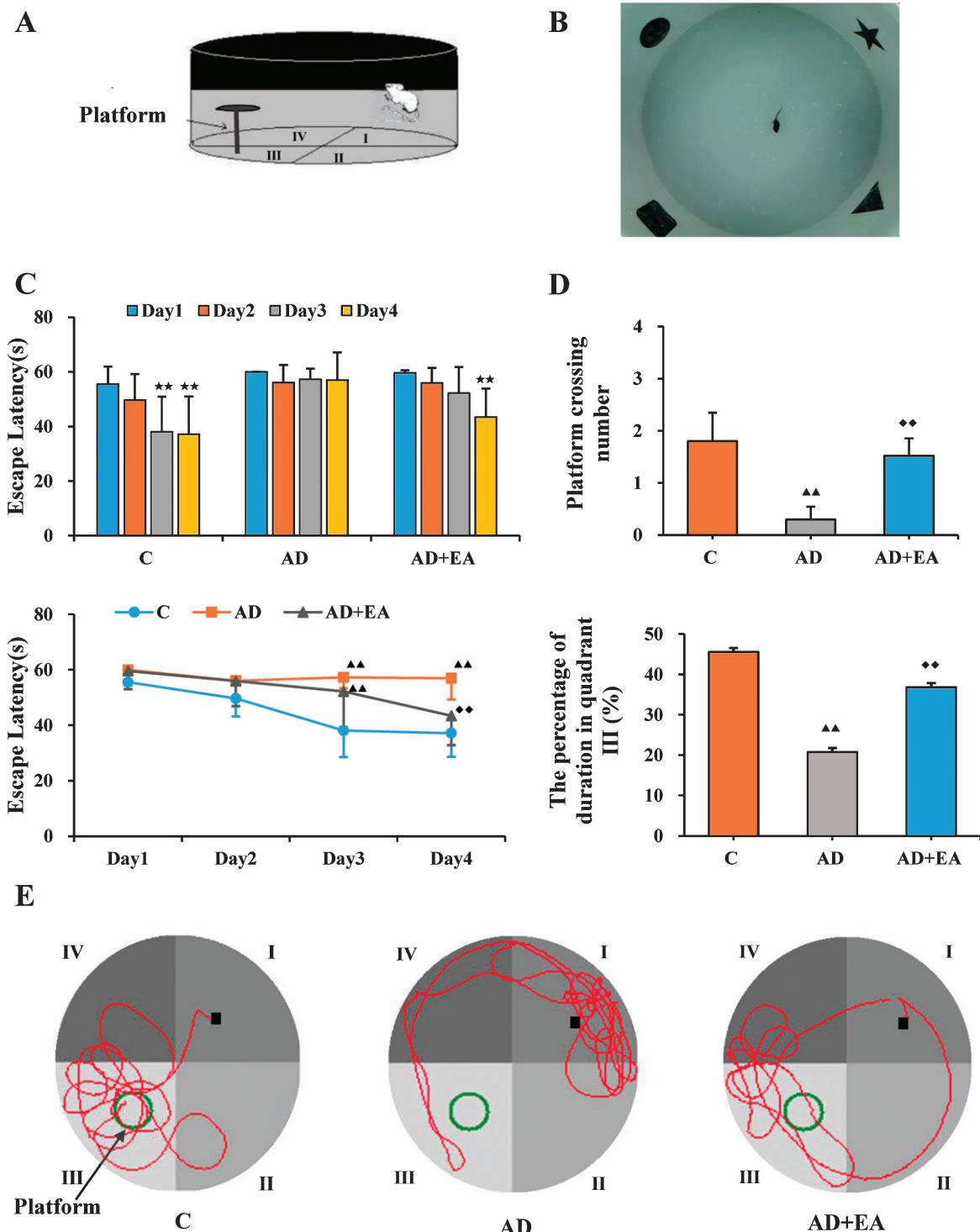


Fig. 3. The MWM device and the results of the MWM test. A) The MWM device. B) The mouse was tested with a MWM apparatus. C) Training trial results. D) Space probe trial results. E) Representative swimming trajectories of the three groups (the black squares indicate the water entry points of mice.)  $n = 10$  per group, the results are presented as the means  $\pm$  SD.  $^{**}$  Compared with Day 1,  $p < 0.01$ ;  $^{\wedge}$  Compared with C group,  $p < 0.05$ ;  $^{\ddagger}$  Compared with AD group,  $p < 0.01$ .

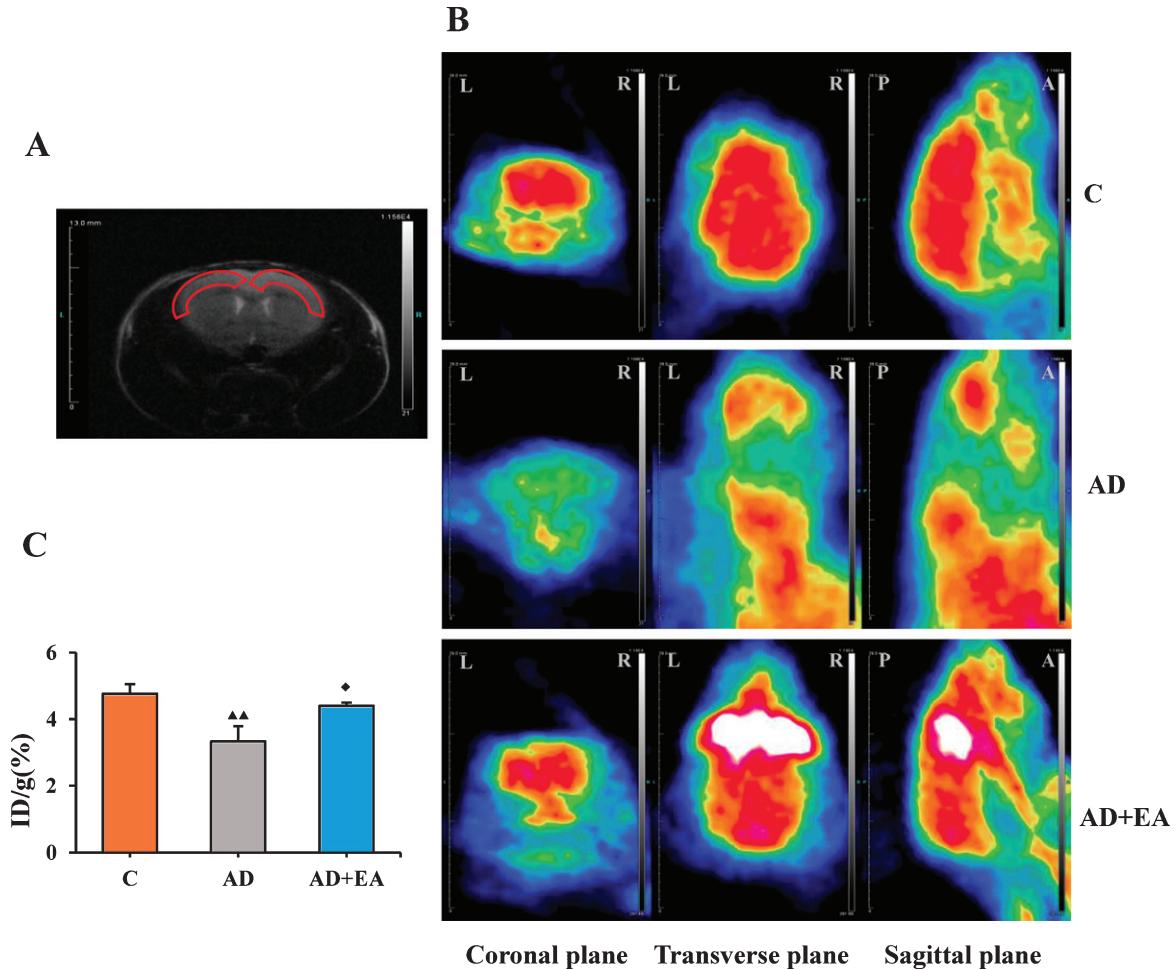


Fig. 4. The frontal lobe of APP/PS1 mice in each group using <sup>18</sup>F-FDG PET scanning images. A) ROIs of the frontal lobe of mice are shown in red. B) The frontal lobe of <sup>18</sup>F-FDG PET scanning image of each group. Color code: min = 0, max = 6.5. C) Comparison of the uptake rate of <sup>18</sup>F-FDG per gram in the frontal lobe of each group.  $n=4$  per group, the results are presented as the means  $\pm$  SD. ▲▲ Compared with C group,  $p < 0.01$ ; ♦Compared with AD group,  $p < 0.05$ .

#### *EA treatment inhibited inflammatory response in the frontal lobe of APP/PS1 mice*

We hypothesized that the accumulation of A $\beta$  could be due to an inflammatory response. The abnormal activation of microglia in the brain appeared crucial to the pathogenesis of A $\beta$  [6, 15]. As expected, we clearly observed the localization of microglia, Iba-1 immunopositive areas in the frontal lobe were detected in the mice of the AD group (Fig. 6A). Western blot results showed that Iba-1 of the frontal lobe in the AD group were obviously higher than that in the C group, which decreased after EA stimulation in the AD+EA group (Fig. 6B). These results suggested that microglial activation were present in

the brains of 6-month-old APP/PS1 mice. We further confirmed the inflammatory response-inhibiting effect of EA by measuring inflammatory cytokines. Activated microglia was determined by repertoire of pro-inflammatory cytokines in brain environment. Therefore, we next examined the effect of EA treatment on the key cytokines, such as IL-1 $\beta$ . The results showed that compared with AD group, the level of pro-inflammatory cytokine IL-1 $\beta$  in AD+EA group was decreased when treated with EA (Fig. 7), and reversely, the level of anti-inflammatory cytokine IL-10 in AD+EA group was significantly increase after EA intervention (Fig. 8). Taken together, these findings showed that EA treatment inhibited inflammation in the frontal lobe of APP/PS1 mice.

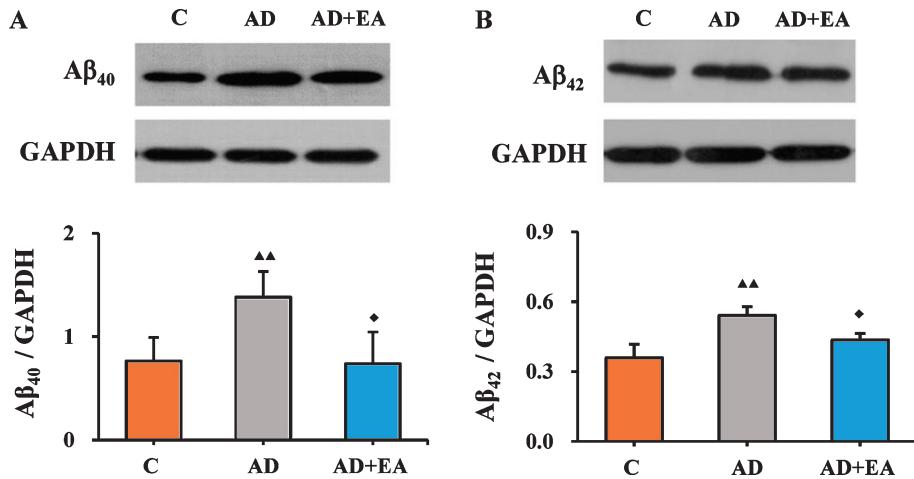


Fig. 5. Effects of EA on  $\text{A}\beta$  deposition in the frontal lobe of APP/PS1 mice in each group. A) The expression levels of  $\text{A}\beta_{40}$  were determined by western blotting analysis. B) The expression levels of  $\text{A}\beta_{42}$  were determined by western blotting analysis. GAPDH was used as a loading control.  $n=6$  per group, the results are presented as the means  $\pm$  SD. <sup>▲▲</sup>Compared with C group,  $p < 0.01$ ; <sup>♦</sup>Compared with AD group,  $p < 0.05$ .

## DISCUSSION

In the current work, our findings supported the notion that EA therapy exerted beneficial effects on cognition in AD mice by stimulating GV acupoints, at least partially through the mechanism that involved glucose metabolism elevation and neuroinflammation inhibition in the frontal lobe.

Despite growing acknowledgement of AD, effective and safe therapeutic interventions remain to be found. Many people focus now on the prevention of cognitive impairments that may lead to AD. EA is one of the complementary alternative medicine techniques, which shows clinically neuroprotective effects in patients with mild cognitive impairment [16]. According to the theory of meridians and collaterals, brain function is closely related to the GV [17]. Recent studies have shown that regulating GV can promote the compensation and recombination of brain function [18, 19]. Most studies chose GV20 as the main acupoint, which might be related to the specific neural response pattern induced by EA at acupoints [20]. Herein, we use GV20, GV26, and GV29, which are attached to the GV. The function of these acupoints are restoring consciousness, regulating mentality, and benefiting intelligence, and in clinical practice, they are often used to treat diseases of the central nervous system [21–23]. The combination of these acupoints, which we call the method of “dredging the governor vessel to wake up the mind” in accordance with the theory of TCM, is an effective therapy for cognitive decline.

Cognitive dysfunction is the core symptom of AD patients. The cognition process in the brains of animals could not be observed directly. The changes could be predicted only according to the observed stimulus response. Cognitive abilities can be analyzed by measuring the performance or reaction time of the model at a specific interval after learning or performing a certain task [24]. In this experiment, the mice were trained to learn to find the hidden platform in the fixed position underwater for stable spatial position cognition [25, 26]. AD mice performed worse than controls and showed a random search strategy for the target (platform) without the obvious inclination. This phenomenon indicated that the learning and memory abilities of APP/PS1 mice at the age of 6 months were markedly degraded, enthusiasm to avoid water was decreased, and physical fitness and activity were reduced. After EA treatment, they performed better, thereby suggesting that EA promoted the cognition of mice.

However, it should be emphasized that the cognitive function is a very complex process that is the result of whole brain activity. Therefore, the complex cognitive process should not be simply located in a specific brain region [27, 28]. Previous studies on AD have focused on the hippocampus [29]. Recent studies have found that the transcriptional levels of some factors closely related to brain aging change with age (in months) only in the frontal lobe [30]. It reported that early cognitive deficits in AD mice were related to the frontal cortex before hippocampus-dependent impairments [31]. This suggests that there may be

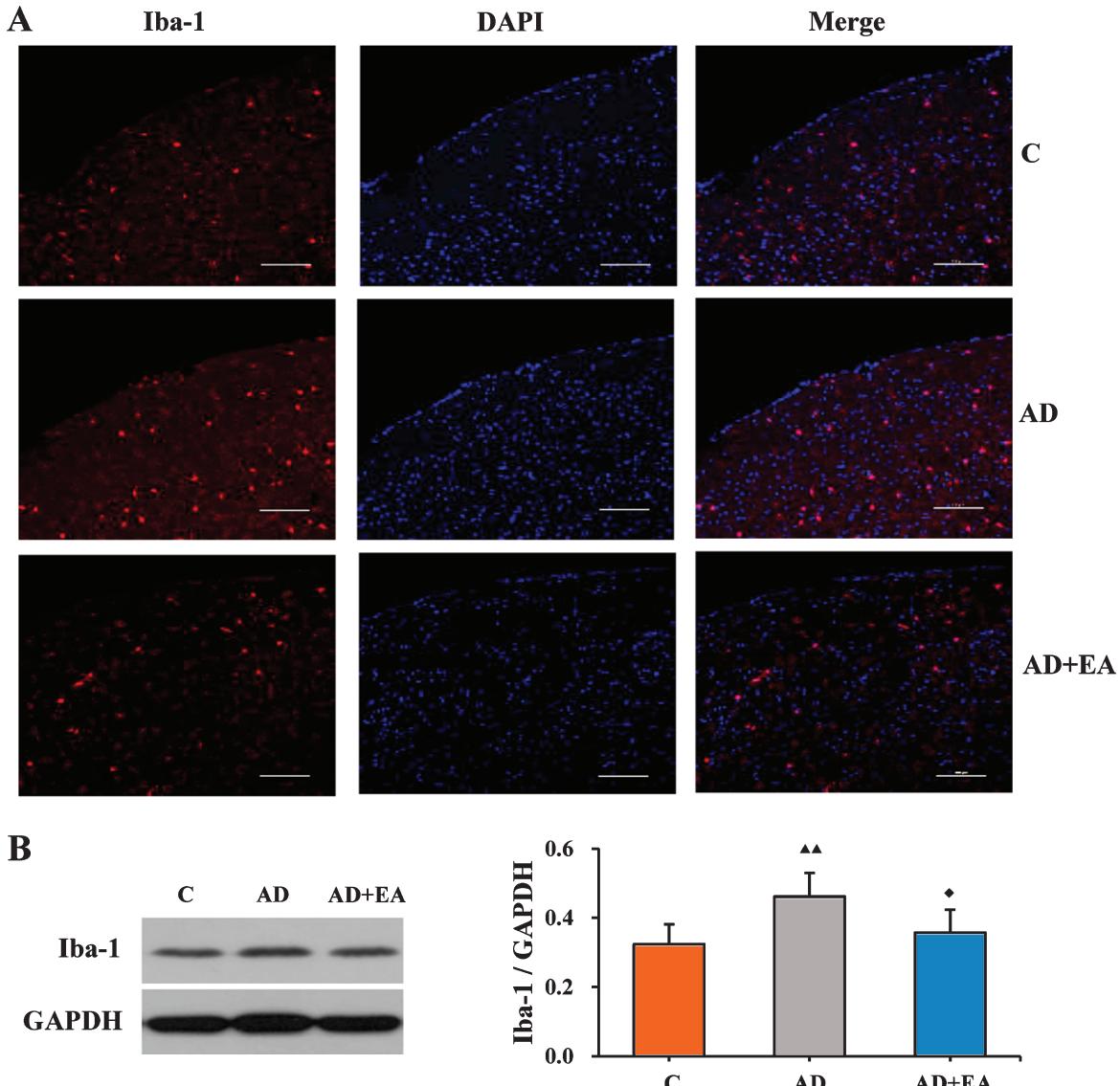


Fig. 6. Effects of EA on microglial activation in the frontal lobe of APP/PS1 mice in each group. A) Microglia (red) were detected in the frontal lobe region using immunofluorescence.  $n=4$  per group. Scale bar = 100  $\mu$ m, magnification  $\times 200$ . B) The expression levels of Iba-1 were determined by western blotting analysis. GAPDH was used as a loading control.  $n=6$  per group, the results are presented as the means  $\pm$  SD. \*\*Compared with C group,  $p < 0.01$ ; \*Compared with AD group,  $p < 0.05$ .

some important changes in the frontal lobe different from the hippocampus and basal forebrain that are not yet known. The frontal lobe, which makes up roughly one-third of the cerebral lobe, is the most recently developed and highly evolved part of the brain and is responsible for the integration of all senses and perceptions. The prefrontal lobe is closely related to higher cognitive function [32]. The frontal lobe contains many neurotransmitters and receptors, such as 5-HT [33], adenosine receptors [34]. Also, prefrontal lobe function is regulated by dopamine

neurons [35], which can improve the excitability of frontal cortex pyramidal neurons and plays an important role in synaptic plasticity [36, 37]. The receptor of dopamine in the frontal lobe is mainly D1, which can reduce the release of glutamate in L5 pyramidal cells of the prefrontal cortex when stimulated [38]. They are related to attention, prediction, short-term memory tasks, reasoning, decision-making, emotion and other high-level psychological activities, and play a very important role in the long-term memory maintenance of nontasks. In previous work, we conducted

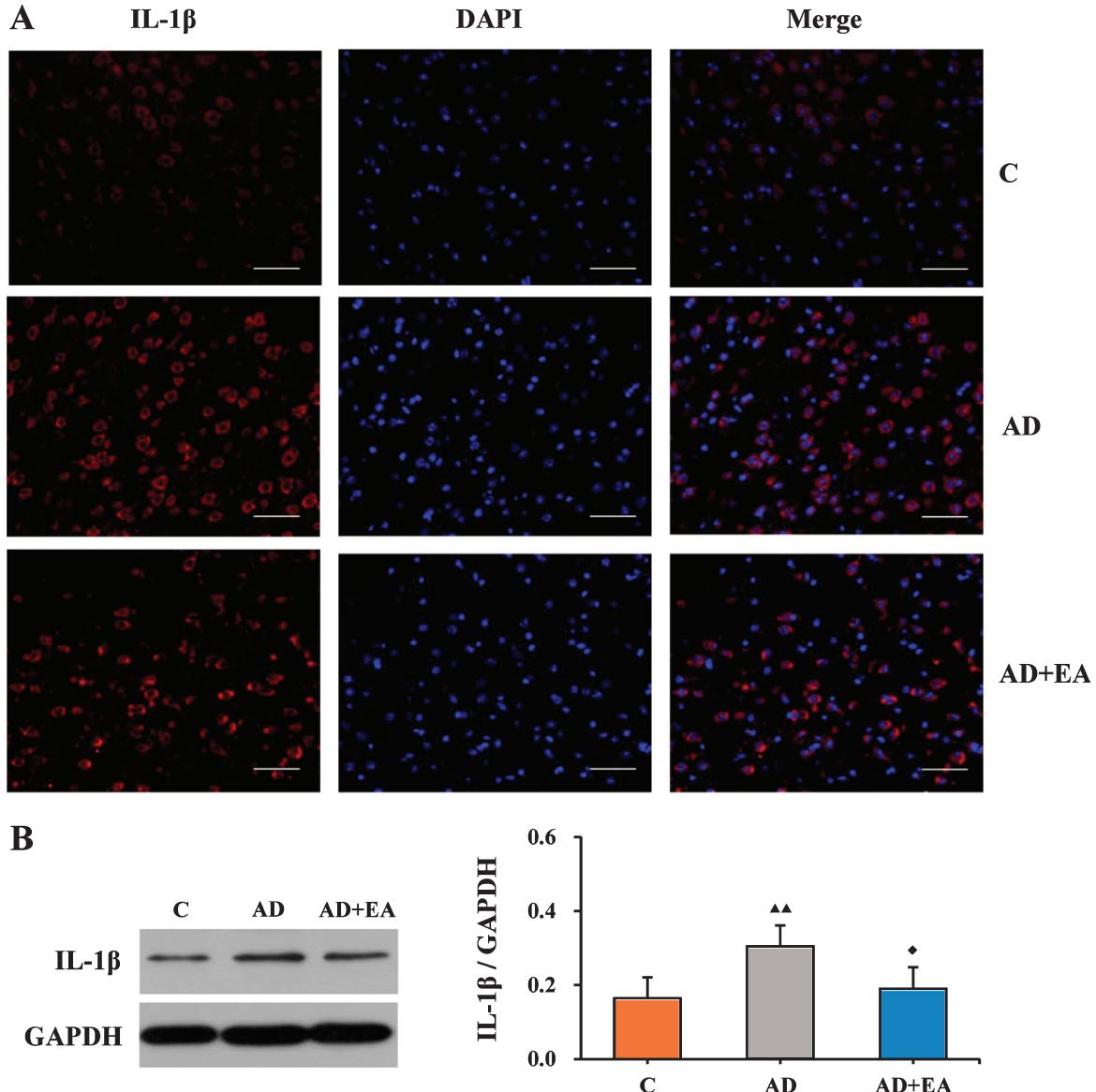


Fig. 7. Effects of EA on expression of pro-inflammatory cytokine IL-1 $\beta$  in the frontal lobe of APP/PS1 mice in each group. A) IL-1 $\beta$  (red) were detected in the frontal lobe region using immunofluorescence.  $n=4$  per group. Scale bar = 50  $\mu$ m, magnification  $\times 400$ . B) The expression levels of IL-1 $\beta$  were determined by western blotting analysis. GAPDH was used as a loading control.  $n=6$  per group, the results are presented as the means  $\pm$  SD. \*\*Compared with C group,  $p < 0.01$ ; \*Compared with AD group,  $p < 0.05$ .

extensive studies of the hippocampus. Therefore, our focus in this study is on the frontal lobe.

Glucose is a key source of energy for brain tissue in mammals and maintains the normal function of neurons, including cognition. However, neurons cannot synthesize or store glucose, so they depend only on glucose input. Cognitive deficits in AD are usually associated with changes in glucose metabolism in the brain [14]. PET provides a noninvasive way to quantify brain glucose uptake, and  $^{18}\text{F}$ -FDG is the

most commonly used radioactive tracer of brain glucose [39]. In this study, we used  $^{18}\text{F}$ -FDG PET as an approach to assess the effects of EA on the frontal lobe. In the AD group,  $^{18}\text{F}$ -FDG metabolism in the frontal lobe was lower than that in the C group but increased after EA treatment. Reduced glucose utilization in specific brain regions in AD mice may be due to A $\beta$  deposition and excessive neuronal loss; the former is not conducive to the use of glucose, and the latter leads to a direct decrease in glucose metabolism

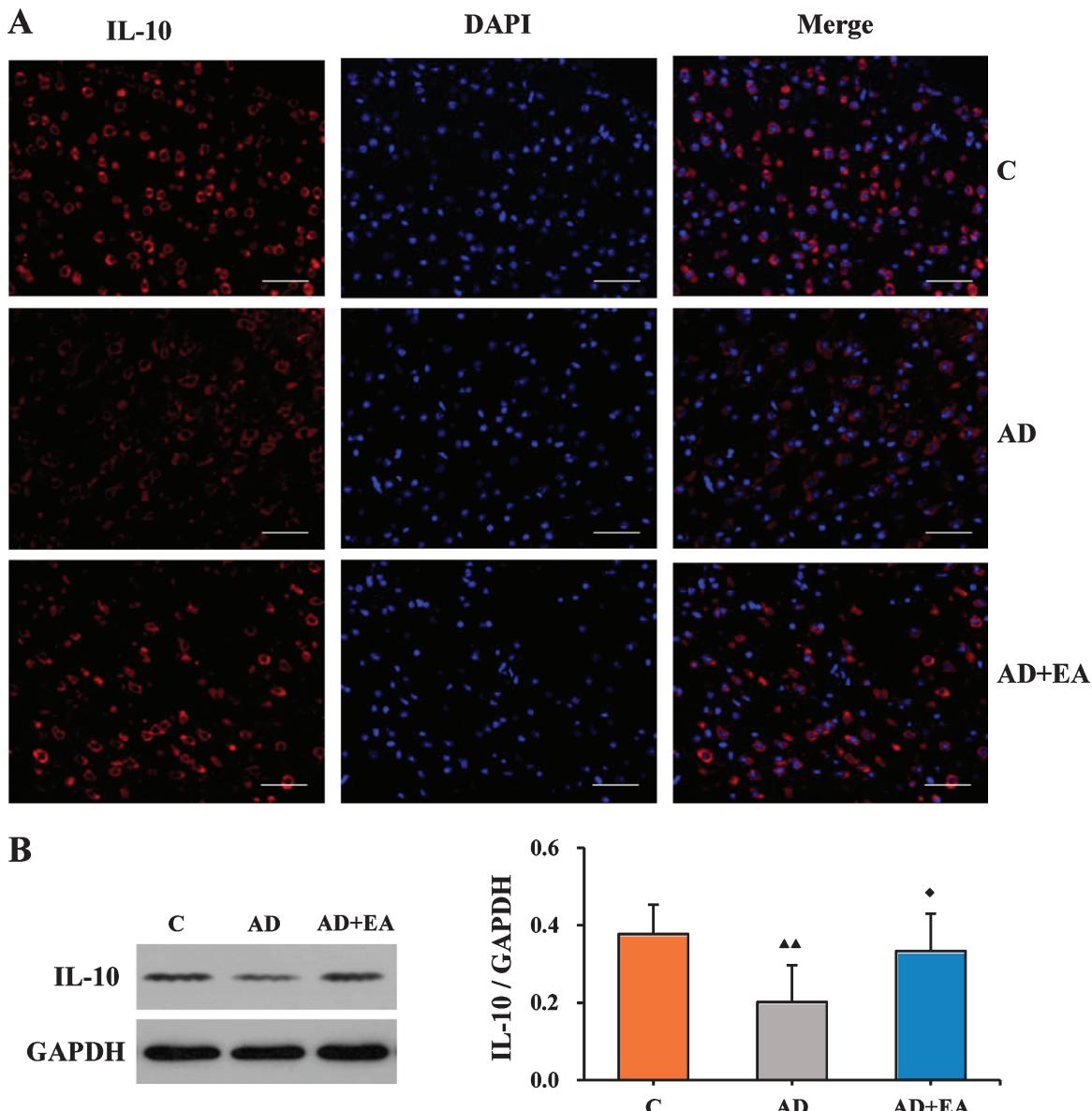


Fig. 8. Effects of EA on expression of anti-inflammatory cytokine IL-10 in the frontal lobe of APP/PS1 mice in each group. A) IL-10 (red) were detected in the frontal lobe region using immunofluorescence.  $n=4$  per group. Scale bar = 50  $\mu$ m, magnification  $\times 400$ . B) The expression levels of IL-10 were determined by western blotting analysis. GAPDH was used as a loading control.  $n=6$  per group, the results are presented as the means  $\pm$  SD. \*\*Compared with C group,  $p < 0.01$ ; ♦Compared with AD group,  $p < 0.05$ .

[40]. In turn, abnormal glucose metabolism can lead to an increase in the production of A $\beta$ , which is a vicious cycle. In our previous study, EA therapy was demonstrated to reduce A $\beta$  in the hippocampus [11]. Insulin resistance exists in the AD brain, so that the brain tissue's ability to absorb glucose is significantly reduced. The above results indicated that EA had a protective effect on AD directly or indirectly by promoting glucose metabolism in the frontal lobe, which

might be related to the regulation of the PI3K/AKT signaling pathway [41].

Growing evidence suggested that inflammatory processes driven by microglia contribute to the pathogenesis of AD. We discovered that EA treatment inhibited inflammatory response. It is known that microglia plays a detrimental role in AD. Inflammatory response mediated by activated microglia is a major contributor to neurodegeneration [42].

Additionally, microglial activation can secrete pro-inflammatory cytokine, such as IL-1 $\beta$  [43], which reduces LTP by inhibition of glutamate release in aged rats [44]. It is worth noting that A $\beta$  is the main component of senile plaques, the characteristic pathological product of AD [45], and its excess production or aggregation is the key for the pathogenesis of AD [46]. Studies found that A $\beta$  constantly stimulated the activation of microglia and released a large number of cytokines, which led to continuous inflammation in the brain and eventually led to neurodegeneration [47, 48]. Additionally, activated microglia can release toxic substances such as peroxides, NO, and reactive oxygen radicals, thus damaging cellular mitochondria, triggering oxidative stress injury, and inducing cell apoptosis [49], which will aggravate the disorder of glucose metabolism in the brain [50]. Actually, A $\beta$  may be the key mediator between neuroinflammation and glucose metabolism in the brain [5]. Chronic inflammation is known to exacerbate insulin resistance associated with systemic disease-states. Neuroinflammation have an important role in the brain insulin and insulin-like growth factor-1 resistances that occur in AD and PD [51]. Insulin can block A $\beta$  binding to synapses, thereby preventing the ensuing neurotoxicity and A $\beta$ -induced loss of insulin receptors from neuronal dendrites [52]. Thus, the insulin-related signaling pathway may be a key pathway for EA to improve cognitive ability.

In the last few years, the number of publications about the ketogenic diet (KD) for neurological diseases have been progressively increasing. KD has been of interest as a treatment for potential neurodegenerative diseases such as AD [53]. It has been shown in AD, ketone bodies become the alternative energy source to glucose for the brain due to their ability to cross the blood-brain barrier carried by specific transporters that are not downregulated during AD [54]. Besides, KD was shown to decrease the production of amyloid- $\beta$  protein precursor and A $\beta$  [55]. Moreover, KD was related to the activation of peroxisome proliferator-activated receptor gamma and then to the decrease of inflammation [56].

In conclusion, the results of the current study demonstrate that EA stimulation has a useful degree of efficacy in AD mice. The effects of EA treatment on cognition are at least partially dependent on enhancing glucose metabolism and inhibiting inflammatory response in the frontal lobe of APP/PS1 mice, which may be closely related to the regulation of GV. Results from this study imply that EA may represent a potential therapeutic strategy for the prevention of

cognitive decline in AD. Indeed, there are many other valuable acupoints to improve cognition. More work in future studies is needed to explore the positive role of EA treatment in age-related neurodegenerative diseases, such as AD. In addition, clinical trials should be considered for future studies.

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