Electroacupuncture stimulation of the brachial plexus trunk on the healthy side promotes brain-derived neurotrophic factor mRNA expression in the ischemic cerebral cortex of a rat model of cerebral ischemia/reperfusion injury

Zongjun Guo¹, Lumin Wang²

¹Department of Special Health Care, Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, Shandong Province, China
²Department of Emergency Treatment, Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, Shandong Province, China

Abstract
A rat model of cerebral ischemia/reperfusion was established by suture occlusion of the left middle cerebral artery. In situ hybridization results showed that the number of brain-derived neurotrophic factor mRNA-positive cells in the ischemic rat cerebral cortex increased after cerebral ischemia/reperfusion injury. Low frequency continuous wave electroacupuncture (frequency 2–6 Hz, current intensity 2 mA) stimulation of the brachial plexus trunk on the healthy (right) side increased the number of brain-derived neurotrophic factor mRNA-positive cells in the ischemic cerebral cortex 14 days after cerebral ischemia/reperfusion injury. At the same time, electroacupuncture stimulation of the healthy brachial plexus trunk significantly decreased neurological function scores and alleviated neurological function deficits. These findings suggest that electroacupuncture stimulation of the brachial plexus trunk on the healthy (right) side can greatly increase brain-derived neurotrophic factor mRNA expression and improve neurological function.

Key Words
ischemia/reperfusion; brain-derived neurotrophic factor; electroacupuncture; brachial plexus trunk; cerebral cortex; in situ hybridization; neural regeneration

Research Highlights
Electroacupuncture stimulation of the brachial plexus trunk on the healthy side can significantly improve the neurological function of a rat model of cerebral ischemia/reperfusion injury and enhance brain-derived neurotrophic factor mRNA expression in the ischemic cerebral cortex 14 days after ischemia/reperfusion injury.

Abbreviations
MCAO, middle cerebral artery occlusion

INTRODUCTION
There is evidence that brain-derived neurotrophic factor plays an important role in neural cell injury and repair after cerebral ischemia⁹. After cerebral ischemia, increased brain derived neurotrophic factor protein expression in the cerebral cortex, striatum, and hippocampus can greatly alleviate ischemic brain injury (such as cerebral edema) and inhibit neuronal apoptosis, resulting in a protective effect³⁻⁵. Studies have shown that electroacupuncture
can alleviate cerebral edema, decrease calcium ion influx, and reduce inflammation and cell apoptosis in the brain tissue of rats after middle cerebral artery occlusion (MCAO)\cite{6-10}. Moreover, electroacupuncture also exhibits good curative effects in the clinical treatment of acute cerebral ischemia\cite{11}. Electroacupuncture stimulation of the nerve trunk can also improve the neurological function of patients with stroke\cite{12,13}. Little information is known about whether electroacupuncture stimulation of the nerve stem can influence brain derived neurotrophic factor mRNA expression levels in the brain tissue of rats subjected to MCAO. In this study, we monitored brain derived neurotrophic factor mRNA expression levels over time in the cerebral cortex of a rat model of cerebral ischemia/reperfusion injury established by MCAO. In addition, we monitored brain derived neurotrophic factor mRNA expression levels after electroacupuncture stimulation of the brachial plexus trunk on the healthy side.

RESULTS

Quantitative analysis of experimental animals

Sixty-eight healthy female adult Wistar rats were divided into a sham surgery group (n = 4), cerebral ischemia/reperfusion group (n = 32) and a brachial plexus trunk electroacupuncture group (electroacupuncture group, n = 32). Rats from the ischemia/reperfusion and electroacupuncture groups were subjected to suture occlusion of the left middle cerebral artery. Rats from the electroacupuncture group received electroacupuncture stimulation of the brachial plexus trunk after MCAO. Four rats from each group at each time point (2, 6, 12, 24 hours and 2, 3, 7 and 14 days after ischemia/reperfusion) were selected for subsequent examination and all rats were included in the final analysis.

**Electroacupuncture stimulation of the brachial plexus trunk improved MCAO rat neurological function**

After MCAO, rats exhibited varying degrees of neurological function deficits. At 12 hours after ischemia/reperfusion, there was a significant difference in neurological function deficit scores between the ischemia/reperfusion group and the electroacupuncture group (P < 0.05). In the following time periods, neurological function deficit scores in the electroacupuncture group were significantly reduced when compared with the ischemia/reperfusion group (P < 0.05; Table 1).

**Electroacupuncture stimulation of the brachial plexus trunk on the healthy side improved brain-derived neurotrophic factor mRNA expression in the ischemic cerebral cortex of MCAO rats**

*In situ* hybridization showed that brain derived neurotrophic factor mRNA expression in the cerebral cortex was lower in the sham surgery group compared with the electroacupuncture and ischemia/reperfusion groups. The number of brain derived neurotrophic factor mRNA-positive cells in the ischemic rat cerebral cortex increased after ischemic injury for 1 hour and reperfusion for 2 hours, and peaked after reperfusion for 2 and 24 hours. Levels recovered to normal following reperfusion for 7 days (P < 0.05). After reperfusion for 6 hours, the number of brain derived neurotrophic factor mRNA-positive cells in the ischemic cerebral cortex in the electroacupuncture group were significantly greater than those in the ischemia/reperfusion group (P < 0.05), peaked after reperfusion for 24 hours, and decreased thereafter until reperfusion for 14 days (Table 2, Figure 1).

---

**Table 1** Effects of electroacupuncture stimulation of the brachial plexus trunk on neurological function deficit scores in rats subjected to cerebral ischemia/reperfusion injury

| Group         | After reperfusion |
|---------------|-------------------|
|               | 2 hours | 6 hours | 12 hours | 24 hours | 2 days | 3 days | 7 days | 14 days |
| Ischemia/reperfusion | 2.96±0.98 | 2.76±0.56 | 2.80±0.48 | 2.75±0.42 | 2.62±0.56 | 2.40±0.46 | 2.05±0.41 | 1.47±0.38 |
| Electroacupuncture | 2.75±0.73 | 2.53±0.49 | 2.14±0.53 | 2.18±0.38 | 1.84±0.49 | 1.58±0.34 | 1.22±0.26 | 1.02±0.21 |

All data are expressed as mean ± SD of four rats from each group at each time point. Higher scores indicate more severe neurological function deficits. *P < 0.05, vs. ischemia/reperfusion group (two sample t-test).

**Table 2** Effects of electroacupuncture stimulation of the brachial plexus trunk on brain-derived neurotrophic factor mRNA-positive cells (400-fold visual field) in the ischemic rat cerebral cortex

| Group         | After reperfusion |
|---------------|-------------------|
|               | 2 hours | 6 hours | 12 hours | 24 hours | 2 days | 3 days | 7 days | 14 days |
| Sham surgery  | 13.53±6.35 |
| Ischemia/reperfusion | 64.50±5.45a | 53.50±6.99a | 54.13±3.94a | 90.50±12.39a | 72.00±5.17a | 81.75±9.40a | 17.00±4.29 | 10.75±3.19 |
| Electroacupuncture | 67.13±5.94ab | 81.75±2.94ab | 88.25±5.20ab | 111.63±9.06ab | 103.88±4.14ab | 103.25±4.27ab | 32.00±6.93ab | 16.75±2.95ab |

All data are expressed as mean ± SD of four rats at each time point per group. *P < 0.01, vs. sham surgery group; *P < 0.05, vs. ischemia/reperfusion group (two sample t-test).
DISCUSSION

Brain derived neurotrophic factor exhibits properties to enable the survival of multiple neurons, reduce the death of motor neurons, and directly promote axon growth, neuron repair and regeneration\(^{14-16}\). There is evidence that when cerebral ischemia/reperfusion injury occurs, the degree of injury of brain nerve cells is closely related to brain derived neurotrophic factor expression\(^{1, 14, 17-18}\). Results from this study showed that brain derived neurotrophic factor mRNA expression in the ischemic cerebral cortex was significantly increased at the early stage of ischemia/reperfusion, peaked after reperfusion for 24 hours, maintained high levels up to reperfusion for 3 days, and decreased to baseline levels after reperfusion for 7 days. Results also showed that brain derived neurotrophic factor mRNA expression in the cerebral cortex exhibited the first peak after reperfusion for 2 hours, and that the appearance of the first peak may be caused by the protective reaction of the cerebral cortex under the stressed state\(^{15-16}\); the second peak appears after reperfusion for 24 hours, and this peak is much higher and lasts for a longer period than the first peak, considering the self-repair process of neural cells is possibly related to the second peak\(^{5, 15, 18}\).

Clinical evidence suggests that acupuncture exhibits better therapeutic effects in patients with acute cerebral ischemia\(^{6}\), while electroacupuncture stimulation can interfere with the process of brain tissue injury after cerebral ischemia/reperfusion, alleviate cerebral edema after acute cerebral ischemia, and regulate intracellular calcium ion concentrations\(^{20-21}\). Electroacupuncture can increase the expression of nerve growth factor and brain derived neurotrophic factor in the peri-infarct cerebral cortex, exhibiting certain protective effects on cerebral ischemia\(^{20-23}\).

In this study, we used electroacupuncture stimulation of the brachial plexus trunk. Results showed that after ischemia/reperfusion for 6 hours, electroacupuncture stimulation of the brachial plexus trunk can significantly increase brain derived neurotrophic factor mRNA expression until reperfusion for 14 days; at the same time, electroacupuncture stimulation of the brachial plexus trunk can significantly decrease the degree of neurological function deficits in rats with cerebral ischemia/reperfusion and thereby decrease neurological function scores. These findings suggest that electroacupuncture stimulation of the brachial plexus trunk exhibits similar therapeutic effects to acupoint electroacupuncture, and that direct stimulation of the brachial plexus trunk is easy to operate and be accepted by patients\(^{7, 11, 13}\).

Increased brain derived neurotrophic factor levels in vivo exhibit brain protective effects by enhancing the activity.
of the γ-aminobutyric acid system and counteracting the neurotoxicity of glutamate\textsuperscript{[24-25]}. The phenomenon of propagation sensation along channels may be partly related to increased brain derived neurotrophic factor expression\textsuperscript{[26]}. After repeated electroacupuncture of the forelimbs of animals, the topographical boundaries of the forelimb motor areas in the cerebral cortex were found to move towards whisker motor areas in the cerebral cortex, accompanied by alteration in movement threshold\textsuperscript{[27-28]}, suggesting that the properties of output motor cortex neurons changed rapidly with an alteration in afferent information. Based on this, electroacupuncture stimulation of the brachial plexus trunk can cause alteration of neuron output in the cerebral cortex, and thereby lead to alteration of brain derived neurotrophic factor mRNA expression. Nevertheless, the underlying mechanism requires further investigation.

Taken together, electroacupuncture stimulation of the brachial plexus trunk can greatly increase brain derived neurotrophic factor mRNA expression in the cerebral cortex in rats subjected to cerebral ischemia/reperfusion injury. Nevertheless, the underlying mechanism requires further investigation.

**Materials and Methods**

**Design**
A randomized controlled animal experiment.

**Time and setting**
This study was performed at the Central Laboratory, Affiliated Hospital of Qingdao University Medical College from March 2009 to December 2012.

**Materials**
Sixty-eight healthy female Wistar rats of clean grade, aged 5 months, weighing 253 ± 15 g, were provided by the Animal Cultivation Center, Qingdao Institute for Drug Control (license No. SCKK (Lu) 20100167). All experiments were performed at the Central Laboratory, Affiliated Hospital of Qingdao University Medical College, China. The environment temperature was kept at 22°C. Rats had free access to food (standard solid feedstuff produced by the Laboratory Animal Center, Qingdao University Medical College, China) and water. All experimental protocols were performed according to the *Guidance Suggestions for the Care and Use of Laboratory Animals* issued by the Ministry of Science and Technology of China\textsuperscript{[29]}.  

**Methods**

**Preparation of MCAO models**
According to a modification of a previously described method\textsuperscript{[30-31]}, following anesthesia by 10% (v/v) chloral hydrate (300 mg/kg), a surgical nylon suture with a blunt end was inserted into the left external carotid artery, and entered into the inner segment of the internal carotid artery via the bifurcation of the common carotid artery and the outer segment of internal carotid artery, and advanced up to the bifurcation of the middle cerebral artery. After occlusion of the left middle cerebral artery for 60 minutes, the nylon suture was drawn out. In the sham surgery group, occlusion of the middle cerebral artery was omitted. After consciousness recovery for 30 minutes, neurological function deficits were scored according to a method described by Longa *et al.*\textsuperscript{[30-31]}. Rats with a score of 1–3 were considered to have undergone successful cerebral ischemia/reperfusion injury; otherwise, rats were refused for further experimentation. The lost rats were supplemented in time to ensure equal animal numbers in each group at each time point. Neurological function scoring was performed by an investigator who was blinded to group management.

**Electroacupuncture stimulation of the brachial plexus trunk on the healthy side improved MCAO rat neurological function**

After ischemia for 1 hour and reperfusion for 2, 6, 12, and 24 hours, and 2, 3, 7 and 14 days, rats from the electroacupuncture group were anesthetized and received electroacupuncture stimulation of the brachial plexus trunk. Precisely, a stainless steel acupuncture needle 0.4 mm in diameter, 25 mm in length (Suzhou Medical Supplies Factory Co., Ltd., Suzhou, Jiangsu Province, China) was punctured 6 mm towards the collarbone side from the right axillary fossa along the right midaxillary line until the rat brachial plexus trunk tissue was connected with a positive electrode. Another acupuncture needle was punctured 1 mm posterior to bregma and 1 mm left lateral to the midline\textsuperscript{[32]}, and was connected with a negative electrode. Each stimulation lasted for 30 minutes. Low-frequency continuous wave (frequency 2–6 Hz, current intensity 2 mA) electroacupuncture stimulation was performed using an electroacupuncture apparatus (G680522: Shanghai Tiancheng Science and Technology Co., Ltd, Shanghai, China).

**Sample collection**
Following anesthesia with 10% (v/v) chloral hydrate, a cannula was inserted from the left ventricle up to the ascending aorta for cardiac perfusion of approximately 300 mL of 0.9% (w/v) NaCl and approximately 400 mL 4% (w/v) paraformaldehyde (prepared with 0.01 M PBS). After perfusion, a craniotomy was performed for harvesting brain tissue. The brain tissue was resected in the region 1 mm anterior to bregma and 2 mm posterior to bregma to ensure collection of the infarcted cortex\textsuperscript{[32-33]}.  

---

1621
Thereafter, the brain tissue sample was fixed in pre-cooled 4 % (w/v) paraformaldehyde for 24 hours, routinely embedded in paraffin, and cut into 5 μm coronal sections.

Detection of brain derived neurotrophic factor mRNA expression in the rat cerebral cortex
After dehydration in a serious of gradient ethanol, paraffin sections were treated with 3% (w/v) H₂SO₄ for 10 minutes, washed three times with distilled water, each for 2 minutes. The oligonucleotide probe (sequence 5’-GCA ACC AAA GTA TGA AAT AAC CAT AGT AAG-3’) was provided by Boster Biotechnology, Wuhan, China. The sections were treated with diluted pepsin at 37°C and 20 minutes later, washed with 0.5 M PBS three times, each for 5 minutes. Subsequently, these sections were washed with double distilled water for 5 minutes, reacted with 20 μL prehybridization solution for 4 hours in the hybridization box, then with 20 μL hybridization solution for 10–12 hours at 40°C, and finally washed with 2 x sodium citrate for 10 minutes, 0.5 x sodium citrate for 15 minutes, and with 0.2 x sodium citrate for 15 minutes[34]. Diaminobenzidine coloration time was controlled through the use of an optical microscope (Shenzhen Winner Optical Co., Ltd., Shenzhen, Guangdong Province, China). PBS (0.01 M) rather than the oligonucleotide probe was used as a negative control. A high power lens (×400) on the Olympus microscope camera (Wuxi Precision Machinery Co., Ltd., Wuxi, Jiangsu Province, China) was used for sample observation. Eight visual fields were randomly selected from the cerebral cortex on the ischemic side for counting brain derived neurotrophic factor mRNA-positive cells. The number of brain derived neurotrophic factor mRNA-positive cells per visual field was calculated[35-36].

Statistical analysis
All data were expressed as mean ± SD. The two sample t-test was performed using SPSS 10.0 software (SPSS, Chicago, IL, USA). A level of P < 0.05 was considered statistically significant.

Acknowledgments: We are grateful to Professor Shoubiao Wang from the Laboratory of Anatomy, Qingdao University Medical College, China for his guidance and help during sample harvesting and pathological analysis.

Funding: This study was supported by the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period, No. 2006BAI01A00; a grant from Science and Technology Department of Shandong Province, No. 22130109; a grant from Science and Technology Bureau of Qingdao City, No. Kzd-03, 09-1-1-33-rsh.

Author contributions: All authors participated in experimental design, conduction and data analysis.

Conflicts of interest: None declared.

Ethical approval: All experimental protocols received full approval from the Animal Ethical Committee of Qingdao University, China.

REFERENCES

[1] MacLellan CL, Keough MB, Granter-Button S, et al. A critical threshold of rehabilitation involving brain-derived neurotrophic factor is required for poststroke recovery. Neurorehabil Neural Repair. 2011;25(8):740-748.

[2] Zhang J, Yu Z, Yu Z, et al. rAAV-mediated delivery of brain-derived neurotrophic factor promotes neurite outgrowth and protects neurodegeneration in focal ischemic model. Int J Clin Exp Pathol. 2011;4(5):496-504.

[3] Airavaara M, Shen H, Kuo CC, et al. Mesencephalic astrocyte-derived neurotrophic factor reduces ischemic brain injury and promotes behavioral recovery in rats. J Comp Neurol. 2009;515(1):116-124.

[4] Cadet JL, Brannock C, Ladenheim B, et al. Methamphetamine preconditioning causes differential changes in striatal transcriptional responses to large doses of the drug. Dose Response. 2011;9(2):165-181.

[5] Shi Q, Zhang P, Zhang J, et al. Adenovirus-mediated brain-derived neurotrophic factor expression regulated by hypoxia response element protects brain from injury of transient middle cerebral artery occlusion in mice. Neurosci Lett. 2009;465(3):220-225.

[6] Ng SS, Hui-Chan CW. Transcutaneous electrical stimulation on acupoints combined with task-related training to improve motor function and walking performance in an individual 7 years poststroke: a case study. J Neurol Phys Ther. 2010;34(4):208-213.

[7] Chen AL, Li XP, Yu JL, et al. Acupuncture neural stem and rehabilitation training for hand function in stroke patients. Zhongguo Kangfu. 2008;23(6):416.

[8] Kim WS, Kim IS, Kim SJ, et al. Effect of electroacupuncture on motor recovery in a rat stroke model during the early recovery stage. Brain Res. 2009;1248:176-183.

[9] Peng L, Huang XI, Han XH. The effects of electroacupuncture combined with transcranial magnetic stimulation on the expression of NGF and BDNF in different cerebral regions of rats with cerebral ischemia. Zhongguo Kangfu. 2009;24(6):363-365.

[10] Zhang D, Sun Y, Wang LQ. Effects of electroacupuncture at point Baihui and Fengfu on the expressions of BDNF and the cognitive function in dementia rats. Zhongyiyao Xuebao.2011;39(3):15-18.

[11] Wang WC, Song QJ, Wang Q, et al. Randomized controlled trial of treatment of hemiplegia and spasticity between electro-acupuncture at antagonistic muscular motor points and at acupoints in stroke patients. Zhongguo Kangfu Yixue Zazhi. 2011;26(5):438-442.

[12] Liu B, Lin JH. Different frequency electroacupuncture with facilitation techniques in the treatment of hemiplegic
patients after cerebral apoplexy. Disi Junyi Daxue Xuebao. 2007;28(16):1503-1505.

[13] Huang J, Peng ZL, Ding P. Effect of electroacupuncture and Xingnao Kaiqiao needling method on patients with poststroke hemiplegia. Shizhen Guoyi Guoyao. 2011; 22(6):1506-1507.

[14] Tan YX, Li XM, Yu JX, et al. Effects of intracerebroventricular administration of brain-derived neurotrophic factor at different time intervals on cerebral ischemia-reperfusion injuries in rats. Zonghua Shenjing Yixue Zazhi. 2010;9(1):38-42.

[15] Schmidt-Kastner R, Truettner J, Lin B, et al. Transient changes of brain-derived neurotrophic factor (BDNF) mRNA expression in hippocampus during moderate ischemia induced by chronic bilateral common carotid artery occlusions in the rat. Brain Res Mol Brain Res. 2001;92(1-2):157-166.

[16] Jiang Y, Wei N, Zhu J, et al. Effects of brain-derived neurotrophic factor on local inflammation in experimental stroke of rat. Mediators Inflamm. 2010;2010:372423.

[17] Han Q, Li B, Feng H, et al. The promotion of cerebral ischemia recovery in rats by laminin-binding BDNF. Biomaterials. 2011;32(22):5077-5085.

[18] Narantuya D, Nagai A, Sheikh AM, et al. Human microglia transplanted in rat focal ischemia brain induce neuroprotection and behavioral improvement. PLoS One. 2010;5(7):e11746.

[19] Wang J, Hong Y, Zhen C, et al. Changes in expression levels of brain-derived neurotrophic factor and tyrosine kinase B receptor in hippocampal CA1 region of rats with permanent forebrain ischemia. Beijing Zhongyi Yao Daxue Xuebao. 2009;32(1):30-35.

[20] Huang XL, Han XH, Li CF, et al. The effects of electroacupuncture combined with transcranial magnetic stimulation on water content and extracellular calcium content of brain tissues after cerebral ischemia in rats. Zhonghua Wuli Yixue yu Kangfu Zazhi. 2003;25(4):206-208.

[21] Shi Y. Effect of electro-acupuncture on expression changes of BDNF protein in hippocampus of rats with ischemia-reperfusion injury. Zhejiang Yixue. 2010;32(10):1514-1517.

[22] Hwang IK, Chung JY, Yoo DY, et al. Effects of electroacupuncture at Zusanli and Baihui on brain-derived neurotrophic factor and cyclic AMP response element-binding protein in the hippocampal dentate gyrus. J Vet Med Sci. 2010;72(11):1431-1436.

[23] Manni L, Aloé L, Fiore M. Changes in cognition induced by social isolation in the mouse are restored by electro-acupuncture. Physiol Behav. 2009;98(5):537-542.

[24] Sommer C, Kollmar R, Schwab S, et al. Exogenous brain-derived neurotrophic factor prevents postischemic downregulation of [3H]muscimol binding to GABA(A) receptors in the cortical penumbra. Brain Res Mol Brain Res. 2003;111(1-2):24-30.

[25] Gan P, Guo JC, Yang R. The role of GABA in electro-acupuncture effect on cerebral ischemia. Shanghai Zhenjiu Zazhi. 2003;22(9):3-6.

[26] Kim EH, Kim YJ, Lee HJ, et al. Acupuncture increases cell proliferation in dentate gyrus after transient global ischemia in gerbils. Neurosci Lett. 2001;297(1):21-24.

[27] Li XF, Yun JT, QiHX, et al. Repeated electrical stimulation of forelimb nerves changing plasticity of adult rat motor cortex. Jichu Yixue yu Linchuang. 1994;14:181-184.

[28] Zheng SX, Xu JS, Pan XH, et al. Influences of electroacupuncture in different acupuncture points on evoked potential map of primary somatosensory area of cerebral cortex. Fujian Zhongyi Yao Daxue Xuebao. 2011;21(3):1-3.

[29] The Ministry of Science and Technology of the People’s Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

[30] Longa EZ, Weinstein PR, Carlson S, et al. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989;20(1):84-91.

[31] Boyko M, Zlotnik A, Gruenbaum BF, et al. Pyruvate’s blood glutamate scavenging activity contributes to the spectrum of its neuroprotective mechanisms in a rat model of stroke. Eur J Neurosci. 2011;34(9):1432-1441.

[32] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 5th ed. London: Academic Press. 2005.

[33] Guo ZJ, Feng YQ, Guo YL, et al. Effect of nerve growth factor on expression of growth associated protein and synaptophysim after cerebral ischemic reperfusion in rats. Zhonghua Wuli Yixue yu Kangfu Zazhi. 2005;22(4):201-203.

[34] Yin XM, Yang XW, Zhu GY, et al. Effects of mild hypothermia on expression of brain-derived neurotrophic factor mRNA in neonatal rats with hypoxic-ischemic brain damage. Zhongguo Xinshenger Ke Zazhi. 2008;23(2):88-91.

[35] Jin LY, Guo ZJ, Du F, et al. Coexist expression and relation of estrogen receptor α and β-site β-amyloid precursor protein cleaving enzyme in rat’s brain with fimbria/ fornix transection and ovariectomy. Zhongguo Kanglu Yixue Zazhi. 2007;22(9):782-785.

[36] Guo ZJ, Jin LY, Yin JJ, et al. Expressive variety of choline acetyltransferase in different cerebral regions of rats with fimbria/ fornix transection. Zhongguo Linchuang Kanglu. 2006;10(2):176-178.

(Edited by Guo GQ, Qi JP/Song LP)