The Effects of Electroacupuncture on the BDNF/TrkB/PI3K/Akt Pathway and Hippocampal Neuronal Protection in Rats with Learning and Memory Impairment after Ischemia-Reperfusion: A Postprint

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Abstract

Background Learning and memory impairment is a common functional disorder following stroke, severely affecting patients' overall rehabilitation. Electroacupuncture at Shenting (GV24) and Baihui (GV20) has demonstrated definite therapeutic efficacy for patients with post-stroke cognitive dysfunction, but its mechanism of action remains unclear. Objective To investigate the protective effect of electroacupuncture on hippocampal neurons in rats with learning and memory impairment after middle cerebral artery occlusion/reperfusion (MCAO/R) and its influence on the brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB)/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway. Methods From May 2021 to March 2022, 60 healthy male Sprague-Dawley (SD) rats aged 8 weeks were selected. Twenty-four rats were divided into a blank group (n=12) and a sham operation group (n=12), while the remaining 36 rats were used to establish the MCAO/R model. The 24 rats with successful model establishment were randomly assigned to a model group (n=12) and an electroacupuncture group (n=12). The electroacupuncture group received electroacupuncture treatment at Shenting (GV24) and Baihui (GV20) acupoints. The Zea-Longa score was used to assess neurological deficit severity, the spatial probe test was employed to evaluate spatial learning and memory capacity, and TTC staining was utilized to measure cerebral infarct volume. Real-time fluorescent quantitative polymerase chain reaction (RT-PCR) was applied to determine mRNA levels of BDNF, TrkB, PI3K, and Akt, while Western blot was used to detect protein levels of BDNF and TrkB and phosphorylation levels of PI3K and Akt proteins. Results At 2 hours post-surgery, the Zea-Longa scores of

the model and electroacupuncture groups differed significantly from those of the blank and sham operation groups (P<0.05). On treatment days 7 and 14, the Zea-Longa scores of the model group differed significantly from those of the blank, sham operation, and electroacupuncture groups (P<0.05). From treatment days 9 to 13, the escape latency of the model group exceeded that of the sham operation group (P<0.05), while that of the electroacupuncture group was lower than that of the model group (P<0.05). The frequency of crossing the platform effective area in the model group was lower than that in the blank and sham operation groups (P<0.05), whereas that in the electroacupuncture group was higher than that in the model group (P<0.05). Cerebral infarct volumes in the blank, sham operation, model, and electroacupuncture groups were 0, 0, (36.7$±6.3)±$2.2)%, respectively, with the electroacupuncture group showing lower infarct volume than the model group (P<0.05). The mRNA levels of BDNF, TrkB, PI3K, and Akt in the model group were lower than those in the blank and sham operation groups (P<0.05), while those in the electroacupuncture group were higher than those in the model group (P<0.05). Compared with the sham operation group, the model group exhibited decreased protein levels of BDNF and TrkB (P<0.01; P<0.001) and reduced phosphorylation levels of PI3K and Akt proteins (P<0.01; P<0.05). Compared with the model group, the electroacupuncture group showed elevated protein levels of BDNF and TrkB and increased phosphorylation levels of PI3K and Akt proteins (P<0.05). Conclusion Electroacupuncture may alleviate neurological deficit symptoms and improve learning and memory ability after cerebral ischemia-reperfusion by upregulating the expression of proteins associated with the BDNF/TrkB/PI3K/Akt pathway.

Full Text

Effect of Electroacupuncture on the BDNF/TrkB/PI3K/Akt Pathway and Hippocampal Neuronal Protection in Rats with Learning and Memory Impairment after Ischemia-Reperfusion

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Abstract

Background
Learning and memory impairment is a common post-stroke dysfunction that

severely affects patient recovery. Electroacupuncture at the Shenting (GV24) and Baihui (GV20) acupoints demonstrates efficacy in treating post-stroke cognitive dysfunction, though its mechanism of action remains unclear.

Objective

To investigate the neuroprotective effects of electroacupuncture on hippocampal neurons and its influence on the brain-derived neurotrophic factor (BDNF)/tyrosine receptor kinase B (TrkB)/phosphatidylinositol-3-hydroxykinase (PI3K)/protein kinase B (Akt) signaling pathway in rats with learning and memory impairment following middle cerebral artery occlusion/reperfusion (MCAO/R).

Methods

From May 2021 to March 2022, sixty 8-week-old healthy male Sprague-Dawley rats were selected. Twenty-four rats were allocated to blank (n=12) and sham operation (n=12) groups, while the remaining thirty-six underwent MCAO/R model induction. Twenty-four successfully modeled rats were randomly divided into model (n=12) and electroacupuncture (n=12) groups. The electroacupuncture group received treatment at Shenting and Baihui acupoints. Neurological deficits were assessed using the Zea-Longa score, spatial learning and memory were evaluated via the Morris water maze, and cerebral infarct volume was measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining. RT-PCR was employed to determine mRNA levels of BDNF, TrkB, PI3K, and Akt, while Western blotting was used to detect BDNF and TrkB protein levels and PI3K and Akt phosphorylation levels.

Results

At 2 hours post-surgery, Zea-Longa scores in the model and electroacupuncture groups differed significantly from the blank and sham groups ($P<0.05$). On treatment days 7 and 14, the model group showed significantly higher scores compared to the blank, sham, and electroacupuncture groups ($P<0.05$). During treatment days 9–13, escape latencies in the model group exceeded those of the sham group ($P<0.05$), while the electroacupuncture group demonstrated shorter latencies than the model group ($P<0.05$). Platform crossing frequency in the model group was lower than in the blank and sham groups ($P<0.05$), but higher in the electroacupuncture group compared to the model group ($P<0.05$). Cerebral infarct volumes were 0%, 0%, (36.7$±6.3)±$2.2)% in the blank, sham, model, and electroacupuncture groups, respectively, with the electroacupuncture group showing significantly reduced infarct volume versus the model group ($P<0.05$). The model group exhibited lower BDNF, TrkB, PI3K, and Akt mRNA levels compared to the blank and sham groups ($P<0.05$), while the electroacupuncture group showed elevated mRNA levels relative to the model group ($P<0.05$). Compared with the sham group, the model group displayed decreased BDNF and TrkB protein levels ($P<0.01$; $P<0.001$) and reduced PI3K and Akt phosphorylation ($P<0.01$; $P<0.05$). The electroacupuncture group showed increased BDNF and TrkB protein levels and enhanced PI3K and Akt phosphorylation compared to the model group ($P<0.05$).

Conclusion
Electroacupuncture may alleviate neurological deficits and improve learning and memory following cerebral ischemia-reperfusion by upregulating BDNF/TrkB/PI3K/Akt pathway-related protein expression.

[**Key words**] Stroke; Reperfusion injury; Memory disorders; Electroacupuncture therapy; CA1 region, hippocampal; Nerve growth factors

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1. Introduction

Stroke is the leading cause of reduced life expectancy in China, with ischemic stroke accounting for approximately 87% of cases. Stroke patients commonly experience varying degrees of functional impairment, with about two-thirds exhibiting cognitive decline in at least one domain—including executive function/attention, memory, or visuospatial abilities—which severely impacts rehabilitation compliance and overall recovery. Our previous research identified Shenting and Baihui as frequently used acupoints in acupuncture treatment for post-stroke cognitive impairment, and demonstrated that electroacupuncture at these points effectively improves cognitive function and self-care ability in stroke patients, though the underlying mechanisms require further investigation.

Brain-derived neurotrophic factor (BDNF) plays a crucial role in neuronal development and maturation, maintaining neuronal survival and increasing neurotransmitter synthesis. Upon binding to its specific receptor tyrosine kinase B (TrkB), BDNF activates the downstream phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) signaling pathway, which is closely associated with apoptosis, autophagy, and synaptic plasticity. Based on these findings, this study established a middle cerebral artery occlusion/reperfusion (MCAO/R) model in rats with learning and memory impairment to investigate the neuroprotective effects of electroacupuncture on hippocampal neurons and its impact on the BDNF/TrkB/PI3K/Akt signaling pathway.

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2. Materials and Methods

2.1 Experimental Animals

Sixty 8-week-old healthy male Sprague-Dawley rats weighing 180–220 g, specific-pathogen-free grade, were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. [License No. SCXK (Lu) 2019003]. Animals were housed at the Experimental Animal Center of the First Affiliated Hospital of Henan University of Chinese Medicine under controlled conditions (temperature 20–26°C, humidity 50–70%, 12-hour light/dark cycle) with ad libitum access to food and water. All experimental procedures strictly adhered to animal ethics regulations and were approved by the Experimental Animal Welfare Ethics Review Com-

mittee of the First Affiliated Hospital of Henan University of Chinese Medicine (Approval No. YFYDW2021030).

2.2 Main Reagents and Instruments

Reagents: Pentobarbital sodium (Shanghai Fude Chemical Co., Ltd., Cat. No. 21642-83-1), gentamicin sulfate [Shanghai Modern Hasen (Shangqiu) Pharmaceutical Co., Ltd., Cat. No. 1405-41-0], suture for MCAO (Beijing Xilong Technology Co., Ltd., Cat. No. 2036A6), paraformaldehyde (Biosharp, Cat. No. BL539A), neutral balsam (China National Pharmaceutical Group Co., Ltd., Cat. No. 96949-21-2), 2% 2,3,5-triphenyltetrazolium chloride (TTC) staining solution (Beijing Solarbio Science & Technology Co., Ltd., Cat. No. T8170), total RNA extraction kit (Beijing Solarbio, Cat. No. R1200), protein extraction kit (Beijing Solarbio, Cat. No. R0010), BCA protein concentration assay kit (Beijing Solarbio, Cat. No. PC0020), rainbow spectral protein marker (Beijing Solarbio, Cat. No. PR1920), enhanced chemiluminescence (ECL) developing solution (Beijing Solarbio, Cat. No. PE0010), reverse transcription kit [Yeasen Biotechnology (Shanghai) Co., Ltd., Cat. No. 14601ES03], primers (Genewiz Biotech), rabbit anti-BDNF (Abcam, Cat. No. ab108319), TrkB polyclonal antibody (Abcam, Cat. No. ab187041), horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody (Abcam, Cat. No. ab150077), rabbit anti-phosphorylated PI3K (p-PI3K) (Abcam, Cat. No. ab278545), PI3K (Abcam, Cat. No. ab133595), phosphorylated Akt (p-Akt) (Wuhan Sanying Biotechnology, Cat. No. 80455-1-RR), Akt (Wuhan Sanying, Cat. No. 6-2-3-2-lg), GAPDH polyclonal antibody (Wuhan Sanying, Cat. No. 60004-1-lg), and PAGE gel kit (Shanghai Yase Biomedical Technology, Cat. No. PG112).

Instruments: Morris water maze (Shenzhen Reward Life Technology Co., Ltd.), acupuncture needles and electroacupuncture apparatus (Suzhou Medical Supplies Factory), pathological microtome (Leica, Model RM2245), dehydrator (Leica, Model ASP200S), embedding machine (Wuhan Junjie Electronics, Model JB-L7), high-speed tissue grinder (Wuhan Servicebio, Model SWE-FP), PCR instrument (Hangzhou Biggerfly, Model 96B), protein concentration microassay system (Thermo, Model NanoDropOne), real-time fluorescent quantitative PCR instrument (Xi'an Tianlong Technology, Model Gentier), microplate reader (Thermo, Model MK3), electrophoresis and transfer apparatus (Bio-Rad, Models 1658001 and 170-3930), and gel imaging system (Bio-Rad, Model Gel-Doc XR+).

2.3 Animal Modeling and Grouping

After one week of acclimation, rats were weighed and grouped according to a random number table generated from body weight. Twenty-four rats were assigned to blank (n=12) and sham operation (n=12) groups, while the remaining thirty-six rats underwent MCAO/R model establishment using the Zea Longa suture method. Rats were fasted for 12 hours prior to surgery. Following anesthesia with 3% pentobarbital sodium (30 mg/kg intraperitoneal), rats

were placed in supine position. The common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were isolated. The ECA distal end was ligated, and the CCA proximal end and ICA distal end were clamped. A "V"-shaped incision was made at the ECA bifurcation, and a silicone-coated nylon suture (0.26$±$0.02 mm) was inserted through the incision to the ICA clamp, which was then released. The suture was advanced until slight resistance was felt at a depth of approximately 1.8–2.0 cm. The incision was sutured after recording the occlusion time. Postoperatively, gentamicin (2 U per rat) was administered intraperitoneally for three consecutive days. After 2 hours of ischemia, the suture was gently withdrawn approximately 1 cm to allow reperfusion. The blank group received only handling under identical conditions, while the sham group underwent anesthesia and vessel isolation without suture insertion.

Upon full recovery from anesthesia, all rats underwent Zea-Longa scoring (criteria shown in). Rats scoring 0 or 4 were excluded. At 24 hours post-surgery, the sham group and remaining modeled rats underwent Morris water maze navigation testing. Using the sham group's mean escape latency as a reference, model rats with mean escape latency less than 80% of this value were considered successfully modeled. Ultimately, twenty-four successfully modeled rats were randomly divided into model (n=12) and electroacupuncture (n=12) groups.

2.4 Electroacupuncture Intervention

The electroacupuncture group received treatment at Shenting and Baihui acupoints, located according to *Experimental Acupuncture*. Rats were placed on a specially designed treatment frame. Acupuncture needles (0.3 mm × 25 mm) were inserted obliquely backward to a depth of 3 mm and connected to a GM01 electroacupuncture apparatus. Sparse-dense wave was applied at 6 V peak voltage, 2–10 Hz frequency, and 1–3 mA intensity for 30 minutes per session, once daily for 14 consecutive days. During treatment, rats were monitored to prevent needle dislodgement or falls. The model, blank, and sham groups received identical handling without intervention.

2.5 Assessment of Neurological Deficits and Spatial Learning-Memory

Neurological deficits were evaluated using the Zea-Longa score on treatment days 7 and 14 by personnel blinded to group assignments. Spatial learning and memory were assessed via Morris water maze, comprising: (1) **Place navigation test**: Conducted at 8:00 and 18:00 on treatment days 9–13. Rats were placed in the pool starting from quadrant 1 and proceeding clockwise. The time to locate the escape platform (escape latency) was recorded. Rats failing to find the platform within 90 seconds were guided to it, allowed to rest for 10 seconds, and assigned a latency of 90 seconds. (2) **Spatial probe test**: On treatment day 14, the platform was removed while other conditions remained unchanged. Rats were placed facing the pool wall in each of the four quadrants, and the

number of crossings through the former platform area within 90 seconds was recorded.

2.6 TTC Staining for Cerebral Infarct Volume

After intervention completion, three rats from each group were randomly selected, anesthetized, and decapitated. Brains were extracted, rinsed, rapidly frozen at -20°C for 20 minutes, and coronally sectioned into five 2-mm-thick slices. Sections were stained in 2% TTC solution at 37°C in darkness. After staining, slices were photographed and infarct volume was calculated using Image J software.

2.7 Hematoxylin-Eosin (HE) Staining of Hippocampal CA1 Neurons

Following intervention, three rats per group were anesthetized and perfused transcardially with saline and paraformaldehyde. Brains were extracted, fixed in paraformaldehyde, dehydrated, and paraffin-embedded. Sections were cut, baked at 56°C for 1 hour, dewaxed in xylene (8 minutes × 4), and dehydrated through graded ethanol. After hematoxylin staining for 5–10 minutes, sections were rinsed until water ran clear, differentiated in 1% hydrochloric acid alcohol for 2–3 seconds, rinsed, blued in ammonia water, and rinsed again. Following 1% eosin staining for 5–10 minutes and 1-minute rinsing, sections were dehydrated in ethanol and mounted with neutral balsam.

2.8 RT-PCR Quantification of BDNF, TrkB, PI3K, and Akt mRNA

Post-intervention, three rats per group were deeply anesthetized, and hippocampal tissue was dissected on ice, placed in enzyme-free cryovials, and stored at -80°C. Total RNA was extracted according to kit instructions. Primer sequences are listed in . Reverse transcription was performed at 37°C for 15 minutes and 98°C for 5 minutes, with products stored at -20°C. RT-PCR was conducted following kit protocols, and relative gene expression was calculated using the 2^(-ΔΔCT) method.

2.9 Western Blot Analysis of BDNF, TrkB Protein Levels and PI3K, Akt Phosphorylation

Post-intervention, three rats per group were anesthetized, and hippocampal tissue was harvested as described above. Tissue was weighed and homogenized in lysis buffer (RIPA lysis solution: protease inhibitor: phosphatase inhibitor = 100:1:1) at low temperature. After centrifugation at 12,000 rpm for 15 minutes at 4°C, supernatants were collected. Protein concentration was determined by BCA assay, and samples were denatured after adding loading buffer. SDS-PAGE was performed, followed by wet transfer and blocking for 1.5–2 hours. Primary antibodies (BDNF 1:7,000, TrkB 1:5,000, p-PI3K 1:1,000, PI3K 1:1,000, p-Akt 1:1,000, Akt 1:1,000, GAPDH 1:10,000) were incubated overnight at 4°C. Secondary antibody (1:10,000) was applied for 1 hour at room temperature.

ECL development and gel imaging were performed. Relative protein expression was quantified using Image J software, with PI3K and Akt phosphorylation levels expressed as p-PI3K/PI3K and p-Akt/Akt ratios, respectively.

2.10 Statistical Analysis

Normally distributed continuous data are presented as mean ± standard deviation ($\bar{x} \pm s$). Inter-group comparisons were performed using one-way ANOVA with LSD-t test for pairwise comparisons. Repeated measures data were analyzed using two-way repeated measures ANOVA. Ranked data were compared using Kruskal-Wallis H test with non-parametric tests for pairwise comparisons. Statistical significance was set at $P<0.05$.

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3. Results

3.1 Neurological Function Deficits

At 2 hours post-surgery, Zea-Longa scores in the model and electroacupuncture groups differed significantly from the blank and sham groups ($P<0.05$). On treatment days 7 and 14, the model group exhibited significantly higher scores compared to the blank, sham, and electroacupuncture groups ($P<0.05$) .

3.2 Learning and Memory Performance

Repeated measures ANOVA of place navigation data revealed significant main effects of time and group, and a significant time × group interaction. Simple effects analysis showed significant differences in escape latency across treatment days 9–13 ($P<0.05$). Between-group comparisons indicated significant differences in escape latency on days 9–13 ($P<0.05$), with the model group showing longer latencies than the sham group ($P<0.05$) and the electroacupuncture group showing shorter latencies than the model group ($P<0.05$) . Spatial probe test results demonstrated significant between-group differences in platform crossing frequency ($P<0.05$), with the model group crossing fewer times than the blank and sham groups ($P<0.05$) and the electroacupuncture group crossing more times than the model group ($P<0.05$) .

3.3 Cerebral Infarct Volume

Cerebral infarct volumes were 0%, 0%, (36.7$±6.3)±$2.2)% in the blank, sham, model, and electroacupuncture groups, respectively. The electroacupuncture group showed significantly reduced infarct volume compared to the model group ($P<0.05$) [Figure 1: see original paper].

3.4 Hippocampal CA1 Neuron Morphology

HE staining revealed tightly arranged, uniformly stained neurons with clear nuclei in the blank and sham groups. The model group showed sparse neurons with varied sizes and shapes, poor membrane integrity, deformation, swelling, rupture, uneven cytoplasmic staining, and deeply stained, condensed nuclei with indistinct nucleoli. The electroacupuncture group exhibited increased neuronal density, more regular arrangement, intact membranes and nucleoli, and evenly distributed cytoplasm compared to the model group [Figure 2: see original paper].

3.5 Hippocampal mRNA Expression

Significant between-group differences were observed in BDNF, TrkB, PI3K, and Akt mRNA levels ($P<0.05$). The model group showed lower expression than the blank and sham groups ($P<0.05$), while the electroacupuncture group exhibited higher expression than the model group ($P<0.05$) .

3.6 Hippocampal Protein Expression and Phosphorylation

No significant differences in BDNF, TrkB protein levels or PI3K, Akt phosphorylation were observed between the blank and sham groups ($P>0.05$). Compared with the sham group, the model group showed decreased BDNF and TrkB protein levels ($P<0.01$; $P<0.001$) and reduced PI3K and Akt phosphorylation ($P<0.01$; $P<0.05$). The electroacupuncture group demonstrated elevated BDNF and TrkB protein levels and increased PI3K and Akt phosphorylation compared to the model group ($P<0.05$) [FIGURE:3, TABLE:7].

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4. Discussion

Post-stroke cognitive impairment corresponds to “dementia” and “forgetfulness” in traditional Chinese medicine. Modern practitioners localize the disease to the brain, with pathogenesis involving obstructed cerebral vessels and malnourished brain marrow. Our research team, based on the academic principle of “disease location in the brain, first select Governor Vessel points,” developed the Tongdu Xingshen acupuncture method focusing on electroacupuncture at Shenting and Baihui, which has demonstrated significant clinical efficacy. Shenting, the intersection of the Governor Vessel with Foot-Taiyang and Yangming meridians, governs mental disorders, while Baihui, located at the vertex, connects meridians throughout the body and serves as a crucial point for regulating cerebral function. Our previous studies confirmed that electroacupuncture at these points improves post-stroke cognitive function and self-care ability.

The hippocampus is intimately associated with higher brain functions including learning, memory, and emotion. Under normal conditions, short-term memory is stored in the hippocampus before being transferred to permanent storage

through repetition and reinforcement. Hippocampal damage leads to learning and memory deficits, making it a key region for studying memory mechanisms. Additionally, hippocampal neurons are exceptionally sensitive to ischemia and hypoxia, rendering the hippocampus the most studied brain region in post-stroke cognitive impairment. Our findings revealed extensive neuronal dissolution, reduced cell numbers, and blurred cellular architecture in the CA1 region of MCAO/R rats, correlating with impaired learning and memory performance.

BDNF, the most widely distributed neurotrophin in the mammalian central nervous system, critically regulates neuronal formation, functional remodeling, and cognitive functions. Abnormal BDNF expression restricts axonal and dendritic growth, impairs synaptic transmission, and contributes to neurological disorders including neurodegeneration, neuropathic pain, depression, and epilepsy. Hippocampal BDNF expression directly correlates with structural and functional integrity. BDNF knockout induces cognitive dysfunction and even mortality in mice, while intracerebroventricular BDNF administration prevents ischemic CA1 neuronal death, promotes dentate gyrus granule cell maturation, and enhances postsynaptic receptor activity to improve neurological function.

BDNF exerts its central nervous system effects through high-affinity binding to TrkB receptors, inducing dimerization and subsequent PI3K/Akt pathway activation. The BDNF/TrkB-mediated PI3K/Akt cascade plays vital roles in synaptic remodeling, neurogenesis, and neurodevelopmental processes related to emotion, learning, and memory. The PI3K/Akt pathway represents a crucial intracellular survival signaling cascade involved in cell proliferation and differentiation, exerting significant neuroprotective effects in nervous system injury. LI et al. demonstrated that modulating the PI3K pathway improved cognitive function in focal cerebral ischemia rats. Our results showed that MCAO/R reduced hippocampal BDNF, TrkB, PI3K, and Akt levels, impaired learning and memory, and caused neuronal damage, while electroacupuncture upregulated these pathway components and improved cognitive performance, likely by enhancing hippocampal synaptic plasticity. However, further research is needed to confirm these mechanisms. Additionally, mTOR, a key downstream effector of PI3K/Akt and critical regulator of autophagy, may mediate electroacupuncture's effects through the BDNF/TrkB/PI3K/Akt/mTOR cascade, warranting investigation into the relationship between autophagy and synaptic plasticity.

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5. Conclusion

Electroacupuncture may alleviate neurological deficits and improve learning and memory following cerebral ischemia-reperfusion by upregulating BDNF/TrkB/PI3K/Akt pathway-related protein expression.

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Author Contributions: SU Kaiqi designed the study and drafted the manuscript. SU Kaiqi, LYU Zhuan, LUO Meng, NIE Chenchen, and LIU Hao performed animal experiments and data collection. WU Mingli and GAO Jing managed experiments and analyzed data. FENG Xiaodong supervised quality control and revised the final manuscript.

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[Tables and Figures]

Zea-Longa scoring criteria
RT-PCR primer sequences
Comparison of Zea-Longa scores among 4 groups
Comparison of escape latency among 4 groups ($\bar{x} \pm s$, s)
Comparison of platform crossing times among 4 groups ($\bar{x} \pm s$, times)
Comparison of BDNF, TrkB, PI3K, Akt mRNA levels in hippocampal tissues ($\bar{x} \pm s$)
Comparison of BDNF, TrkB, PI3K, Akt protein relative expression levels in hippocampal tissues ($\bar{x} \pm s$)

[Figure 1: see original paper] TTC staining of brain tissue in 4 groups
[Figure 2: see original paper] HE staining in hippocampal CA1 region of 4 groups ($\times$200)
[Figure 3: see original paper] Protein bands of BDNF, TrkB, PI3K, Akt, p-PI3K, p-Akt in hippocampus of 4 groups by Western Blotting

Note: Figure translations are in progress. See original paper for figures.