Journal of Traditional and Complementary Medicine 13 (2023) 588–599

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Effect of electrode configuration in electroacupuncture on ischemic stroke treatment in rats

Chung-Hsiang Liu [a], Huong Thi Mai Nguyen [b], Der-Yen Lee [c], Ching-Liang Hsieh [b, d, e, *]

[a] Department of Neurology, China Medical University Hospital, Taichung, Taiwan
[b] Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan
[c] Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan
[d] Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan
[e] Chinese Medicine Research Center, China Medical University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 3 March 2023
Received in revised form
22 August 2023
Accepted 15 September 2023
Available online 20 September 2023

Keywords:
Electroacupuncture
Ischemic stroke
Electrode configuration
Neuroprotection

ABSTRACT

Background and aim: This study investigated the effect of the electrode configuration on EA treating ischemic stroke.
Experimental procedure: An ischemic stroke rat model was established. In the EA-P group, the anodes of EA were placed on the BL7 and BL8 acupoints of the lesioned, and the cathodes were placed on the BL7 and BL8 acupoints of the nonlesioned hemispheres; by contrast, in the EA-N group.
Results: The difference in neurological deficit scores between the first and fourth days and the difference in Rotarod test time between the fourth and first days after reperfusion were greater in the EA-P and EA-N groups than in the sham group (all $p < 0.001$). In the lesioned hemisphere, neuronal nuclei (NeuN), γ-aminobutyric acid-A (GABA)-A, postsynaptic density 95 (PSD95), and astrocyte glutamate transporter 1 (GLT-1) expression and microtubule-associated protein 2 (MAP2)/glyceraldehyde 3-phosphate dehydrogenase (GADPH) ratios were greater and the glial fibrillary acid protein (GFAP)/GADPH ratios were smaller in the EA-P than in the sham group (all $p < 0.05$), but these ratios in the EA-N group were similar to those in the sham group (all $p > 0.05$); serum adrenaline and serotonin levels in the sham group were lower than those in the normal and EA-P groups (both $p < 0.05$), and cerebrospinal fluid (CSF) glutamate levels were higher in the EA-P group than in the sham group ($p < 0.05$).
Conclusion: EA improved neurological function through multiple pathways. However, placing the anode on the lesioned hemisphere can provide more neuroprotection.

1. Introduction

In 2016, approximately 13.7 million people experienced stroke worldwide, and stroke is the second leading cause of disability and death.[1] According to a report, the incidence of stroke increased between 2001 and 2013 in Taiwan in contrast to a decline in Western countries owing to the more efficient control of risk factors.[2] According to the National Health Insurance Research Database in Taiwan, the incidence rate of first-ever ischemic stroke ranges from 142.3 to 129.5 per 100,000 population in people aged ≥18 years.[3] The treatments in patients with acute ischemic stroke are reperfusion therapy, involving the administration of intravenous recombinant tissue plasminogen activator (IV TPA), within 4.5 h or endovascular thrombectomy (EVT) within 24 h after stroke onset. However, IV TPA treatment increases the risk of cerebral hemorrhage and has constraints in terms of time and conditions. EVT limits the anterior circulation of internal carotid and proximal middle cerebral arterial occlusions.[4] Thus, a more effective and safe treatment strategy for ischemic stroke is urgently required.

Interhemispheric inhibition in the brain plays a dynamic role. After stroke, the inhibition from the lesioned hemisphere and the excitability of the region around the infarction decrease, resulting in stronger inhibition from the nonlesioned hemisphere around the infarction zone. The regulation of the excitability of the region

* Corresponding author. Graduate Institute of Acupuncture Science, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan.
E-mail address: clhsieh0826@gmail.com (C.-L. Hsieh).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

https://doi.org/10.1016/j.jtcme.2023.09.003

Abbreviations

BL7	Tongtian
BL8	Luoque
CMU	China Medical University
CSF	cerebrospinal fluid
D1	first day after reperfusion
D4	fourth day after reperfusion
DAPK1	death-associated protein kinase1
DTT	dithiothreitol
EA	electroacupuncture
EA-P	EA anode group
EA-N	EA cathode group
ELC	electrochemiluminescence
EVT	endovascular thrombectomy
GABA-A	γ-aminobutyric acid-A
GADPH	glyceraldehyde 3-phosphate dehydrogenase
GFAP	glial fibrillary acid protein
GLT-1	astrocyte glutamate transporter 1
GV14	Dazhui
GV20	Baihui
IL-10	interleukin-10
lLC/ESI-MS/MS	liquid chromatography–electrospray ionization –tandem mass spectrometry
IV TPA	intravenous recombinant tissue plasminogen activator
MAP2	microtubule-associated protein 2
MCAo	middle cerebral artery occlusion
MRM	multiple reaction monitoring
NC	nitrocellulose
NeuN	neuronal nuclei
NMDARs	*N*-methyl-D-aspartic acid receptors
nNOS	neuronal nitric oxide synthase
PAGE	polyacrylamide
PSD95	postsynaptic density 95
SD	Sprague Dawley
SDS	sodium dodecyl sulphate
TDCS	Transcranial direct current stimulation
TRPV1	transient receptor potential cation channel subfamily V member 1
TTC	2,3,5-triphenyltetrazolium chloride
UPLC	ultra-performance liquid chromatography

around the infarction zone is a target in the treatment of stroke[5] Transcranial direct current stimulation (TDCS) uses a mobile battery-operated direct current stimulator connects to two electrodes. An active electrode is placed on the C3 of scalp, and the other electrode is placed on the contralateral supraorbital region service as a reference 6. Electroacupuncture (EA) uses an electrostimulator operated mild electrical current to two acupuncture needles inserted into acupoint.[7] A study that employed TDCS for stroke treatment determined that anodal stimulation increased the excitability of the cerebral cortex, whereas cathodal stimulation reduced excitability.[8] Several studies have found that cortical stimulation in rats with cerebral ischemia increases the density of microtubule-associated protein2 (MAP2) around the lesion and the activity of dendritic processes.[9] γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter that regulates motor cortical plasticity, and it plays a crucial role in poststroke recovery.[10] Studies have found reduced GABA levels in the periphery of the infarction area, indicating the inhibition of GABA generation from distant brain zones after stroke.[11] Glutamate is an excitatory neurotransmitter released after cerebral ischemia. The excess extracellular glutamate leads to the overactivation of glutamate receptors, especially *N*-methyl-D-aspartic acid receptors (NMDARs), and induces excitotoxicity.[12] Although NMDARs play a central role in ischemic excitotoxic neuronal death, NMDAR channel blockers have not been successfully translated to clinical stroke therapy. Recent studies have identified important NMDAR-associated signaling complexes that are linked to death-signaling pathways, and the inhibition of these pathways does not need to block NMDARs. NMDARs play a dual role in cell survival and death, and activated NMDARs can send prosurvival or prodeath signals depending on their subcellular location or subtype.[13] Cerebral ischemic injury can promote the binding of death-associated protein kinase1 (DAPK1) and NMDAR, and disrupting this binding can reduce brain damage.[14] DAPK1 and NMDAR NR2B interact at extrasynaptic sites, and this interaction promotes brain damage.[15] Based on the aforementioned findings, increasing the GABA concentration around the infarction area and disrupting the binding between DAPK1 and NMDAR NR2B are beneficial for the treatment of ischemic stroke.

Our previous studies have demonstrated that EA at Baihui (GV20) and Dazhui (GV14) can reduce the infarction volume and neurological deficit in rats with transient middle cerebral artery occlusion (MCAo). This effect of EA is consistent with an increase in brain-derived neurotrophic factor and a decrease in S100B levels.[16,17] EA at Baihui can also reverse the reduction of long-term potentiation induced by transient MCAo in rats and reduce the NR1 and transient receptor potential cation channel subfamily V member 1 (TRPV1) receptors in the hippocampus.[18] Our study revealed that EA at Luoque (BL8, anode) and Tongtian (BL7, cathode) in the nonlesioned hemisphere can reduce the infarction volume, improve neurological status, and increase GABA-A levels around the infarction brain region in rats with transient MCAo.[19] Therefore, the present study investigated the effect of the electrode configuration in EA on the treatment of ischemic stroke. We compared the effects between the placement of anodes and cathodes of EA in lesioned and nonlesioned hemispheres in rats with ischemic stroke.

2. Materials and methods

2.1. Animals

Male Sprague Dawley (SD) rats, weighing 250–350 g, were purchased from BioLASCO Taiwan Co. Ltd. and were bred at the Animal Center of China Medical University (CMU). The rats were reared in a 12/12-h light–dark environment. Room temperature was maintained at 20°C-24 °C through air conditioning, humidity was maintained between 50% and 70%, and adequate food and drinking water were given. The design of the experimental protocol complied with the regulations of the Animal Experiment Ethics Committee of CMU, and the procedures were conducted in a manner that minimized pain or discomfort in the animals. The protocol was approved by the Experimental Animal Committee of CMU (CMUIACUC-2021-076).

2.2. Establishment of the ischemic stroke rat model

The ischemic stroke rat model was established according to our previous study.[19] First, the rats were anesthetized with 2% isoflurane gas, and an incision was made from the midline of the rat's neck to expose the right internal carotid artery, external carotid

artery, and common carotid artery. Then, ligation was performed from the starting point where the pterygoid artery separates from the maxillary artery, and the right internal carotid artery and common carotid artery were clamped at an appropriate location by using arterial clips. The external carotid artery was ligated permanently, and a small opening was made near the common carotid artery by using a pair of scissors, and the wound was then sutured using a 3/0 nylon thread with the tip smoothed at high temperature and the surface coated with poly-L-lysine. The nylon thread was passed from the external carotid artery up to the internal carotid artery through the common carotid artery and was moved approximately 23–25 mm to the orifice of the MCA to block the blood flow. The nylon thread was then fixed with an arterial clip, and after 30 min, the thread was slowly withdrawn to allow gradual reperfusion of blood. After the neck and head wounds of the rat were sutured, the rat was placed back into an iron cage until it woke up.

The neurological deficit score of the rats was measured 24 h after reperfusion. Overall, 36 rats with ischemic stroke with neurological deficit scores of 7–8 were randomly divided into three groups, namely the sham, EA-P, and EA-N groups, with 12 rats in each group. In addition, 12 rats without ischemic stroke were categorized as the normal group. The grouping of the rats referenced previous study[20] described as follows:

2.3. Animal grouping

1. Normal group: Incisions were made from the midline of the neck of the rats to expose the common carotid artery, and the wound was sutured after 30 min. After the neurological deficit score and Rotarod test time were evaluated 24 h later, the rats were anesthetized for another 20 min for 3 consecutive days. On the fourth day, the neurological deficit score and Rotarod test time were evaluated again. Thereafter, the cerebrospinal fluid (CSF) was collected from the cistern magna, and 5 cc of blood was obtained from the heart under isoflurane gas anesthesia. Finally, the rats were sacrificed, and their brains were removed. The brain tissues of six rats were stained with 2,3,5-triphenyltetrazolium chloride (TTC) to calculate the infarction volume, and the brain tissues of other six rats were subjected to Western blot analysis.
2. Sham group: After 24 h of reperfusion, the neurological deficit score and Rotarod test time were evaluated. Four stainless steel acupuncture needles were inserted into the ipsilateral side of the cerebral infarction (lesioned hemisphere) and the opposite side (nonlesioned hemisphere). The side is equivalent to the subcutaneous area of the Tongtian (BL7) and Luoque (BL8) acupoints on the human head. The needles were connected to the EA machine (with the anode on the lesioned hemisphere and the cathode on the nonlesioned hemisphere), but no electrical stimulation was performed, once a day, 20 min each time, for 3 consecutive days, after which, the neurological deficit score and Rotarod test time were evaluated again on the fourth day after reperfusion. The CSF was then collected from the cistern magna, and 5 cc of blood was obtained from the heart under isoflurane gas anesthesia. Finally, the rats were sacrificed, and their brains were removed. The brain tissues of six rats were stained with TTC to calculate the infarction volume, and the brain tissues of other six rats were subjected to Western blot analysis.
3. EA anode group (EA-P): The stimulation method was the same as that in the sham group, except that four stainless steel acupuncture needles were inserted into the ipsilateral and opposite sides of the lesioned hemisphere. The needles were connected to the EA machine (Trio 300, Japan; modulation wave; 150 μs in width of wave) to conduct 15-Hz electrical stimulation that was based on our pilot study. The stimulation intensity is mainly based on slight muscle contraction.
4. EA cathode group (EA-N): The stimulation method was the same as that in the EA-P group, except that the anode was placed on the nonlesioned-hemisphere, whereas the cathode was placed on the lesioned hemisphere.

2.4. Assessment of neurological status

(1) Neurological deficit scores

Neurological deficit was assessed according to the modified neurological severity score[21] and the method described in our previous study.[19] In summary, the modified neurological severity score includes motor function scored from 0 to 6, sensory function scored from 0 to 2, balance function scored from 0 to 6, and reflex function scored from 0 to 4, with a maximum neurological deficit score of 18.

(2) Rotarod test

In this test, first, the condition was set, and the rat was placed on a Rotamex roller treadmill (Columbus Instruments, OH, USA), with the shaft of the roller treadmill rotating at 4 revolutions per minute (rpm); then, the buckle was pressed to start the test. The shaft speed increased by 1 rpm every 8 s from the initial speed of 4 rpm and gradually increased to a maximum of 40 rpm until the rat fell off the shaft. Once the rat fell, the machine automatically stopped and displayed the running time of the rat. In this study, the same method was used to repeat the Rotarod test 5 times, and the best value of the 3 times was used to average, which is the value of the Rotarod test.

2.5. Evaluation of cerebral infarct size: TTC staining

The method of evaluation of the cerebral infarct size was similar to that described previously.[19] In summary, using scissors, incisions were made from the abdominal cavity to the chest of the rats under 2% isoflurane gas anesthesia, and a small hole was cut in the right atrium. Normal saline was rapidly perfused into the left ventricle to wash the blood in the brain tissue. The brain was then removed and placed in a plastic model of the rat brain, and the rat brain was sliced into six 2-mm-thick section from the frontal pole. The slices were stained with TTC (Merck, Germany) for 15 min; the infarction region was visible in white color, whereas the noninfarction area were observed as purple-red stained regions. The cerebral infarction size was calculated using a microscopic image-analysis system (Image-Pro Lito Version 3.0, Media Cybernetics, USA). The ratio of the infarction volume to the total brain volume was measured for each slice, and data are presented as percentages (%).

2.6. Western blot analysis

The protocol for Western blot analysis in this study was similar to that in our previous study.[19] First, 2.5 × tissue weight lysis buffer (50 mM Tris-HCl, 0.5% Triton X-100, and 1 × protease inhibitor) was added to the brain tissue (penumbra region of infraction), and the mixture was sonicated and centrifuged at 15,000 rpm and 4 °C for 10 min. The supernatant was then collected and stored at −80 °C.

Tissue extracts were obtained using sodium dodecyl sulphate (SDS) sample buffer (62.5 mM Tris-HCl at pH 6.8, 2% SDS, 10% glycerol, 50 mM dithiothreitol [DTT], and 0.1% bromophenol blue). Thereafter, 10% SDS-polyacrylamide (PAGE) analysis was

conducted, and 20 μg of protein was obtained and transferred to a nitrocellulose (NC) membrane.

The NC membrane was blocked using 5% skimmed milk for 1 h, and primary interleukin-10 (IL-10; Thermo, 1:1000), glial fibrillary acid protein (GFAP); Calbiochem, 1:1000), neuronal nuclei (NeuN; Millipore, 1:1000), astrocyte glutamate transprer-1 (GLT-1; Abcam, 1:1000), postsynaptic density protein95 (PSD95; Abcam, 1:1000), γ-aminobutyric acid A (GABA-A; Millipore, 1:1000) receptor, MAP2 (Abcam, 1:1000), DAPK (Proteintech, 1:1000), and integrin-β1 (Abcam, 1:1000) antibodies were then added. The samples were then incubated overnight at 4 °C, and secondary antibodies were added. The mixture was incubated at room temperature for 1 h. Finally, the microfluidic electrochemiluminescence (ECL) color rendering system (Amersham) was applied for color development, and the film was exposed and developed under cold light. AlphaEaseFC software was used in the present study to calculate the integrated density value, and the result was divided by the value for actin to obtain the value of Western blot data.

2.7. Liquid chromatography–electrospray ionization–tandem mass spectrometry analysis for the measurement of neurotransmitters

Blood was collected from the rat heart and transferred to a 1.5-mL microtube and incubated at room temperature for 30 min and then at 4 °C for 2 h. The serum was collected after blood coagulation and centrifugation.

Before analysis, 30 μL of serum or CSF samples were completely mixed with 120 μL of 100% methanol through vigorous vortexing. The sample–methanol mixture was centrifuged at 13,000×*g* for 10 min, and 120 μL of the supernatant was collected and dried in a vacuum concentrator. The dried samples were dissolved in 50 μL of ultrapure water, and 45 μL of the supernatant was collected after centrifugation at 13,000×*g* for 10 min and subjected to liquid chromatography–electrospray ionization–tandem mass spectrometry (LC/ESI-MS/MS) analysis.

The LC/ESI-MS/MS analysis was established by referring to previous study[22] performed using the Xevo TQ-XS system (Waters, Miliford, MA, USA). The samples were resolved through reverse-phase ultra-performance liquid chromatography (UPLC) on the Acquity UPLC BEH C18 1.7-μm column (2.1 mm × 50 mm; Waters) at 30 °C. Elution was started using 99% mobile phase A (0.1% HCOOH in ultrapure water) and 1% mobile phase B (0.1% HCOOH in methanol), held at 1% B for 0.5 min, increased to 90% B in 2.5 min, held at 90% B for 0.5 min, and then decreased to 1% B in 0.5 min. The column was equilibrated by pumping 1% B for 2 min. The flow rate was set at 0.2 mL/min, and the injection volume was 7.5 μL. Mass spectra and chromatograms were acquired in the ES+ with the multiple reaction monitoring (MRM) mode and processed using Mass Lynx software (Waters). Neurotransmitter signals in each sample were identified and quantified by summarizing the LC peak area of the corresponding ion mass transitions and retention times for each target.

2.8. Statistical analysis

All values are presented as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance followed by a post hoc Tukey test. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Effect of EA on the neurological state of rats with ischemic stroke

The neurological deficit score on the first day (D1) after reperfusion in the normal group was lower than those in the sham, EA-P, and EA-N groups (all $p < 0.001$; Table 1); however, no significant differences were observed in the neurological deficit scores among the sham, EA-P, and EA-N groups (all $p > 0.05$; Table 1). The difference in the neurological deficit scores between D1 and the fourth day (D4) after reperfusion in the normal group was smaller than those in the EA-P and EA-N groups (both $p < 0.001$) but similar to that in the sham group ($p > 0.05$). The difference in the neurological deficit score between D1 and D4 in the sham group was smaller than those in the EA-P and EA-N groups (both $p < 0.001$; Table 1), but the difference was not significant between the EA-P and EA-N groups ($p > 0.05$; Table 1).

The Rotarod test time on D1 after reperfusion was more in the normal group than in the sham, EA-P, and EA-N groups (all $p < 0.001$; Table 1), but it was not significantly different among the sham, EA-P, and EA-N groups (all $p > 0.05$; Table 1). The difference in the Rotarod test time between D4 and D1 was greater in the EA-P and EA-N groups than in the normal and sham groups (all $p < 0.001$; Table 1), whereas the difference in the Rotarod test time between D4 and D1 was similar between the normal and sham groups and between the EA-P and EA-N groups (both $p > 0.05$; Table 1).

3.2. Effect of EA on infarction volume in rats with ischemic stroke

The ratio of the infarction volume in the rats with ischemic stroke in the normal group was lower than that in the sham ($p < 0.01$; Fig. 1A and B), EA-P ($p < 0.05$; Fig. 1A and B), and EA-N ($p < 0.01$; Fig. 1A and B) groups; the ratio of the infarction volume in the sham group was higher than that in the EA-P group ($p < 0.001$; Fig. 1A and B) and the EA-N group ($p < 0.01$; Fig. 1A and B), whereas the ratio of the infarction volume was similar between the EA-P and EA-N groups ($p > 0.05$; Fig. 1A and B).

3.3. Effect of EA on GLT-1, IL-10, GABA-A, NeuN, PSD95, GFAP, integrin-β1, MAP2, and DAPK1 expression in rats with ischemic stroke: Western blot analysis

1. Right cerebral hemisphere (lesioned hemisphere)

The GLT-1/glyceraldehyde 3-phosphate dehydrogenase (GAPDH) ratio in the normal group was higher than that in the sham group ($p < 0.001$: Figs. 2 and 3) but was similar to those in the EA-P and EA-N groups (both $p > 0.05$; Figs. 2 and 3). In addition, the GLT-1/GAPDH ratio in the EA-P group was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3) but similar to that in the EA-N group ($p > 0.05$; Figs. 2 and 3). Furthermore, the GLT-1/GAPDH ratio was similar between the EA-N and the sham groups ($p > 0.05$; Figs. 2 and 3).

The Iinterleukin-10 (IL-10)/GAPDH ratio in the normal group was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3) but was similar to those in the EA-P and EA-N groups (both $p > 0.05$; Figs. 2 and 3). However, the IL-10/GAPDH ratio did not significantly differ among the sham, EA-P, and EA-N groups (all $p > 0.05$; Figs. 2 and 3).

The GABA-A/GAPDH ratio in the normal group was higher than those in the sham and EA-N groups ($p < 0.001$ and < 0.05,

Table 1
Effect of electroacupuncture on the neurological state of rats with ischemic stroke.

	Normal	Sham	EA-P	EA-N
Neurological deficit score (score)				
D1	0.00 ± 0.00	7.25 ± 0.45***	7.17 ± 0.39***	7.17 ± 0.39***
D4	0.00 ± 0.00	7.08 ± 0.29***	4.25 ± 0.75***###	4.67 ± 0.49***###
D1-D4	0.00 ± 0.00	0.17 ± 0.39	2.92 ± 0.67***###	2.50 ± 0.52***###
Rotarod test time (second)				
D1	98.60 ± 8.00	20.54 ± 6.02***	23.54 ± 5.50***	23.27 ± 6.61***
D4	109.46 ± 14.69	29.72 ± 7.67***	74.84 ± 4.71***###	66.51 ± 15.61***###
D4-D1	10.85 ± 13.45	9.18 ± 8.79	51.3 ± 3.95***###	43.24 ± 15.84***###

Data are presented as mean ± standard deviation. Normal: normal group; sham: sham group; EA-P: EA-P group; EA-N: EA-N group; D1: first day after reperfusion; D4: fourth day after reperfusion; D1-D4: the difference in score between the first day and fourth day after reperfusion; D4-D1: the difference in time between the fourth day and first day after reperfusion ***$p < 0.001$ compared with normal; ###$p < 0.001$ compared with sham; n = 12.

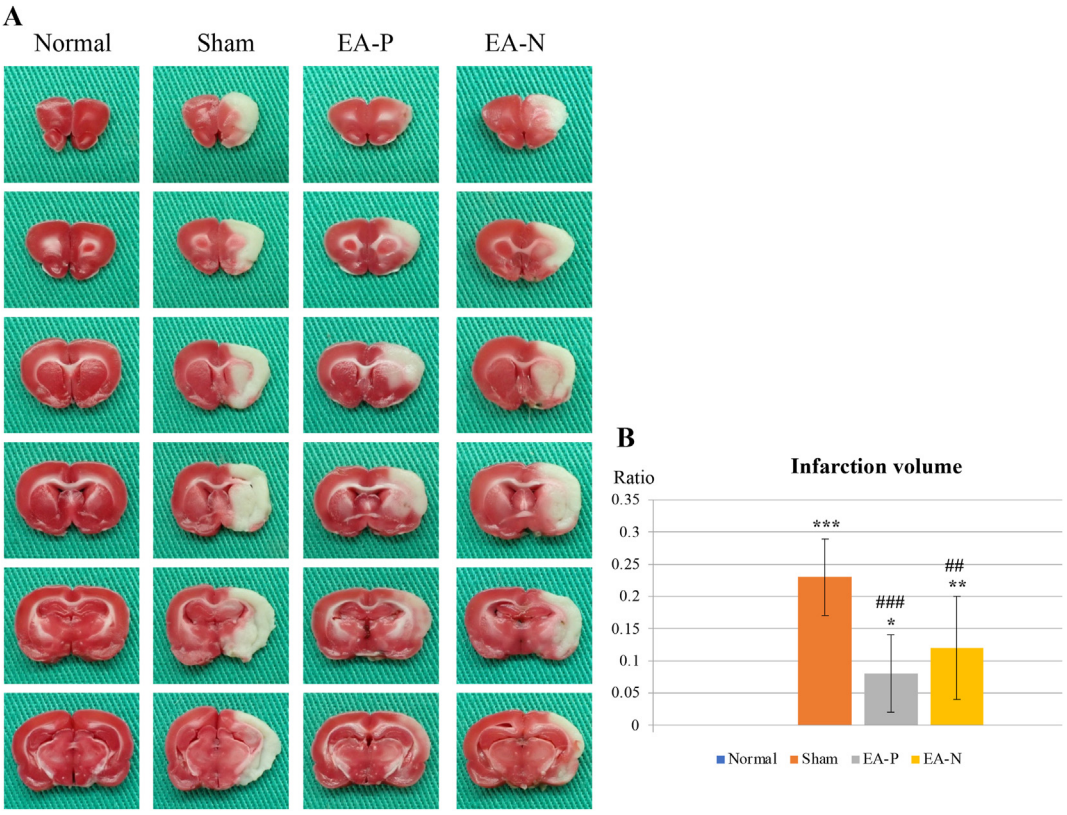

Fig. 1. Effect of electroacupuncture on infarction volume in rats with ischemic stroke. Normal: normal group; Sham: sham group; EA-P: EA-P group, EA-N: EA-N group; red-purple color: normal brain tissue; white color: cerebral infarction area. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with normal; ##$p < 0.01$ and ###$p < 0.001$ compared with sham; n = 6.

respectively; Figs. 2 and 3) but was similar to that in the EA-P group ($p > 0.05$; Figs. 2 and 3). Moreover, the GABA-A/GAPDH ratio in the EA-P group was higher than that in the sham group ($p < 0.001$; Figs. 2 and 3) but was similar to that in the EA-N group ($p > 0.05$; Figs. 2 and 3). Furthermore, the GABA-A/GAPDH ratio in the EA-N group was similar to that in the sham group ($p > 0.05$; Figs. 2 and 3).

The NeuN/GAPDH ratio in the normal group was higher than that in the sham group ($p < 0.01$; Figs. 2 and 3) but was similar to those in the EA-P and EA-N groups (both $p > 0.05$; Figs. 2 and 3). The NeuN/GAPDH ratio in the EA-P group was similar to that in the EA-N group ($p > 0.05$) but was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3); the NeuN/GAPDH ratio in the sham group was similar to that in the EA-N group ($p > 0.05$; Figs. 2 and 3).

The PSD95/GAPDH ratio in the normal group was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3) but was similar to those in the EA-P and EA-N groups (both $p > 0.05$; Figs. 2 and 3). Moreover, the PSD95/GAPDH ratio in the EA-P group was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3) but was similar to that in the EA-N group ($p > 0.05$; Figs. 2 and 3). Furthermore, the PSD95/GAPDH ratio in the EA-N group was similar to that in the sham group ($p > 0.05$; Figs. 2 and 3).

The GFAP/GAPDH ratio in the normal group was lower than those in the sham and EA-N groups ($p < 0.001$ and < 0.01, respectively; Figs. 2 and 3) but was similar to that in the EA-P group ($p > 0.05$; Figs. 2 and 3). Moreover, the GFAP/GAPDH ratio in the EA-P group was lower than that in the sham group ($p < 0.001$; Figs. 2 and 3). Furthermore, the GFAP/GAPDH ratio in the EA-N group was similar to that in the sham group ($p > 0.05$; Figs. 2 and 3) as well as similar to that in the EA-P group ($p > 0.05$; Figs. 2 and 3).

The integrin-β1/GAPDH ratio in the normal group was higher than those in the sham and EA-N groups ($p < 0.05$ and < 0.01, respectively; Figs. 2 and 3) but similar to that in the EA-P group

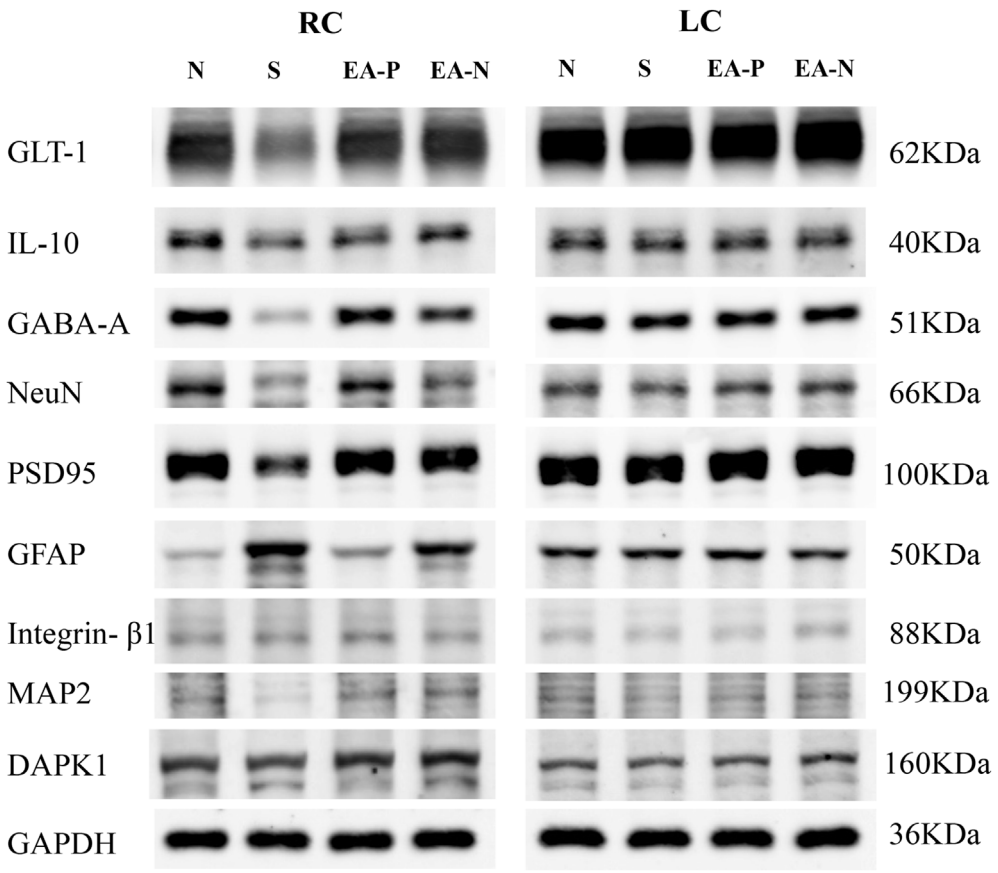

Fig. 2. Effect of electroacupuncture on levels of astrocyte glutamate transporter-1(GLT-1), interleukin-10 (IL-10), γ-aminobutyric acid-A (GABA-A), NeuN, postsynaptic density95 (PSD95), glial fibrillary acid protein (GFAP), Integrin-β1, microtubule-associated protein2 (MAP2), and death-associated protein kinase1 (DAPK1) in rats with ischemic stroke: Western blot analysis. RC: right cerebral hemisphere; LC: left cerebral hemisphere; N: normal group; S: sham group; EA-P: EA-P group; EA-N: EA-N group; n = 6.

($p > 0.05$; Figs. 2 and 3). Moreover, the integrin-β1/GAPDH ratio was similar among the sham, EA-P, and EA-N groups (all $p > 0.05$; Figs. 2 and 3).

The MAP2/GAPDH ratio in the normal group was higher than those in the sham and EA-N groups ($p < 0.01$ and < 0.05, respectively; Figs. 2 and 3) but similar to that in the EA-P group ($p > 0.05$; Figs. 2 and 3); the MAP2/GAPDH ratio in the EA-P group was higher than that in the sham group ($p < 0.05$; Figs. 2 and 3) but similar to that in the EA-N group ($p > 0.05$; Figs. 2 and 3).

The DAPK1/GAPDH ratio was not significantly different among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Figs. 2 and 3).

2 Left cerebral hemisphere (nonlesioned hemisphere)

GLT-1, IL-10, GABA-A, NeuN, PSD95, GFAP, integrin-β1, and MAP2 expression and DAPK1/GAPDH ratios were not significantly different among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Figs. 2 and 4).

3.4. Effect of EA on neurotransmitter levels in the serum and CSF of rats with ischemic stroke

1. Serum neurotransmitter levels

Serum dopamine levels were not significantly different among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 5).

Serum adrenaline levels were higher in the normal group than in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-P and EA-N groups (both $p > 0.05$; Fig. 5). Moreover, serum adrenaline levels in the EA-P group were higher than those in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-N group ($p > 0.05$; Fig. 5). Furthermore, serum adrenaline levels were similar between the sham and EA-N groups ($p > 0.05$; Fig. 5).

Serum noradrenaline levels in the normal group were similar to those in the sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 5). Serum noradrenaline levels were higher in the EA-P group than in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-N group ($p > 0.05$; Fig. 5). Furthermore, serum noradrenaline levels in the sham group were similar to those in the EA-N group ($p > 0.05$; Fig. 5).

Serum glutamate levels were not significantly different among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 5).

Serum GABA levels did not significantly differ among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 5).

Serum serotonin levels were higher in the normal group than in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-P and EA-N groups (both $p > 0.05$; Fig. 5). Moreover, serum serotonin levels in the EA-P group were higher than those in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-N group ($p > 0.05$; Fig. 5). Furthermore, serum serotonin levels were similar between the sham and EA-N groups ($p > 0.05$; Fig. 5).

2 CSF neurotransmitter levels

The CSF dopamine, adrenaline, noradrenaline, GABA, and

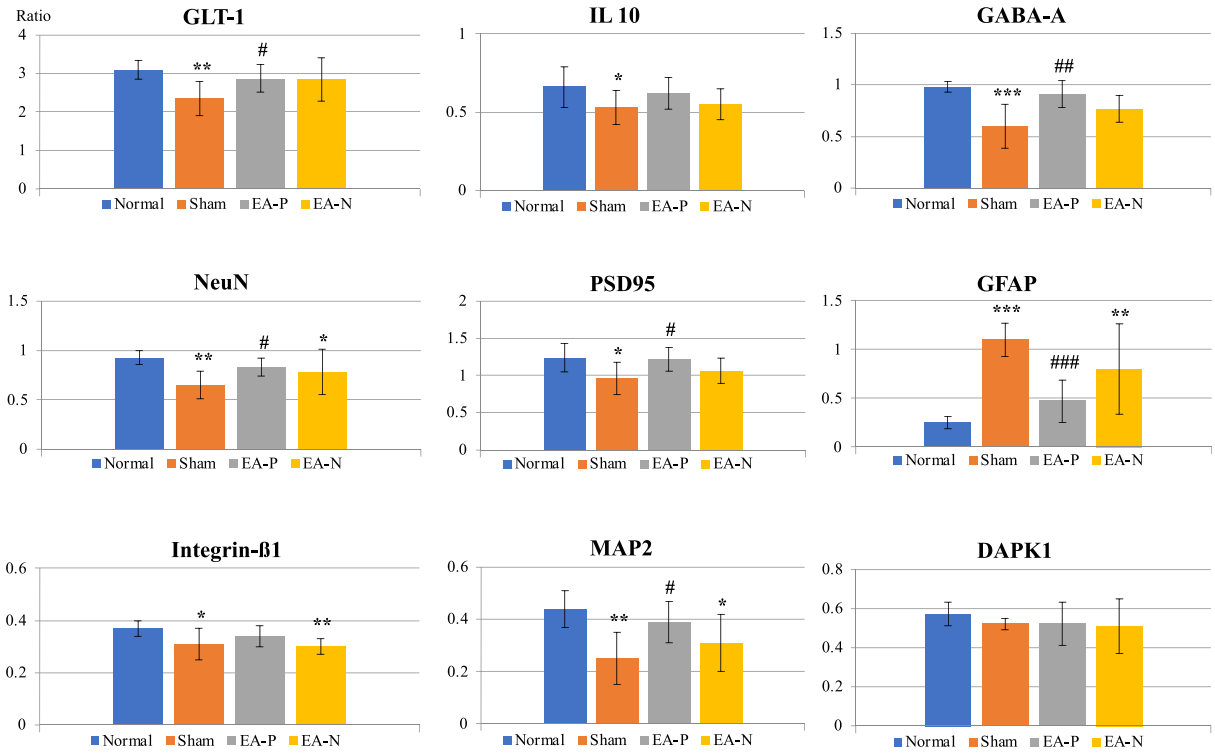

Fig. 3. Effect of electroacupuncture on levels of astrocyte glutamate transporter-1(GLT-1), interleukin-10 (IL-10), γ-aminobutyric acid-A (GABA-A), NeuN, postsynaptic density95 (PSD95), glial fibrillary acid protein (GFAP), Integrin-β1, microtubule-associated protein2 (MAP2), and death-associated protein kinase1 (DAPK1) in rats with ischemic stroke: right cerebral hemisphere. Normal: normal group; sham: sham group; EA-P: EA-P group; EA-N: EA-N group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with normal; #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ compared with sham; n = 6.

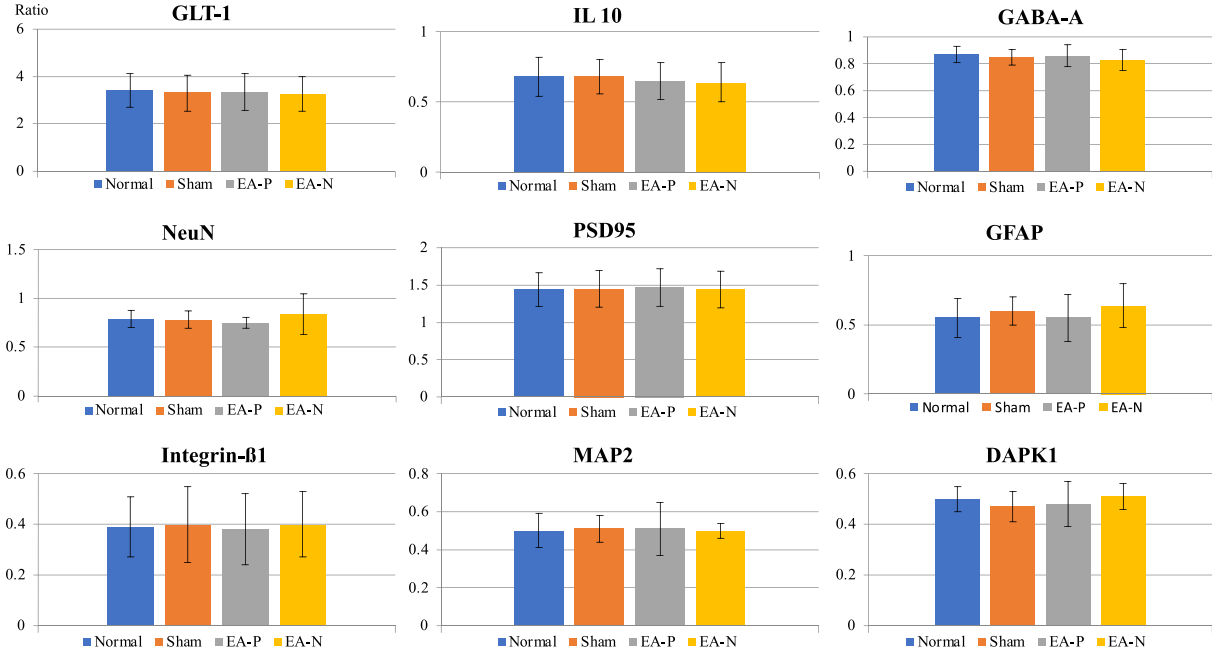

Fig. 4. Effect of electroacupuncture on levels of astrocyte glutamate transporter-1(GLT-1), interleukin-10 (IL-10), γ-aminobutyric acid-A (GABA-A), NeuN, postsynaptic density95 (PSD95), glial fibrillary acid protein (GFAP), Integrin-β1, microtubule-associated protein2 (MAP2), and death-associated protein kinase1 (DAPK1) in rats with ischemic stroke: left cerebral hemisphere. Normal: normal group; sham: sham group; EA-P: EA-P group; EA-N: EA-N group; n = 6.

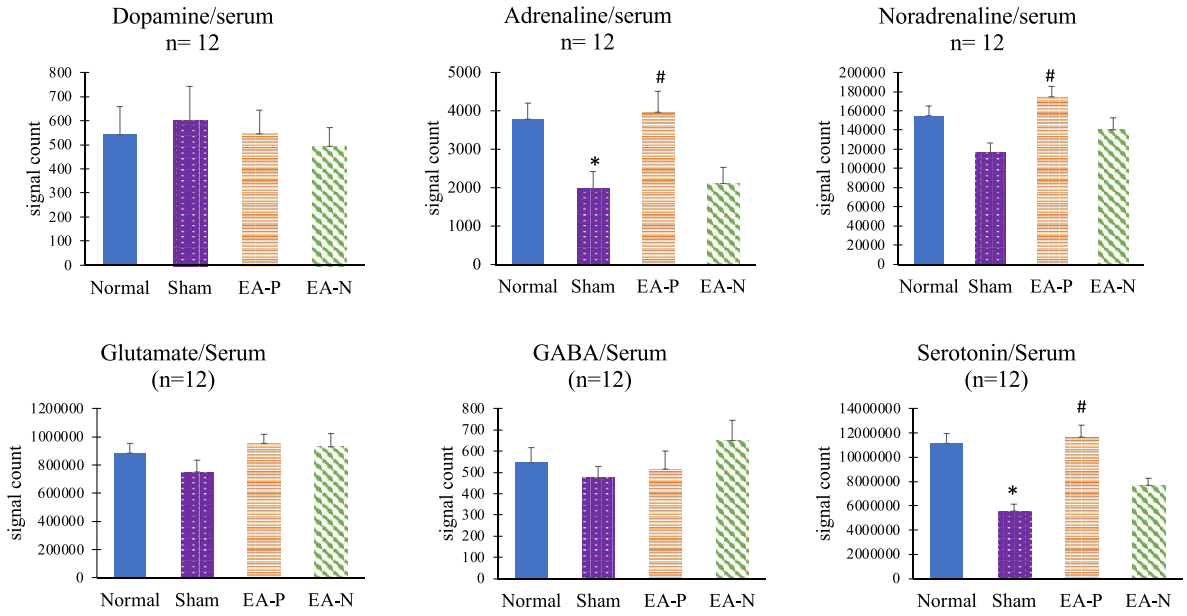

Fig. 5. Effect of electroacupuncture on serum dopamine, adrenaline, noradrenaline, glutamate, γ-aminobutyric acid (GABA), and serotonin in rats with ischemic stroke: metabolomic analysis. Normal: normal group; sham: sham group; EA-P: EA-P group; EA-N: EA-N group; *$p < 0.05$ compared with normal; #$p < 0.05$ compared with sham, n = 12.

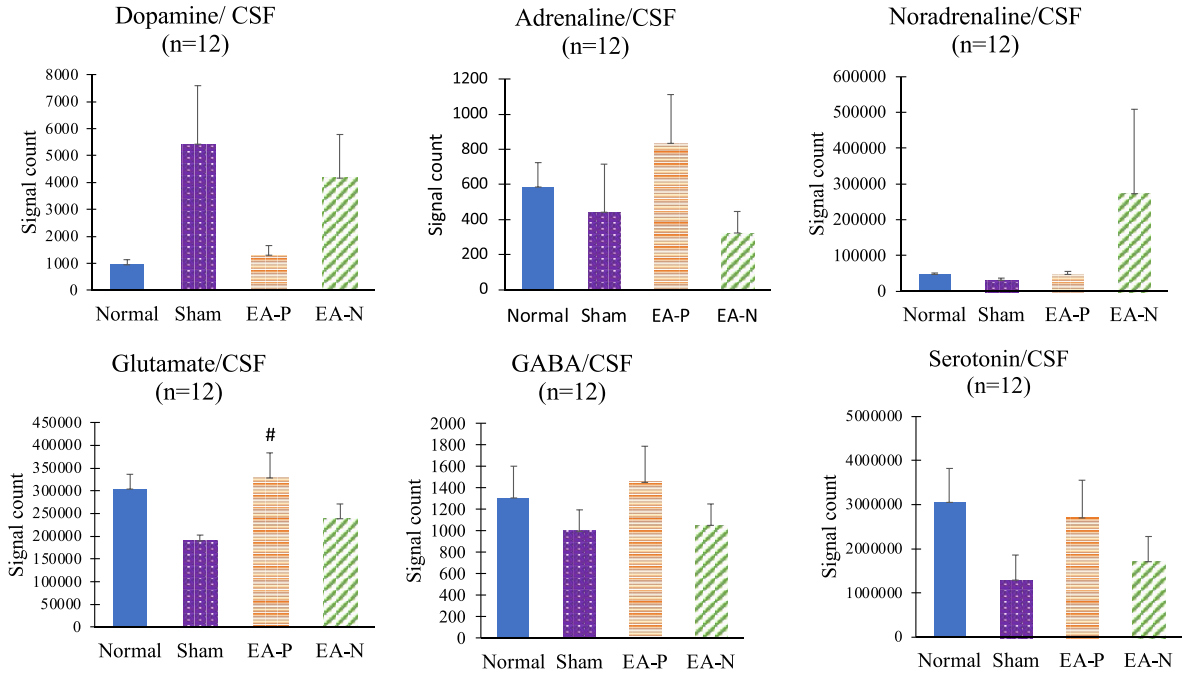

Fig. 6. Effect of electroacupuncture on cerebrospinal fluid (CSF) dopamine, adrenaline, noradrenaline, glutamate, γ-aminobutyric acid (GABA), and serotonin in rats with ischemic stroke: metabolomic analysis. Normal: normal group; sham: sham group; EA-P: EA-P group; EA-N: EA-N group; #$p < 0.05$ compared with sham, n = 12.

serotonin levels were not significantly different among the normal, sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 6).

The CSF glutamate levels in the normal group were similar to those in the sham, EA-P, and EA-N groups (all $p > 0.05$; Fig. 5); the CSF glutamate levels in the EA-P group were higher than those in the sham group ($p < 0.05$; Fig. 5) but similar to those in the EA-N group ($p > 0.05$; Fig. 5); and the CSF glutamate levels in the sham group were similar to those in the EA-N groups ($p > 0.05$; Fig. 5).

The above results were summarized in Table 2.

4. Discussion

The results of the present study revealed that the neurological deficit score decreased and the Rotarod test time increased in both the EA-P and EA-N groups. Moreover, the ratio of the cerebral infarction volume decreased in the rats with ischemic stroke in

Table 2
Effect of electroacupuncture on brain tissue, serum and cerebrospinal fluid in rats with ischemic stroke.

item	EA-P	EA-N
Right cortex		
GLT-1	↑	—
IL-10	—	—
GABA-A	↑	—
NeuN	↑	—
PSD95	↑	—
GFAP	↓	—
Integrin-β1	—	—
MAP2	↑	—
DAPK1	—	—
Serum		
Dopamine	—	—
Adrenaline	↑	—
Noradrenaline	↑	—
Glutamate	—	—
GABA	—	—
Serotonin	↑	—
Cerebrospinal fluid		
Dopamine	—	—
Adrenaline	—	—
Noradrenaline	—	—
Glutamate	↑	—
GABA	—	—
Serotonin	—	—

EA-P: EA-P group; EA-N: EA-N group; ↑represented increase, ↓represented decrease, represented —no significant difference, the value compared with the value of sham group without electrical stimulation; DAPK1: death-associated protein kinase1; GABA-A: γ-Aminobutyric acid-A; GFAP: glial fibrillary acid protein. GLT-1: astrocyte glutamate transprer-1; IL-10: interleukin-10; MAP2: microtubule-associated protein2; NeuN: neuronal nuclei; PSD95: postsynaptic density protein95.

both the groups. These results suggest either the anode placed on the lesioned and cathode placed on the nonlesioned hemispheres in EA, or anode placed on the nonlesioned and cathode on the lesioned hemispheres plays a neuroprotective role in rats with ischemic stroke. Our results were partly different from anodal stimulation increased the excitability of the cerebral cortex, whereas cathodal stimulation reduced excitability in the TDCS for stroke treatment.[6,8] Therefore, suggesting there are at least some differences in the mechanism of treating stroke between EA and TDCS. Our results also indicated that the expression of NeuN increased and that of GFAP decreased in the lesioned hemisphere (right hemisphere) in the EA-P group, but not in the EA-N group. NeuN is a sensitive and specific neuronal cell biomarker,[23] whereas GFAP is a biomarker of astrocyte activation, and GFAP levels in the serum and CSF can be used as an indicator of neurodegeneration.[24,25] Taken together, the results indicate the placement of the anode in EA on the lesioned hemisphere can protect against and reduce neuronal cell damage in rats with ischemic stroke.

The results of the present study also revealed that the expression of GABA-A, GLT-1, PSD95, and MAP2 increased in the EA-P group, but not in the EA-N group, in the lesioned hemisphere in the rats with ischemic stroke. The release of glutamate and the activation of NMDAR lead to Ca^{2+} overload, which results in neuronal cell death after cerebral ischemia. In response to this overload and to inhibit the excitotoxic effect of glutamate, GABA levels are increased.[11,26] A study reported that GABA levels decreased around the infarction brain region in the early stage after stroke, possibly to reduce the excitotoxic effect of amino acid; therefore, GABA plays a crucial role in reducing peripheral infarction.[11] GLT-1 can reduce the excess release of glutamate after cerebral ischemia and therefore prevent neuronal cell death as well as promote the recovery of neurological function in rats with MCAo.[27] GLT-1 levels are reduced in the lesioned hippocampus, and this decrease reduces the clearance of synaptic glutamate, resulting in neuronal damage in rats with MCAo.[28] PSD95 is a scaffolding protein that binds to NMDAR and neuronal nitric oxide synthase (nNOS).[29,30] The activation of NMDAR and the release of nitric oxide are a mediator of neuronal injury after ischemic cerebral damage.[31] Although the overstimulation of NMDARs and the activation of nNOS play crucial roles in causing neuronal damage after stroke, the direct inhibition of NMDAR or nNOS causes severe side effects. If the combination of PSD95 and nNOS can be blocked, a neuroprotective effect may be produced.[32] A study reported that blocking the interaction between NMDAR and PSD95 can prevent the downstream neurotoxic signaling of NMDAR without blocking the activity of synapses or the influx of calcium ions into the cells.[33] Such promotion of dissociation of the NMDAR–PSD95–nNOS complex and the consequent prevention of PSD95 downregulation can exert a neuroprotective effect.[34] The results of this study revealed an increase in PSD95 levels in the EA-P group, presumably because EA promotes the dissociation of the NMDAR–PSD95-nNOS complex, thereby increasing the levels of PSD95, which is beneficial for recovery after stroke. However, this assumption requires further research in the future. MAP2 is a major component of the neuronal cytoskeleton and is mainly expressed in dendrites; decreased MAP2 levels have been reported in the ischemic area of mice and rats with stroke,[35,36] which is consistent with the results of the present study. After cerebral ischemia, the reduction of brain MAP2 levels indicates the initial stage of neurological dysfunction, and consequent dendrite destruction may be the first sign of neurodegeneration.[37] Therefore, an increase in MAP2 levels in the lesioned hemisphere in the EA-P group is speculated to be beneficial for preventing damage to neuronal dendrites after stroke. Taken together, the anode was placed on the lesioned hemisphere and the cathode was placed on the nonlesioned hemisphere in EA treating ischemic stroke could increase the expression of GABA-A, GLT-1, PSD95, and MAP2 of cerebral cortex in the lesioned hemisphere. Therefore, generated greater neuroprotection.

The results of this study also revealed that IL-10 and integrin-β1 levels in the lesioned hemisphere were lower in the sham group than in the normal group; however, these levels were not higher in the EA-P or EA-N groups. IL-10 is an anti-inflammatory cytokine. Intraventricular or systemic injection of IL-10 can reduce the infarct volume in rats with MCAo, indicating the neuroprotective effect of IL-10 in ischemic stroke.[38] IL-10 reduces the infarct volume in acute stroke by downregulating proinflammatory signaling cascades.[39] Integrin-β1 is a cell surface molecule that plays a key role in endothelial cell adhesion, migration, and survival during angiogenesis. Neurovascular remodeling and recovery of function are crucial after stroke.[40] Integrin-β1 promotes the adhesion of neuroblast cells to laminin and effectively mediates the translocation of the cell body to the damaged site in mice with MCAo.[41] Taken together, the aforementioned findings indicate that the improvement in neurological function in the EA-P and EA-N groups may not be attributable to anti-inflammation or the promotion of angiogenesis.

The results of this study also revealed that DAPK1 levels in the lesioned hemisphere did not significantly differ among the normal, sham, EA-P, and EA-N groups. DAPK1 is a vital modulator of apoptosis and autophagy.[42] Studies have suggested that the combination of DAPK1 and the NR2B subunit of NMDAR at the extrasynaptic site causes brain damage after stroke.[15] Therefore, inhibiting the activation of the DAPK1–NMDAR complex can prevent brain damage after stroke.[43] Accordingly, the effect of DAPK1 on ischemic stroke treatment should be further investigated. In addition, the results of the present study indicated that IL-10, GFAP, NeuN, GABA-A, PSD95, GLT-1, Integrin-β1, DAPK1, and MAP2 levels in the nonlesioned hemisphere were not significantly different among the normal, sham, EA-P, and EA-N groups, suggesting that the effect of EA is prominent in the lesioned hemisphere in rats with ischemic stroke; however, further study is required to validate this finding.

Our results also showed that serum adrenaline and serotonin levels were lower in the sham group than in the normal and EA-P groups, and serum noradrenaline levels in the sham group were lower than those in the EA-P group. A study reported that the mean plasma levels of noradrenaline, adrenaline, and dopamine were higher in patients with cerebral infarction than in controls without stroke. The high plasma norepinephrine levels may reflect an increase in peripheral sympathetic activity, which can cause cardiac abnormalities in patients with cerebral infarction.[44] Primary catecholamines including noradrenaline and adrenaline can contribute to myocardial ischemia and calcium overload, which can cause cardiac damage in patients with cerebrovascular diseases.[45] However, circulating catecholamine response stress against ischemic brain damage plays a neuroprotective role.[46] Transcutaneous stimulation of the auricular vagus nerve can affect the locus coeruleus–norepinephrine system,[47] and noradrenaline is generated in the locus coeruleus of the brainstem.[48] Several studies have reported that noninvasive vagus nerve stimulation can reduce the infarction volume and neurological deficits in rats with ischemic stroke.[49] In addition, the effectiveness of noradrenaline reuptake inhibitors has been demonstrated for the rehabilitation of stroke patients.[50] Depression is the most common complication after ischemic stroke. Neurological functional deficits and body weight loss are more severe in rats with ischemic stroke and depression, and these rats also exhibit reduced serotonin levels in the brain.[51] A double-blinded randomized controlled trial reported that the selective serotonin reuptake inhibitor (SSRI) fluoxetine can enhance motor recovery in stroke patients without depression.[52] The SSRIs fluoxetine and sertraline can promote the recovery of neurological function in mice with photothrombotic cortical ischemia by increasing the expression of heme oxygenase-1 and hypoxia-inducible factor-1α proteins in the ischemic region.[53] Taken together, the aforementioned results indicate that the increase in the serum levels of noradrenaline, adrenaline, and serotonin in the EA-P group is beneficial for poststroke recovery.

Our results also indicated that CSF glutamate levels were higher in the EA-P group than in the sham group. Glutamate is released from neuronal cells in the ischemic region after cerebral ischemia, and a decrease in the astrocyte-to-glutamate uptake leads to an increase in the extracellular concentration of glutamate, causing neuronal cell death.[54] High concentrations of glutamate in the blood and CSF have been reported in humans within 24 h of stroke onset.[55] A study reported that extracellular glutamate accumulation is localized to the gray matter, and that glutamate diffuses into the adjacent white matter structures and gradually into the CSF. We assume that the placement of the anode in EA on the lesioned hemisphere can enhance glutamate clearance from the extracellular fluid of the ischemic core into the CSF; therefore, increasing CSF glutamate levels is beneficial for stroke recovery. Our assumptions are supported by our result of the reduction of the infarction volume and neurological deficits in the EA-P group. However, whether glutamate increases simultaneously in brain tissue and CSF needs further study in the future.

Our results also indicated that serum and CSF dopamine and GABA levels did not significantly differ among the normal, sham, EA-P, and EA-N groups. Dopamine is a neurotransmitter that plays a crucial role in brain sensorimotor function, and it is essential for recovery after stroke.[56] GABA is an inhibitory neurotransmitter, and GABAergic can promote the decrease of cerebral ischemia–reperfusion injury and thereby prevent the damage of peripheral organs. However, the release and transmission of GABA involve complex pathological process.[57] Therefore, the relationship of EA with dopamine and GABA warrants further study.

Some limitation in the present study as follows: 1) the study lacks direct evidence to explain EA may promote the dissociation of the NMDAR-PSD-95-nNOS complex, further research requires in the future; 2) the data lacks glutamate levels of the cerebral cortex, the measurement of cortical glutamate level is need; 3) EA only choosed 15-Hz for study, more EA frequency will be compared; and 4) this manuscript delved multitude of targets or mediators, therefore, making it challenging to comprehend. Based on the results of this study focus on the most logical and well-founded data to elucidate the substantial effects of EA in the future study.

In conclusion, the placement of either the anode or cathode in EA on the lesioned hemisphere of rats with ischemic stroke can improve neurological function and the infarction volume, suggesting that EA can improve neurological function after stroke through multiple pathways. However, the placement of the anode on the lesioned hemisphere can produce more neuroprotective effects.

Consent for publication

This study has not used any individual person's data.

Funding

This study was supported by the grant from China Medical University (CMU111-MF-49), and also by the grant MOST 110-2320-B-039-025 from the National Science and Technology Council in Taiwan. The study was also financially supported by the Chinese Medicine Research Center of China Medical University from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education in Taiwan (**CMRC-CENTER-5**).

Author contributions

C–H Liu performed animal experiments, electroacupuncture and investigated the neurological states, and wrote manuscript; Thi Mai Nguyen performed Western blot analysis; D-Y Lee performed metabolomic analysis; and C-L Hsieh designed the protocol and supervised the entire experimental process, and revised the manuscript. All co-authors have read and agreed on the current version of this manuscript.

Availability of data

The data in this study are available to other researchers upon request, Professor Hsieh should be contact if someone wants to request the data from this study.

Declaration of competing interest

The authors declare that there are no conflicts of interest associated with this manuscript, and no significant financial support was received that would influence the findings.

References

1. Saini V, Guada L, Yavagal DR. Global epidemiology of stroke and access to acute ischemic stroke interventions. *Neurology*. 2021;97(20 Supplement 2):S6–S16.
2. European Society of Cardiology. *Stroke Incidence Rising in Taiwan Contrary to Falls in Western Countries*. European Society of Cardiology; 2018.
3. Lee M, Wu Y-L, Ovbiagele B. Trends in incident and recurrent rates of first-ever ischemic stroke in Taiwan between 2000 and 2011. *J Stroke*. 2016;18(1):60.
4. Hasan TF, Hasan H, Kelley RE. Overview of acute ischemic stroke evaluation and management. *Biomedicines*. 2021;9(10):1486.
5. Boddington L, Reynolds J. Targeting interhemispheric inhibition with neuromodulation to enhance stroke rehabilitation. *Brain Stimul*. 2017;10(2):214–222.
6. Schlaug G, Renga V. Transcranial direct current stimulation: a noninvasive tool to facilitate stroke recovery. *Expet Rev Med Dev*. 2008;5(6):759–768.
7. Lee DY, Jiu YR, Hsieh CL. Metabolism modulation in rat tissues in response to point specificity of electroacupuncture. *Sci Rep*. 2022;12(1):210.
8. Schlaug G, Renga V, Nair D. Transcranial direct current stimulation in stroke recovery. *Arch Neurol*. 2008;65(12):1571–1576.
9. Adkins-Muir DL, Jones TA. Cortical electrical stimulation combined with rehabilitative training: enhanced functional recovery and dendritic plasticity following focal cortical ischemia in rats. *Neurol Res*. 2003;25(8):780–788.
10. Paik N-J, Yang E. Role of GABA plasticity in stroke recovery. *Neural Regen Res*. 2014;9(23):2026.
11. Głodzik-Sobańska L, Słowik A, Kozub J, Sobiecka B, Urbanik A, Szczudlik A. GABA in ischemic stroke: proton magnetic resonance study. *Med Sci Mon Int Med J Exp Clin Res*. 2004;10(suppl 3).
12. Domercq M, Matute C. Excitotoxicity therapy for stroke patients still alive. *EBioMedicine*. 2019;39:3–4.
13. Wu QJ, Tymianski M. Targeting NMDA receptors in stroke: new hope in neuroprotection. *Mol Brain*. 2018;11(1):1–14.
14. Martin HG, Wang YT. Blocking the deadly effects of the NMDA receptor in stroke. *Cell*. 2010;140(2):174–176.
15. Tu W, Xu X, Peng L, et al. DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell*. 2010;140(2):222–234.
16. Cheng CY, Lin JG, Su SY, Tang NY, Kao ST, Hsieh CL. Electroacupuncture-like stimulation at Baihui and Dazhui acupoints exerts neuroprotective effects through activation of the brain-derived neurotrophic factor-mediated MEK1/2/ERK1/2/p90RSK/bad signaling pathway in mild transient focal cerebral ischemia in rats. *BMC Compl Alternative Med*. 2014;14(1):1–11.
17. Cheng C-Y, Lin J-G, Tang N-Y, Kao S-T, Hsieh C-L. Electroacupuncture-like stimulation at the Baihui (GV20) and Dazhui (GV14) acupoints protects rats against subacute-phase cerebral ischemia-reperfusion injuries by reducing S100B-mediated neurotoxicity. *PLoS One*. 2014;9(3):e91426.
18. Lin Y-W, Hsieh C-L. Electroacupuncture at Baihui acupoint (GV20) reverses behavior deficit and long-term potentiation through N-methyl-d-aspartate and transient receptor potential vanilloid subtype 1 receptors in middle cerebral artery occlusion rats. *J Integr Neurosci*. 2010;9:269–282, 03.
19. Liu C-H, Liao W-L, Su S-Y, Chen W-L, Hsieh C-L. Electroacupuncture in the contralesional hemisphere improves neurological function involving GABA in ischemia–reperfusion injury rats. *Evid base Compl Alternative Med*. 2021:2021.
20. Chelette CC K, Nichols L, Salyers E, Sawaki L. *Effects of Electrode Configurations in Transcranial Direct Current Stimulation after Stroke*. Natal, Brazil: 2014 IEEE 16th International Conference on e-Health Networking, Applications and Services (Healthcom); 2014, 15-18 October 2014.
21. Chen J, Li Y, Wang L, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001;32(4):1005–1011.
22. Blanco ME, Mayo OB, Bandiera T, Tonelli DD, Armirotti A. LC-MS/MS analysis of twelve neurotransmitters and amino acids in mouse cerebrospinal fluid. *J Neurosci Methods*. 2020:341.
23. Wolf HK, Buslei R, Schmidt-Kastner R, et al. NeuN: a useful neuronal marker for diagnostic histopathology. *J Histochem Cytochem*. 1996;44(10):1167–1171.
24. Verberk IM, Laarhuis MB, van den Bosch KA, et al. Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study. *Lancet Healthy Longevity*. 2021;2(2):e87–e95.
25. Benninger F, Glat MJ, Offen D, Steiner I. Glial fibrillary acidic protein as a marker of astrocytic activation in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *J Clin Neurosci*. 2016;26:75–78.
26. Green AR, Hainsworth AH, Jackson DM. GABA potentiation: a logical pharmacological approach for the treatment of acute ischaemic stroke. *Neuropharmacology*. 2000;39(9):1483–1494.
27. Harvey BK, Airavaara M, Hinzman J, et al. Targeted over-expression of glutamate transporter 1 (GLT-1) reduces ischemic brain injury in a rat model of stroke. *PLoS One*. 2011;6(8):e22135.
28. Ketheeswaranathan P, Turner NA, Spary EJ, Batten TF, McColl BW, Saha S. Changes in glutamate transporter expression in mouse forebrain areas following focal ischemia. *Brain Res*. 2011;1418:93–103.
29. Sattler R, Xiong Z, Lu W-Y, Hafner M, MacDonald JF, Tymianski M. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science*. 1999;284(5421):1845–1848.
30. Cao J, Viholainen JI, Dart C, Warwick HK, Leyland ML, Courtney MJ. The PSD95–nNOS interface: a target for inhibition of excitotoxic p38 stress-activated protein kinase activation and cell death. *J Cell Biol*. 2005;168(1):117–126.
31. Dawson VL, Kizushi VM, Huang PL, Snyder SH, Dawson TM. Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase-deficient mice. *J Neurosci*. 1996;16(8):2479–2487.
32. Zhou L, Li F, Xu H-B, et al. Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. *Nat Med*. 2010;16(12):1439–1443.
33. Aarts M, Liu Y, Liu L, et al. Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science*. 2002;298(5594):846–850.
34. Ugalde-Triviño L, Díaz-Guerra M. PSD-95: an effective target for stroke therapy using neuroprotective peptides. *Int J Mol Sci*. 2021;22(22):12585.
35. Dawson DA, Hallenbeck JM. Acute focal ischemia-induced alterations in MAP2 immunostaining: description of temporal changes and utilization as a marker for volumetric assessment of acute brain injury. *J Cerebr Blood Flow Metabol*. 1996;16(1):170–174.
36. Mages B, Fuhs T, Aleithe S, et al. The cytoskeletal elements MAP2 and NF-L show substantial alterations in different stroke models while elevated serum levels highlight especially MAP2 as a sensitive biomarker in stroke patients. *Mol Neurobiol*. 2021;58(8):4051–4069.
37. Matesic DF, Lin RC. Microtubule-associated protein 2 as an early indicator of ischemia-induced neurodegeneration in the gerbil forebrain. *J Neurochem*. 1994;63(3):1012–1020.
38. Spera PA, Ellison JA, Feuerstein GZ, Barone FC. IL-10 reduces rat brain injury following focal stroke. *Neurosci Lett*. 1998;251(3):189–192.
39. Liesz A, Bauer A, Hoheisel JD, Veltkamp R. Intracerebral interleukin-10 injection modulates post-ischemic neuroinflammation: an experimental microarray study. *Neurosci Lett*. 2014;579:18–23.
40. Lathia JD, Chigurupati S, Thundyil J, et al. Pivotal role for beta-1 integrin in neurovascular remodelling after ischemic stroke. *Exp Neurol*. 2010;221(1):107–114.
41. Fujioka T, Kaneko N, Ajioka I, et al. β1 integrin signaling promotes neuronal migration along vascular scaffolds in the post-stroke brain. *EBioMedicine*. 2017;16:195–203.
42. Gade P, Manjegowda SB, Nallar SC, Maachani UB, Cross AS, Kalvakolanu DV. Regulation of the death-associated protein kinase 1 expression and autophagy via ATF6 requires apoptosis signal-regulating kinase 1. *Mol Cell Biol*. 2014;34(21):4033–4048.
43. Wang S, Shi X, Li H, et al. DAPK1 signaling pathways in stroke: from mechanisms to therapies. *Mol Neurobiol*. 2017;54(6):4716–4722.
44. Myers MG, Norris JW, Hachniski V, Sole MJ. Plasma norepinephrine in stroke. *Stroke*. 1981;12(2):200–204.
45. Du Y, Demillard LJ, Ren J. Catecholamine-induced cardiotoxicity: a critical element in the pathophysiology of stroke-induced heart injury. *Life Sci*. 2021;287:120106.
46. Koide T, Wieloch TW, Siesjö BK. Circulating catecholamines modulate ischemic brain damage. *J Cerebr Blood Flow Metabol*. 1986;6(5):559–565.
47. Ludwig M, Wienke C, Betts MJ, Zaehle T, Hämmerer D. Current challenges in reliably targeting the noradrenergic locus coeruleus using transcutaneous auricular vagus nerve stimulation (taVNS). *Auton Neurosci*. 2021;236:102900.
48. Atzori M, Cuevas-Olguin R, Esquivel-Rendon E, et al. Locus ceruleus norepinephrine release: a central regulator of CNS spatio-temporal activation? *Front Synaptic Neurosci*. 2016;8:25.
49. Tang H, Li J, Zhou Q, et al. Vagus nerve stimulation alleviated cerebral ischemia and reperfusion injury in rats by inhibiting pyroptosis via α7 nicotinic acetylcholine receptor. *Cell Death Discov*. 2022;8(1):1–10.
50. Sternberg Z, Schaller B. Central noradrenergic agonists in the treatment of ischemic stroke—an overview. *Transl Stroke Res*. 2020;11(2):165–184.
51. Zhang G, Chen L, Yang L, et al. Combined use of spatial restraint stress and middle cerebral artery occlusion is a novel model of post-stroke depression in mice. *Sci Rep*. 2015;5(1):1–9.
52. Chollet F, Tardy J, Albucher J-F, et al. Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. *Lancet*

Neurol. 2011;10(2):123–130.
53. Shin TK, Kang MS, Lee HY, et al. Fluoxetine and sertraline attenuate post-ischemic brain injury in mice. *KOREAN J PHYSIOL PHARMACOL*. 2009;13(3):257–263.
54. Belov Kirdajova D, Kriska J, Tureckova J, Anderova M. Ischemia-triggered glutamate excitotoxicity from the perspective of glial cells. *Front Cell Neurosci*. 2020;14:51.
55. Dávalos A, Shuaib A, Wahlgren NG. Neurotransmitters and pathophysiology of stroke: evidence for the release of glutamate and other transmitters/mediators in animals and humans. *J Stroke Cerebrovasc Dis*. 2000;9(6):2–8.
56. Gower A, Tiberi M. The intersection of central dopamine system and stroke: potential avenues aiming at enhancement of motor recovery. *Front Synaptic Neurosci*. 2018;10:18.
57. Chen C, Zhou X, He J, Xie Z, Xia S, Lu G. The roles of GABA in ischemia-reperfusion injury in the central nervous system and peripheral organs. *Oxid Med Cell Longev*. volume 2019, 19 pages.