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Original Article

Electroacupuncture attenuated cerebral ischemia injury correlated with increased glucose consumption and elevated hippocampal GLUT1/3 expression

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Abstract: Objectives: Cerebral ischemic stroke (IS) is a prevalent cerebrovascular accident that can lead to severe consequences. Electroacupuncture (EA) has demonstrated efficacy in alleviating cerebral ischemia/reperfusion (I/R) injury; however, the underlying molecular mechanisms remain poorly understood. It has been reported that the upregulation of glucose transporter gene expressions restores the ability of cerebral cells to consume energy during I/R. However, whether the use of EA affects glucose metabolism remains elusive. This study tries to elucidate the role of EA in regulating glucose metabolism during cerebral I/R process. Methods: Rats with middle cerebral artery occlusion (MCAO) were employed as cerebral I/R injury models, and EA were performed at Baihui and Shenting acupoints in the modeled rats as treatment strategies. Additionally, Magnetic Resonance Imaging (MRI) was employed to measure cerebral infarction volume, Positron Emission Tomography/Computed Tomography (PET/CT) to evaluate glucose uptake in the hippocampus, neurological deficit scoring along with Morris Water Maze (MWM) testing was used to assess neurological deficits, and Western blot and Enzyme-linked Immunosorbent Assay (ELISA) tests determined the expression levels of glucose transporters genes GLUT1 and GLUT3. Results: Neurological deficit scoring and MWM test results indicated that EA at Baihui and Shenting acupoints significantly alleviated neurological deficits caused by I/R injury. MRI results also showed that the treatment reduced the cerebral infarction volume. Moreover, PET/CT outcome revealed that EA at the two acupoints enhanced glucose uptake and metabolism in the injured cerebral hippocampus. At the molecular level, EA at the two acupoints significantly upregulated the expression of glucose transporter genes GLUT1 and GLUT3, as evidenced by Western blot results. Conclusions: EA at Baihui and Shenting acupoints can ameliorate I/R injury and facilitate glucose uptake and metabolism by promoting the expression of hippocampal glucose transporter genes GLUT1 and GLUT3.

Keywords: Electroacupuncture, cerebral ischemia, Baihui (DU20), Shenting (DU24), glucose metabolism, GLUT1/3

Introduction

Stroke is a common cerebrovascular accident, whose prevalence increases significantly with changes in age, making it one of the leading causes of death globally [1-3]. Stroke can be categorized into several subtypes based on varied etiology, including ischemic stroke (IS), intracerebral hemorrhagic stroke, subarachnoid hemorrhagic stroke, and spinal cord stroke [4]. Among these subtypes, IS can occur due to middle cerebral artery occlusion (MCAO), leading to insufficient blood flow to the brain tissue, causing damage and disfunction within the cortex [5]. MCAO can lead to multiple defects, including defective learning, motor control impairment, and facial paralysis [5-7].

Significant advances have been achieved in the prevention, diagnosis, evaluation, and treatment of IS. However, its prognosis relies heavily on the severity of the condition, especially withing the time window from onset to receiving treatment [5]. Many IS patients suffer from disability and other post-stroke symptoms as a consequence of delayed treatment [8-10].

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Hence, novel therapy is urgently needed to enhance the prognosis of IS patients and help them recover from IS-caused symptoms.

Electroacupuncture (EA) is a Traditional Chinese Medicine therapy employed for the treatment of various diseases with evidence showing its efficacy is better than conventional acupuncture [11]. EA has been demonstrated to have a neuroprotective effect on both animal models and human subjects with varied neurological disorders, such as Alzheimer's disease and stroke [12-16]. However, the underlying mechanisms of the neuroprotective effects of EA remain obscure.

The onset of IS could decrease the glucose available to be consumed by cerebral cells due to insufficient blood supply. Consequently, cerebral cells compensate for the lack glucose uptake by upregulating the expressions of glucose transporter genes, such as GLUT1 and GLUT3, thereby restoring their metabolic energy consumption [17, 18]. However, whether increased expression of glucose transporter genes affects the neuroprotective function of EA for IS therapy remains unclear.

In our previous studies, we demonstrated that EA at Baihui (DU20) and Shenting (DU24) acupoints can minimize ultrastructural brain damage, reduce cerebral MMP-2/9 expression in rats with ischemia/reperfusion (I/R) injury, and ameliorate post-stroke learning and memory defects [13]. Recently, it has been reported that EA at Baihui (DU20) and Shenting (DU24) acupoints alleviates cognitive deficits by regulating the Pten/Akt pathway [14]. These findings collectively suggest that EA at Baihui (DU20) and Shenting (DU24) acupoints may hold potential in treating IS. Nevertheless, the underlying mechanism of how EA at Baihui (DU20) and Shenting (DU24) acupoints protects cerebral cells from IS requires further investigation. We therefore explored the effects of EA at the two acupoints on the consumption of glucose and the expression levels of glucose transporter genes GLUT1 and GLUT3 in rat models with I/R injury.

Materials and methods

Animals

Sprague-Dawley rats (male, 260±20 g in weight and 3 months in age) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (China) and housed in a pathogen-free facility at 22°C with free access to food and water, with one rat per cage. All animal procedures were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Lishui Second People's Hospital Affiliated with Wenzhou Medical University, China (Approval notice #20190606-01).

Establishment of the MCAO rat model

The MCAO modeled rats were established following procedures in a previous research [19]. Briefly, rats were fasted for 24 hours before being anesthetized by 5% isoflurane. Then, they were given oxygen masks and placed in a gas chamber receiving 5% isoflurane with 1 mL/min oxygen flow to induce anesthesia. Subsequently, the isoflurane concentration was altered to 2% until the rats lost their tail pinch reflex. Next, the rats were placed on heating pads to maintain their body temperature, and maintained with 2% isoflurane treatment to continue their anesthetic state during operations. After that, a midline cervical incision (MCA) was made to expose their left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). To induce left MCA occlusion, a nylon surgical thread (~18-22 mm) was gently inserted into the ICA until the blunted distal end met slight resistance. After 2 hours, the nylon thread was removed to restore blood flow in the affected MCA area. Sham-operated rats underwent identical operations without the ligation and occlusion procedures. Throughout the surgery, body temperature was monitored and maintained using a heating pad. After recovery from the surgery, neurological deficits in the MCAO rats were scored on days 1, 3, 5, and 7. Rats scoring from 1 to 3 points were assessed as having mild neurological deficits, and were included for further analysis, while those whose score was 0 or 4 points were assessed as having no neurological deficit or severe deficit, respectively, and were excluded from subsequent analysis.

Animal grouping and operations

A total of 80 rats were included and were divided into 4 groups: the sham group, in which the

rats were anesthetized and underwent neck surgery to expose their MCA, after which their wound was closed without left MCA occlusion and they did not undergo EA treatment; the MCAO group, in which the rats were operated on with left MCA occlusion but did not receive EA treatment; the MCAO+EA group, in which the rats were given with both left MCA occlusion and EA treatment; and the MCAO+NA group, in which the rats underwent left MCA occlusion and non-acupoint (NA) treatment. There were 20 rats in each group, with 6 rats used for water content measurements, 6 for hippocampal protein extraction and Western blot analysis, 6 for hippocampal supernatant extraction and ELISA analysis, another 2 as backups in case of the occurrence of accidents during surgery or surgical recovery process. In terms of the euthanasia process, experimental rats were put in a chamber with continuous CO_2 gas until they were sacrificed for harvesting of brain tissues.

EA treatment

Twenty-four hours after surgery, the rats in the MCAO+EA group and the MCAO+NA group received daily acupuncture sessions for one week, using an EA device with #30 acupuncture needles purchased from Suzhou Medical Appliance Factory, China. The needles were inserted to a depth of about 2 mm at the positions of Baihui (DU20) and Shenting (DU24) acupoints or at a NA location near the costal margin with a depth of 5 mm. The NA was located 10-15 mm anterior to the iliac crest where no acupoint was recognized in this area [20, 21]. The following stimulation parameters were used: condensation-rarefaction wave, frequency 1-20 Hz, and a duration of 30 minutes per session. Rats in the sham group and the MCAO group underwent identical treatment except without the application of EA.

Morris Water Maze (MWM)

Three days after surgery, rats in all groups were tested for their spatial learning ability and memory in the MWM. The water maze apparatus (Institute of Materia Medica, Chinese Academy of Medical Sciences, China) consisted of a tank (150 cm in diameter and 60 cm in height) filled with water to a depth of 30 cm and was maintained at a temperature of 25±2°C. A circular escape platform (6 cm in diameter and 28 cm in height) was positioned 2 cm below the water surface. The tank was divided into 4 equal quadrants, monitored by a computer-connected camera installed above the center of the tank. The escape platform was in the third quadrant. Rats were allowed to freely swim in the maze for 2 minutes to familiarize themselves with the environment.

For the positioning cruise experiment, the rats were gently placed in the water, facing the wall of the apparatus, and tested from four different starting positions. Rats reaching the escape platform within 90 seconds and staying there for at least 3 seconds were considered to have found the platform, and their escape latency was recorded as the time it took to find the platform. If the rats failed to find the platform within 90 seconds, they were gently placed on the platform for 10 seconds, and their escape latency was set to 90 seconds. The escape latencies from all 4 quadrants were recorded to calculate the mean value generated in four consecutive days.

On the following day, the platform in the third quadrant was removed, and the number of times the rats passed through the position of the platform within 90 seconds was counted to assess their memory retention ability. The rats were dried with a hairdryer after each test before returning to their cages.

Magnetic resonance imaging (MRI)

To evaluate the cerebral infarction volume, MRI was employed to scan the brains of the rats following the approaches described in previous research with the use of BioSpec 70/20 USR (Bruker, Germany) [22]. Briefly, the rats were kept in a prone position and anesthetized with isoflurane, while their heads were fixed with a rat adaptor. T2-weighted imaging (T2*WI) was performed using the Rapid Acquisition with Relaxation Enhancement (RARE) sequence, with the following scan parameters: repetition time (TR): 2738 ms; echo time (TE): 33 ms; thickness: 0.8 mm; interval: 0.5 mm; field of view (FOV): 30 mm×30 mm; image matrix: 256×256; flip angle: 30° (FA30); slices number: 24; scan time: 5 minutes 50 seconds. For infarction volume analysis, Image J software (NIH, USA) was used to outline both the infarction and whole brain areas.

Positron emission tomography/computed tomography (PET/CT)

PET/CT was performed with the use of MILABS vector+ (Netherlands) following approaches described in a previously study [23]. Briefly, the rats were fasted for 12 hours and kept under anesthesia with isoflurane. A dose of 20±1.84 MBq of ^{18}F-FDG in 200 μl saline was injected into their tail veins, after which the rats were allowed to have a 40-minute rest. Then, the rats were transferred to a scan bed customized for them and positioned in a prone position, with a continuous isoflurane supply to maintain anesthesia. X-ray mode was used to set up the target area, and PET data were acquired under the "list" mode, with the following parameters: energy window: 450-550 KeV; window width: ±20%; 30 min/frame, projection: 84 times. Subsequently, CT scanning was conducted under the "normal" mode, using the following parameters: voltage: 55 KeV; current: 615 μA, with a total scan time of 15 minutes for each rat.

Image reconstruction was performed following methods described in previous study [24]. Briefly, PET images were reconstructed using POSEM software, while CT images were reconstructed using NRecon (v1.6.3 s, Skyscan). To merge PET and CT images, PMOD software (Switzerland) was utilized, and the resulting images were further smoothed with a 3D Gaussian filter (1.0 mm FWHM). Subsequently, the images were merged with the W. Schiffer Brain Atlas to calculate the standardized uptake values (SUVs) of ^{18}F-FDG glucose, which were then used to determine the ratio of glucose uptake in the corresponding hemisphere [25].

Brain water content measurement

The intact brains were carefully dissected and weighed to obtain the wet weight. Subsequently, the brains were wrapped with foil and placed in an oven at 110°C for 24 hours to obtain the dry weight. The water content was then calculated using the formula: Water content (%) = (wet weight - dry weight)/wet weight ×100%.

Enzyme-linked immunosorbent assay (ELISA)

To evaluate ATP, lactate dehydrogenase (LDH) and lactic acid (LA) levels, the left cerebral hippocampal tissues were dissected and immediately weighed, which were then minced into small pieces and mixed with nine times their volume of pre-cooled saline, followed by homogenization. Next, the homogenized tissues were centrifuged at 3,600 rpm for 15 minutes, and the supernatants were collected for ELISA assay, following the manufacturer's instructions. Finally, the OD_{440} values were measured using an ELX800 microplate reader (Bio-Tek, USA). The following ELISA kits were used according to manufacturer's instructions: ATP kit (A095, Nanjing Jiancheng Bioengineering Institute, China), LDH kit (A020-1, Nanjing Jiancheng Bioengineering Institute, China), and LA kit (A019-2, Nanjing Jiancheng Bioengineering Institute, China).

Western blot

Freshly isolated left cerebral hippocampal tissues were homogenized in RIPA lysis buffer containing 1% PMSF at a 1:10 tissue-to-buffer ratio (w/v) using a mechanical homogenizer under ice-cold conditions. The mixtures were then centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was collected, and the protein concentration was determined using a BCA kit. The protein solutions were denatured and separated using SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked in a 5% skim milk solution for 2 hours at room temperature. Subsequently, the membranes were incubated with primary antibodies overnight at 4°C while placed on a shaker. On the following day, the membranes were washed three times with TBS/0.1% Tween 20 solution and then incubated with the HRP-conjugated secondary antibody solution for 1 hour at room temperature. After three additional washes with TBS/0.1% Tween 20, the membranes were incubated with the working solution from an ECL kit to develop signals. The signals were then visualized using a ChemiDoc Imaging machine (Bio-Rad, USA), and the band intensity was quantified using Image J software (NIH, USA). The following antibodies were used: anti-GLUT1 (ab652, 1:1000, Abcam, UK), anti-GLUT3 (ab41525, 1:1000, Abcam, UK), β-ACTIN (HC201-01, 1:1000, TransGen Biotech, China), and HRP-conjugated Anti-rabbit IgG (#7074, 1:5000, Cell Signaling Technology, USA). All Western blot-related experiments were repeated three times.

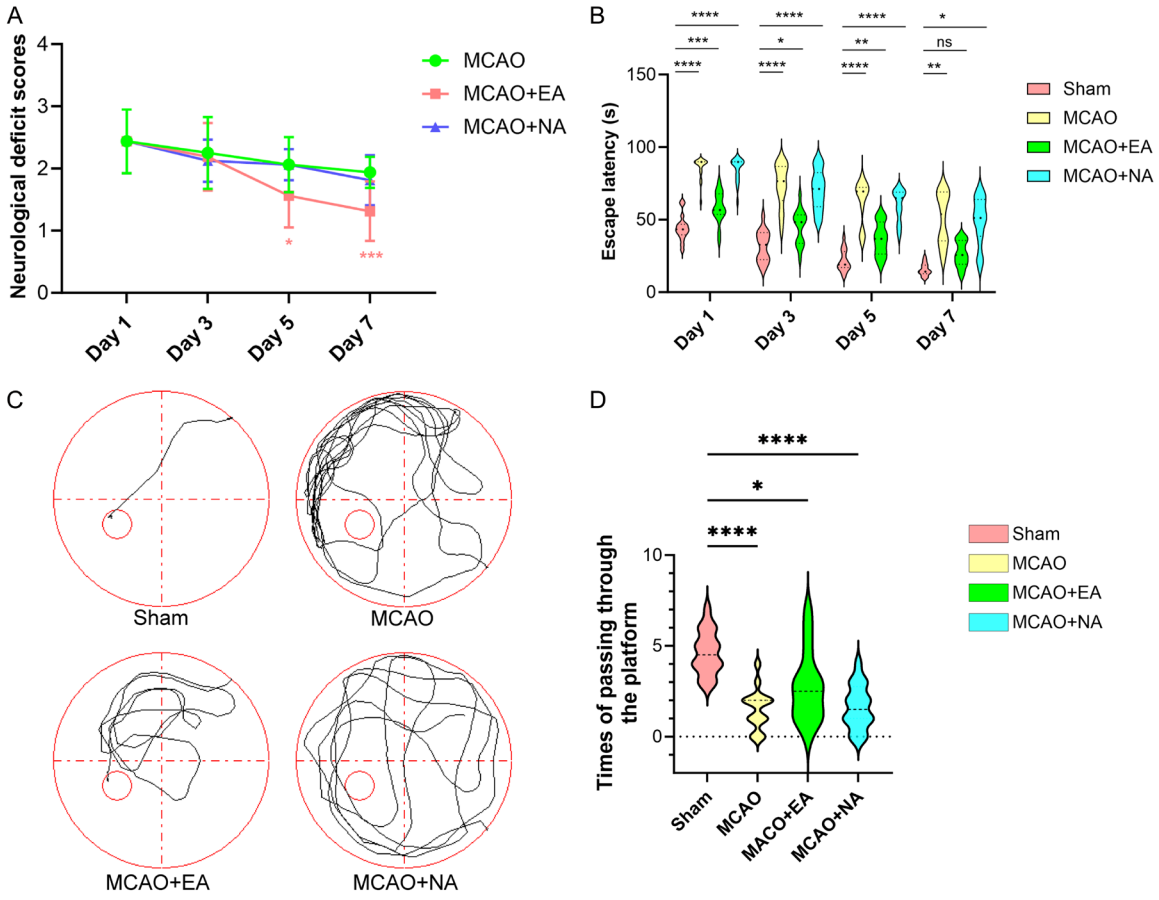

Figure 1. EA at Baihui (DU20) and Shenting (DU24) acupoints improved neurological deficits in the MCAO rat models. A. Changes in neurological deficit scores in the MCAO group, the MCAO+EA group and the MCAO+NA group on days 1, 3, 5, and 7 since initial EA treatment. B. Escape latencies from MWM tests in the sham group, the MCAO group, the MCAO+EA group and the MCAO+NA group on days 3-6 since initial EA treatments. C. Typical trace figures of rat models. D. The number of times the rat models passed through the platforms in MWM test in the sham group and the MCAO group, the MCAO+EA group and the MCAO+NA group on day 7 since initial EA treatments. All twenty rats in each group were tested. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns: not significant.

Statistical analysis

All data were analyzed using Prism software (v8.0, GraphPad, USA). Quantitative data were presented as mean ± standard deviation. ANOVA and Kruskal-Wallis test were used to calculate the between-group differences in data sets. $P < 0.05$ indicated the difference was statistically significant. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significant.

Results

EA at Baihui (DU20) and Shenting (DU24) acupoints improved neurological deficits in MCAO rats

MCAO modeled rats were utilized in the study to investigate the effects of EA at Baihui (DU20) and Shenting (DU24) acupoints on the rats' I/R injury [26, 27]. Additionally, a group of MCAO rats treated with EA at a NA was included as a control for non-specific effects of EA. Our findings demonstrated that rats in the MCAO+EA group exhibited significantly better neurological deficit scores compared to rats in the MCAO+NA group (**Figure 1A**). Moreover, the impaired learning ability and memory observed in MCAO rats were significantly improved by EA at Baihui (DU20) and Shenting (DU24) acupoints, while EA at NA presented no such effect, as supported by the MWM test results (**Figure 1B-D**). These results confirmed that EA at Baihui (DU20) and Shenting (DU24) acupoints could effectively attenuate neurological deficits in the MCAO modeled rats.

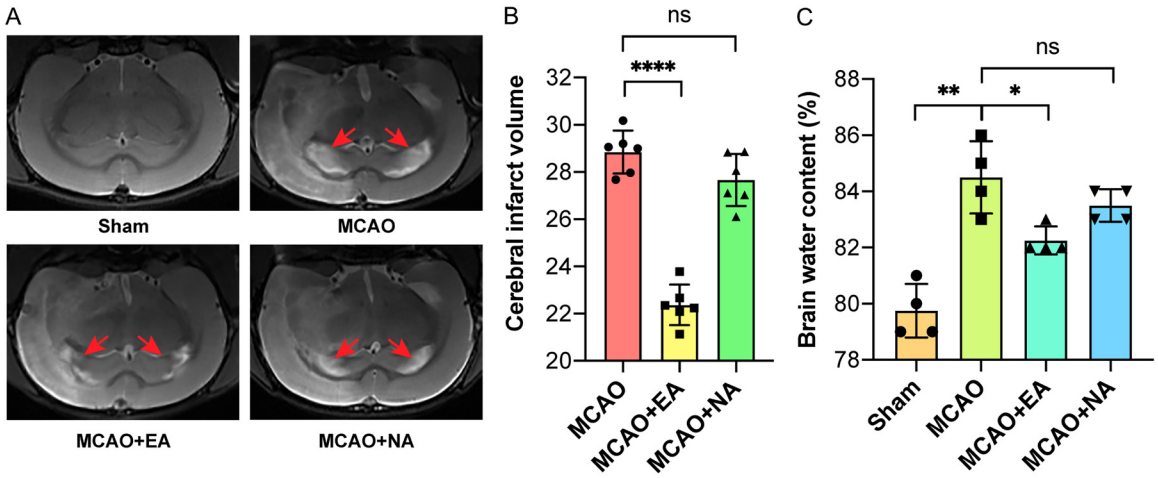

Figure 2. EA at Baihui (DU20) and Shenting (DU24) acupoints reduced cerebral infarction volume. A. MRI images showing cerebral infarction in the sham group, the MCAO+EA group and the MCAO+NA group on day 7 after initial EA treatments. Six rats that were randomly chosen from each group were scanned. Red arrows indicated infarction areas. B. Statistical results of MRI data that show the extent of cerebral infarction in each group on day 7 after initial EA treatments. C. Brain water content that presents the level of brain edema in the sham group, the MCAO+EA group and the MCAO+NA group on day 7 after initial EA treatments. Each data point represents pooled brain tissues from six randomly selected rats from each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$, ns: not significant.

EA at Baihui (DU20) and Shenting (DU24) acupoints reduced cerebral infarction volume

MRI was carried out to examine cerebral infarction volume within the MCAO rats receiving different treatments for comprehensive evaluation of the impacts of EA at Baihui (DU20) and Shenting (DU24) acupoints on the rats' I/R injury. Our findings revealed that EA at Baihui (DU20) and Shenting (DU24) acupoints significantly reduced the cerebral infarction volume in the MCAO rats, while EA at NA generated unmarked effects on this outcome (**Figure 2A**, **2B**).

Consistent with these findings, brain water content, a marker of cerebral edema, was significantly reduced in the MCAO+EA group. In contrast, rats in the MCAO+NA group were observed with brain water content that was comparable to the sham group and the MCAO group (**Figure 2C**). These results indicated that EA at Baihui (DU20) and Shenting (DU24) acupoints yielded specific therapeutic benefits against cerebral infarction in the MCAO modeled rats.

Glucose uptake and metabolism were enhanced by EA at Baihui (DU20) and Shenting (DU24) acupoints

Glucose uptake and metabolism was measured in the MCAO group, the MCAO+EA group and the MCAO+NA group using PET/CT to assess their brain functions [28]. The results revealed that EA at Baihui (DU20) and Shenting (DU24) acupoints significantly enhanced glucose uptake in the affected cerebral cortex of MCAO rats (**Figure 3A**, **3B**). In contrast, rats in the MCAO+NA group exhibited similar glucose uptake in the affected cerebral cortex in comparison to rats in the untreated groups. Consistent with these findings, the ATP level in the left hippocampus increased in the MCAO+EA group more than that in the MCAO+NA group (**Figure 3C**). Similarly, the hippocampal LA level, which was elevated due to hypoxia, was downregulated in rats in the MCAO+EA group, yet this effect was not observed in the MCAO+NA group (**Figure 3D**) [29]. Additionally, the hippocampal LDH level, which promotes the production of LA from glucose, was also reduced in the MCAO+EA group but not in the MCAO+NA group (**Figure 3E**) [30]. Collectively, these results demonstrated that EA at Baihui (DU20) and Shenting (DU24) acupoints enhanced cerebral glucose uptake and metabolism in the MCAO rat models.

Hippocampal GLUT1 and GLUT3 expressions were upregulated by EA at Baihui (DU20) and Shenting (DU24) acupoints after MCAO

To elucidate how EA at Baihui (DU20) and Shenting (DU24) acupoints improve glucose

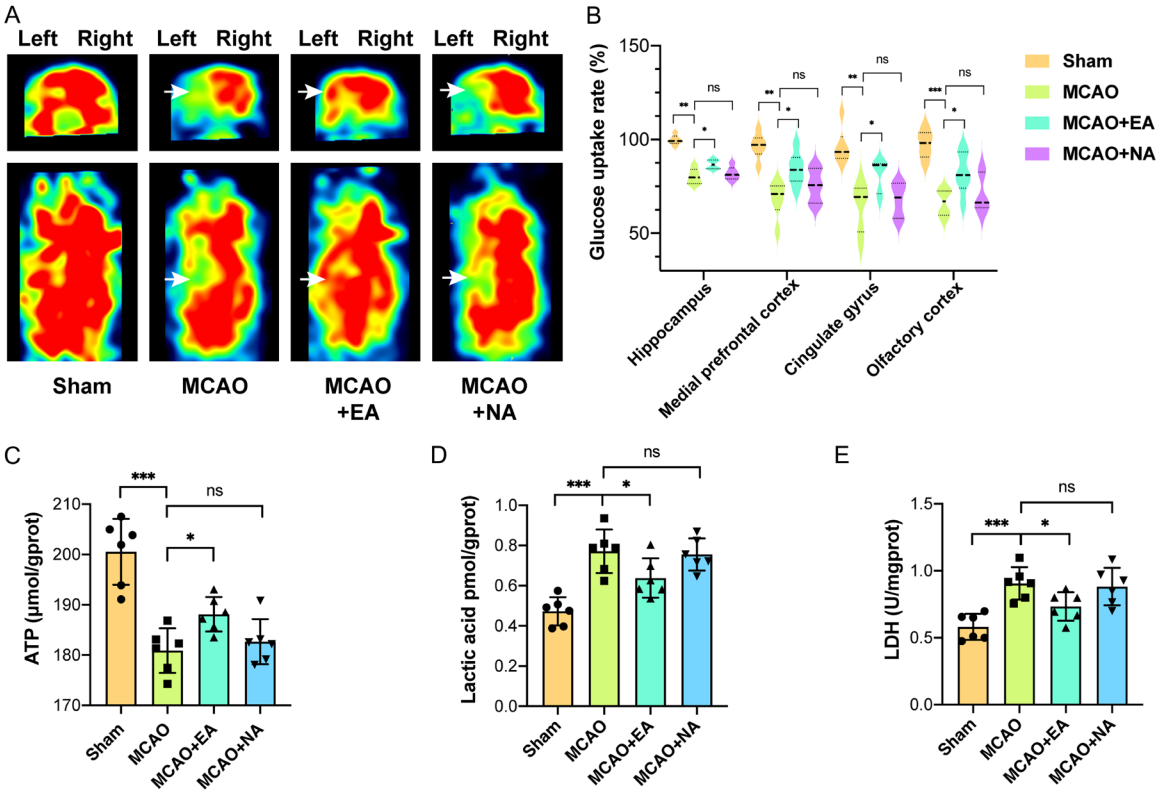

Figure 3. EA at Baihui (DU20) and Shenting (DU24) acupoints enhanced glucose uptake and metabolism. (A) PEC/CT images showing the glucose uptake extent in the sham group, the MCAO+EA group and the MCAO+NA group on day 7 after initial EA treatments. The white arrows indicate affected areas in the MCAO rats. (B) Statistical analysis results of glucose uptake rates that present the extent of cerebral glucose uptake in each group on day 7 after initial EA treatments. (C-E) ELISA data showing the hippocampal ATP (C), Lactic acid (D), and lactate dehydrogenase levels (E), in rats in the sham group, the MCAO+EA group and the MCAO+NA group on day 7 after initial EA treatments. Brain tissues from six randomly selected rats from each group were used for ELISA assay. *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant.

uptake during cerebral infarction progress, we conducted Western blot assays to measure the expression of glucose transporter genes GLUT1 and GLUT3 in the hippocampus. Our findings revealed that the expressions of GLUT1 and GLUT3 were significantly upregulated in the hippocampus of rats in the MCAO+EA group (**Figure 4A-C**). In contrast, there were no significant changes in the expressions of GLUT1 and GLUT3 in the hippocampus of rats in the MCAO+NA group. These data further suggested that EA at Baihui (DU20) and Shenting (DU24) acupoints could improve cerebral glucose uptake by increasing the expressions of GLUT1 and GLUT3.

Discussion

Stroke has been a major threat to public health, despite advances in its prevention and treatments [31]. The key factor to yield a favorable prognosis in stroke cases is receiving treatment within a narrow "time window", typically within a few hours after the initial stroke onset [32]. Unfortunately, a considerable portion of stroke patients still experience various post-stroke symptoms, for instance disability due to delayed treatment [33, 34]. Recovery from these symptoms often necessitates diverse therapeutic and rehabilitation approaches. Therefore, finding novel therapies to improve the post-stroke symptoms remains urgent in this field.

EA has been proved promising in the treatments of various diseases. For instance, EA has been shown to improve the motor function and increase bowel movements in patients with Parkinson's disease [35]. In addition, EA can significantly ease the chronic low back pain

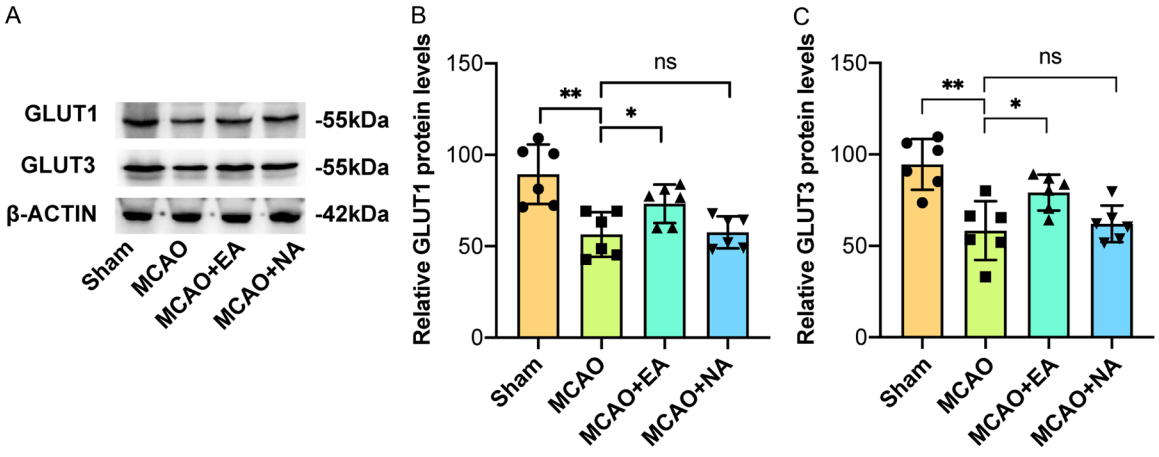

Figure 4. EA at Baihui (DU20) and Shenting (DU24) acupoints upregulated hippocampal GLUT1 and GLUT3 expression. (A) Western blot data showing the expressions of GLUT1 and GLUT3 in the left hippocampus of rats in each group. (B, C) Statistical analysis results in (A). Brain tissues from six randomly selected rats from each group were used for the Western blot assay. $^{*}P < 0.05$, $^{**}P < 0.01$, ns: not significant.

in adults [36]. It has also been found to improve insulin resistance in patients with type II diabetes mellitus [37]. As mentioned earlier, EA also exhibits neuroprotective effects on several neurological disorders by distinct mechanisms. Consistent with these observations, our current data suggested that EA at Baihui (DU20) and Shenting (DU24) acupoints could ameliorate I/R injury by promoting glucose uptake and metabolism via upregulating the expressions of hippocampal GLUT1 and GLUT3, providing supporting that EA can facilitate the recovery of patients from post-stroke events.

Cerebral tissues consume a significant portion of glucose under physiological conditions and are vulnerable to IS-caused deprivation of oxygen and glucose [28, 38]. During the process of cerebral IS, the cells in the affected area employ multiple mechanisms to enhance glucose uptake including upregulating the expressions of glucose transporter genes GLUT1 and GLUT3 [17]. Furthermore, increased expressions of GLUT1 and GLUT3 induced by existing compounds are positively associated with neuroprotective effects against IS [39-41]. In line with these reports, our data showed that the improved neurological deficit by EA at Baihui (DU20) and Shenting (DU24) acupoints was correlated with elevated hippocampal GLUT1 and GLUT3 expressions, providing a new therapeutic option in addition to existing chemicals to induce the expressions of GLUT1 and GLUT3 that facilitate patients' recovery from I/R injury.

However, given that multiple mechanisms might induce the expressions of GLUT1 and GLUT3, and that EA at Huantiao (GB30) and Yanglingquan (GB34) acupoints in rats with neuropathic pain exhibits an inhibitory effect on medial prefrontal cortical glucose metabolism and GLUT3 expression, it would be important to characterize the mechanism by which EA at Baihui (DU20) and Shenting (DU24) acupoints increases GLUT1 and GLUT3 expressions in the hippocampus [17, 42].

In contrast to previous studies, we specifically included a group of MCAO rats that received EA at non-acupoints, as acupuncture had been reported to have non-specific effects [13, 14, 43, 44]. Surprisingly, we did not observe any significant difference in neurological deficit scores, spatial learning ability and memory, cerebral infarction volume, and cerebral glucose uptake and metabolism between the MCAO group and the MCAO+EA group rats. This suggested that the neuroprotective effect of EA was specifically generated due to activation of Baihui (DU20) and Shenting (DU24) acupoints in the MCAO rat models. Interestingly, in an Alzheimer's disease mouse model, EA at Baihui (DU20) and Yintang (DU29) acupoints was found to have a neuroprotective effect as well [45]. It would be meaningful to explore whether EA at other acupoints may help MCAO rat models recover from post-stroke symptoms.

It is noteworthy that the effects of increased glucose level on IS-injured cerebral tissues

remain uncertain. While elevated glucose uptake help cerebral tissues survive from IS attack, a high blood glucose level suggests a poor outcome of the disease [46]. However, the causality between a high circulating glucose level and a poor prognosis of IS remains unclear [46]. Our data support the concept that enhanced glucose consumption was positively related to better recovery from I/R injury. Nevertheless, this might be an indirect effect as EA at Baihui (DU20) and Shenting (DU24) acupoints could improve the survival and function of cerebral cells by employing other aforementioned mechanisms, thereby enhancing their glucose uptake and metabolism. Due to the constraints of experimental conditions such as the MWM test, this study selected only the 7-day time point for mechanistic exploration and did not investigate other time points. Additionally, while changes in GLUI1 and GLUT3 in hippocampal tissues were observed before and after EA treatment, the specific molecular pathway mechanisms were not explored. Further investigations are warranted to clarify the causality between enhanced glucose uptake and attenuated neurological deficits mediated by EA at Baihui (DU20) and Shenting (DU24) acupoints.

In conclusion, our study has suggested that EA at Baihui (DU20) and Shenting (DU24) acupoints has specific effects in improving neurological deficits, reducing cerebral infarction volume, and enhancing cerebral glucose uptake and metabolism in the MCAO modeled rats. These results highlight the potential efficacy of EA as an alternative therapy for treating post-stroke symptoms.

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Disclosure of conflict of interest

None.

Abbreviations

EA, Electroacupuncture; NA, Non-acupoint; DU20, Baihui; DU24, Shenting; IS, Ischemic stroke; I/R, Ischemia/reperfusion; MCAO, Middle cerebral artery occlusion; GLUT1, Glucose Transporter 1; GLUT3, Glucose Transporter 3; MWM, Morris Water Maze; MCA, Midline cervical incision; CCA, Common carotid artery; ECA, External carotid artery; ICA, Internal carotid artery; MRI, Magnetic resonance imaging; PET/CT, Positron emission tomography/computed tomography; ELISA, Enzyme-linked immunosorbent assay; LDH, Lactate dehydrogenase; LA, Lactic acid.

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