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Electrical stimulation of the sciatic nerve improves cognitive function in rats with chronic cerebral ischemia via the PGC-1α/FNDC5/BDNF signaling pathway

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A B S T R A C T

Objective: To investigate the potential mechanism of electrical stimulation (ES) of the sciatic nerve on cognitive function in rats with chronic cerebral ischemia (CCI), and the expression of the peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α)/fibronectin type III domain-containing protein 5 (FNDC5)/irisin/brain-derived neurotrophic factor (BDNF) signaling pathway in the hippocampus and skeletal muscle.

Methods: Twenty-four male Sprague-Dawley rats were randomly assigned to the ES, CCI, and sham groups. CCI models were established via bilateral common carotid artery occlusion. The ES group underwent bilateral sciatic nerve ES (1 mA, 2/20 Hz, 20 min/day) for 4 weeks. Cognitive function, hippocampal morphology, serum irisin levels, and pathway-related protein/mRNA expression were assessed by Morris Water Maze (MWM), HE staining, ELISA, Western blot and RT-PCR, respectively.

Results: The behavioral tests demonstrated that sciatic nerve ES significantly ameliorated CCI-induced cognitive deficits, as evidenced by shorter escape latency, increased platform crossings, and prolonged time in the target quadrant. HE staining revealed that ES alleviated neuronal damage in the hippocampus. The molecular investigations demonstrated that sciatic nerve ES activated the PGC-1α/FNDC5/BDNF signaling pathway: specifically, sciatic nerve ES upregulated PGC-1α expression in skeletal muscle, leading to increased cleavage of FNDC5 and secretion of irisin, elevated irisin levels subsequently enhanced hippocampal BDNF expression, which was associated with improved neuronal survival and reduced apoptotic activity.

Conclusion: Sciatic nerve ES improves CCI-induced cognitive impairment by promoting neuronal repair and reducing apoptosis, which may be mediated by upregulating the PGC-1α/FNDC5 (irisin)/BDNF pathway in the brain and skeletal muscle.

Keywords: chronic cerebral ischemia; cognitive dysfunction; electrical stimulation of the sciatic nerve; irisin; brain-derived neurotrophic factor

1. Introduction

Chronic cerebral ischemia (CCI), a chronic cerebral hypoperfusion, is a prevalent cerebral ischemic injury and a pathological process that contributes to the onset and development of multiple cognitive disorders, including vascular dementia [1]. Currently, the pathophysiological mechanism of cognitive dysfunction caused by CCI remains unknown. Several studies suggest that CCI can result in impaired vascular endothelial function and a damaged blood-brain barrier [2,3]. Additionally, it can induce oxidative stress, increase neuroinflammatory response, and cause neuronal structural damage, which in turn results in permanent neurological damage and cognitive impairment [4–6]. The effectiveness of rehabilitation treatment and the quality of life are significantly impacted by the inability of patients with cognitive dysfunction of CCI to actively participate in such treatment. However, there remains a lack of optimal means and methods for preventing and treating cognitive dysfunction in CCI. Consequently, there has been a growing interest in seeking effective methods to improve cognitive dysfunction after CCI and improve the rehabilitation efficacy and quality of life. Several studies have demonstrated that electrical stimulation (ES) of the sciatic nerve can stimulate glial cell proliferation and promote nerve regeneration in the brain [7-9]; however, the underlying mechanism remains unclear.

Irisin is a muscle factor that is cleaved from fibronectin type III domain protein5 (FNDC5). FNDC5 is a transmembrane precursor protein that is expressed in muscle under the control of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). The irisin levels are closely associated with the degree of activation of the PGC-1α/FNDC5 pathway [10]. Irisin acts as a neuroprotective factor in the body, increasing synaptic plasticity and neurogenesis, and influencing the production of brain-derived neurotrophic factor (BDNF), thereby enhancing cognitive function [11]. Recent studies have demonstrated that exercise can directly stimulate the expression of FNDC5 in the brain, thereby influencing the brain's microstructure and function through the PGC-1α-FNDC5-BDNF signaling pathway [12]. Additionally, muscle contraction during exercise can stimulate skeletal muscle synthesis and the secretion of a significant quantity of irisin, which in turn increases the brain's secretion of BDNF [13,14].

In this study, we investigated the effect of ES of the sciatic nerve on PGC-1α, FNDC5, and BDNF expression in the brains and skeletal muscles of rats with CCI. Furthermore, we aimed to investigate the potential mechanism of ES of the sciatic nerve in improving the learning and memory abilities of rats with CCI and provide a novel theoretical foundation and treatment approach for the rehabilitation of patients with cognitive dysfunction associated with CCI.

2. Materials and methods

2.1. Animals and experimental design

All experimental procedures complied with the Code of Welfare and Ethics for Laboratory Animals and were approved by the Experimental Animal Ethics Committee of Anhui Medical University (approval number: LLSC20252876). Twenty-four specific-pathogen-free (SPF) male Sprague-Dawley (SD) rats (10-12 weeks old, 175-200 g) were obtained from the Experimental Animal Center of Anhui Medical University (license number: SYXK (Anhui) 2022-004) and housed under controlled environmental conditions: ventilated and dry housing, ambient temperature at 20-25 °C, relative humidity maintained at approximately 40%-70%, and a 12-hour light/dark cycle. Rats had free access to food and water and were allowed to acclimate for one week. A summary of the study design is presented in Fig.1.

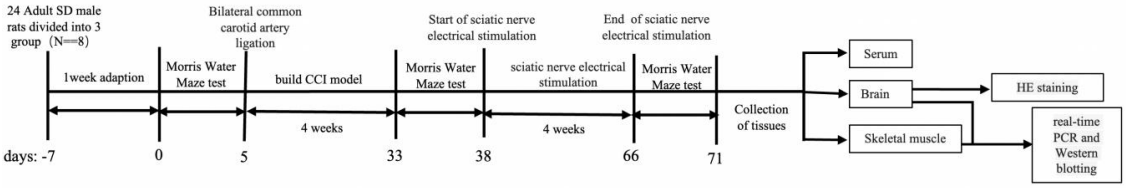


Fig. 1. Experimental timeline for this experiment.

2.2. Grouping of experimental animals

After screening, twenty-four rats were randomly assigned to three groups: the sham operation group (sham, n = 8), the CCI model group (CCI, n = 8), and the ES of the sciatic nerve group (ES, n = 8). Those who could not swim, had a weak physique, poor eyesight, or did not meet the study's inclusion criteria were excluded from the study. The Morris water maze (MWM) was used to assess escape latency, the number of platform crossings, and residence time in the target quadrant for all rats. The statistical analysis was performed to confirm that there was no significant difference in the learning and memory functions of the rats between the two groups ($P > 0.05$).

2.3. Animal model of CCI

From the model and the stimulation groups, sixteen rats each were used to establish a CCI model through bilateral common carotid artery occlusion (BCCAO) [15,16]. The rats were anesthetized intraperitoneally with a 1.25% tribromoethanol solution at a dosage of 10 mL/kg body weight. Subsequently, the neck hair was meticulously shaved to expose the underlying skin. A midline ventral incision was made on the neck, followed by careful dissection and exposure of both common carotid arteries (CCAs) from their sheaths. The bilateral CCAs were permanently occluded using 4-0 silk sutures, and the ligation site was confirmed to have a slowed blood flow and distal blood vessel non-filling. The incision was then sutured layer by layer. After re-disinfection, each rat received an intramuscular injection of 80,000 units of penicillin to prevent infection. Rats in the sham group underwent the same procedures as described above, except that the CCA was not ligated. Throughout the surgical modeling process, strict adherence to operational protocols was maintained. Four rats were excluded due to intraoperative hemorrhage, postoperative infection, or the onset of motor dysfunction, and were promptly replaced.

2.4. MWM test

Four weeks after bilateral CCA ligation, a second MWM-based navigation and spatial exploration test was performed to evaluate spatial learning and memory in the three groups of rats [17]. The circular pool was divided into four quadrants (I-IV), with a hidden platform (10 cm in diameter and 30 cm in height) placed in quadrant IV. Each quadrant was marked by distinct visual cues, consisting of fluorescent cards of different colors attached to the pool walls, and a black curtain was arranged around the outer perimeter to minimize external interference. The pool was filled with water to a level 2 cm above the platform, and the water temperature was maintained at 26 ± 1 °C. The experimental environment was kept quiet and dimly lit throughout the testing period.

2.4.1. Navigational task

The first four days were dedicated to the navigational training. At the beginning of each trial, rats were gently placed into the pool from the midpoint of each quadrant, facing away from the center of the

maze to prevent prior visual identification of the platform. Escape latency was defined as the time from water entry to climbing onto the platform and was recorded using the AnyMaze tracking system. If a rat failed to locate the platform within 90 s, the escape latency was recorded as 90 s, and the rat was gently guided to the platform using a rod. Rats were then allowed to remain on the platform for 10 s to observe the surrounding visual cues before the next trial. The mean escape latency across the four quadrants on day 4 was used for subsequent analysis.

2.4.2. Spatial exploration experiment

The spatial exploration task was conducted on day 5. After removal of the platform, rats were released from the midpoint of the quadrant opposite the original platform location, facing the pool wall. Swimming trajectories were recorded for 90 s, and the number of platform crossings and the time spent in the target quadrant (quadrant IV, the original platform location) were analyzed to evaluate spatial memory performance.

2.5. ES of the sciatic nerve

Following the second Morris water maze test, bilateral sciatic nerve electrical stimulation was initiated in the stimulation group. In rats, the L4-S1 nerve roots emerge from the spinal cord and converge to form the lumbosacral plexus, which continues distally as the sciatic nerve trunk. The sciatic nerve courses anterior to the sacroiliac joint and then passes over the greater trochanter of the femur toward the posterior aspect of the thigh. At the level of the superficial projections of the superior cluneal vessels, the sciatic nerve trunk divides into the sural, tibial, and common peroneal nerves [18,19]. During stimulation, rats were immobilized, and stimulation sites were selected bilaterally at the posterosuperior margins of the hip joints, where the sciatic nerve trunk passes over the greater trochanter, and at points 0.5 cm below the femur, proximal to the nerve bifurcation. These sites were connected to an electroacupuncture device. Stimulation was delivered using dense-sparse waves at frequencies of 2 and 20 Hz, with an intensity of 1 mA adjusted to induce slight muscle twitching without vocalization or struggling. Stimulation was administered once daily for 20 min over a period of 4 consecutive weeks [16,20]. Rats in the sham group were similarly fitted with electrodes but received no electrical stimulation. All animals were maintained under standard housing conditions throughout the intervention period, and no mortality or adverse reactions were observed. Four weeks after the completion of stimulation, a third Morris water maze assessment was conducted in all groups.

2.6. Hematoxylin-eosin (HE) staining

Following the third MWM assessment, rats underwent deep anesthesia. After thoracotomy, a perfusion needle was inserted into the left ventricle, and the right atrium was incised. Cardiac perfusion was performed with 200 mL of normal saline until the liver appeared pale and the effluent blood cleared. This was followed by perfusion with 150 mL of 4% paraformaldehyde at 4 ℃ until limb twitching and rigidity were observed. Following decapitation and brain extraction, the tissues were fixed with 4% paraformaldehyde overnight, then embedded in paraffin, and sectioned into 5-μm-thick slices. The sections were dewaxed following standard histochemical protocols, stained with hematoxylin for 5 min, washed with running water until nuclear bluing was observed, counterstained with eosin for 3 min, cleared, and cover-slipped with neutral resin. The morphological alterations of hippocampal neurons were observed and photographed under light microscopy at 200× magnification.

2.7. Enzyme-linked immunosorbent assay (ELISA)

Following the third MWM assessment, the rats were deeply anesthetized, positioned supine on the operating table, and their incisors and limbs were secured. After disinfection, a layered incision was made from below the xiphoid process along the inferior costal margin to expose the abdominal cavity and liver. The diaphragm was incised to open the chest, and the pericardium was stripped to expose the heart. A blood collection needle was inserted into the cardiac apex, slightly supero-rightward, to collect 3 mL of blood. The blood was centrifuged, and serum was collected and stored in a -80 ℃ ultra-low freezer for subsequent use. For ELISA, the kit was removed from the refrigerator 30 min before use and equilibrated to room temperature. Samples were diluted to various concentrations, and working solutions were prepared sequentially using concentrated biotin and streptavidin-horseradish peroxidase conjugate. Test samples were added to the ELISA plate wells, followed by sequential addition of antibody-enzyme conjugate, tetramethylbenzidine, chromogenic substrate solution, and stop solution. The reaction was terminated when the color changed from blue to yellow, and the optical density (OD) of each well was measured at 450 nm.

2.8. Reverse transcription polymerase chain reaction (RT-PCR) analysis

Brain and muscle tissues from all rats were rapidly harvested as previously described and subsequently detected using RT-PCR. Total RNA was extracted using TRIzol reagent kit (Invitrogen, USA) according to the manufacturer's instructions. Subsequently, chloroform and isopropanol were sequentially added to the samples, followed by centrifugation. To eliminate genomic DNA from total RNA, 1 μL of gDNA digester, 2 μL of gDNA digester buffer, 1 ng–1 μg of total RNA, and DEPC-treated water (DEPC-H_2O) were added to each reaction tube. A total of 10 μL of the reaction mixture from the previous step was collected for further processing. Subsequently, 2 μL of Hifair II enzyme mix, 2 μL of Hifair II buffer plus, 1 μL of $Oligo(dT)_{18}$, and an appropriate volume of nuclease-free water (ddH_2O) were added to the above reaction mixture. PCR reactions were performed using the SYBR® Green PCR Kit (Thermo Scientific, USA) on a LightCycler 480 II RT-PCR system (Roche, Switzerland) under the following thermal cycling conditions: initial denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, and extension at 72 °C for 15 s. The primer sequences used in this study are listed in Table 1. The relative expression levels of target mRNA were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and quantified using the $2^{-\Delta\Delta Ct}$ method.

Table 1 Primer nucleotide sequences for RT-RCR

Gene name	Sequences (5′-3′)
PGC-1α	Forward: ACCACAAACGATGACCCTCC
	Reverse: CCCTTGGGGTCATTTGGTGA
FNDC5	Forward: CTCTTCATGTGGGCAGGTGTC
	Reverse: TGGGCTCGTTGTCCTTGATG
BDNF	Forward: AGTCCCACTGAATCCCCTGT
	Reverse: CCTAATCGACCTGGCCCTTC
GAPDH	Forward: GGTGAAGGTCGGTGTGAACG
	Reverse: CTCGCTCCTGGAAGATGGTG

Note: BDNF: brain-derived neurotrophic factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; FNDC5: fibronectin type III domain-containing protein 5; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1α; RT-RCR: reverse transcription polymerase chain reaction.

2.9. Western blot

Brain and muscle tissues stored in liquid nitrogen were homogenized and lysed, followed by centrifugation to collect the supernatant. Protein concentrations were determined using the bicinchoninic acid (BCA) assay prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Equal amounts of protein were mixed with loading buffer, separated by SDS-PAGE, and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes using constant-current electrophoresis. The membranes were blocked at room temperature for 1 h, washed with Tris-buffered saline containing Tween 20 (TBST), and incubated overnight at 4 °C with primary antibodies, including: PGC-1α (WL02123, 1:1000, Proteintech), FNDC5 (23995-1-AP, 1:1000, Proteintech), BDNF (28205-1-AP, 1:1000, Proteintech), GAPDH (10494-1-AP, 1:5000, Proteintech) as loading controls. After incubation with the corresponding secondary antibodies, protein bands were visualized using chemiluminescent detection.

2.10. Statistical analysis

All statistical analyses were conducted using SPSS version 25.0 (IBM Corp., USA). Data normality was evaluated using the Shapiro-Wilk test, and homogeneity of variance was assessed with Levene's test. Data with a normal distribution are expressed as mean ± standard deviation ($\overline{x} \pm s$). One-way analysis of variance was used for intergroup comparisons across multiple groups, with a least significant difference t-test post hoc for pairwise comparisons. Paired-samples t-tests were performed for within-group pre-post intervention comparisons. Non-normally distributed data are expressed as M (P_{25}, P_{75}). The Kruskal-Wallis test was used for intergroup comparisons. For pairwise multiple comparisons, the Bonferroni correction was used (three comparisons total), and an adjusted $P < 0.05$ was considered significant. For all analyses, the statistical significance was set at $P < 0.05$. Experimental results were visualized using GraphPad Prism software (version 9.4).

3. Results

3.1. Effects of sciatic nerve electrical stimulation on spatial learning and memory

As shown in Fig. 2, the MWM was used to evaluate spatial learning and memory in rats. Before modeling, no significant differences in escape latency were observed among the three groups ($P > 0.05$; Fig. 2a). In the sham group, escape latency progressively decreased across the pre-modeling, pre-intervention, and post-intervention time points. In contrast, the CCI group exhibited a progressive prolongation of escape latency. In the ES group, escape latency was significantly prolonged after modeling compared with the pre-modeling period, whereas it was significantly shortened after intervention compared with the pre-intervention period ($P < 0.05$). During the probe test, the sham group showed significant increases in both the number of platform crossings and the time spent in the target quadrant after modeling ($P < 0.05$; Figs. 2b-c), with no further significant changes following the intervention. In contrast, both the CCI and ES groups exhibited significant reductions in these parameters after modeling ($P < 0.05$). After intervention, the CCI group showed no significant improvement in platform crossings and a further decrease in time spent in the target quadrant ($P < 0.05$). Conversely, rats in the ES group demonstrated significant increases in both the number of platform crossings and the time spent in the target quadrant compared with the pre-intervention period ($P < 0.05$). Intergroup comparisons after intervention are shown in Figs. 2d-f. Compared with the sham group, the ES group exhibited longer escape latency, fewer platform crossings, and reduced time spent in the target quadrant

($P < 0.05$). However, compared with the CCI group, the ES group showed significantly shorter escape latency, increased platform crossings, and prolonged time spent in the target quadrant ($P < 0.05$). Representative swimming trajectories (Fig. 2g) further demonstrated that ES of sciatic nerve improved search strategy efficiency, as rats in the ES group predominantly exhibited target-oriented swimming patterns, whereas rats in the pre-intervention ES phase and the CCI group mainly displayed random and thigmotactic behaviors.

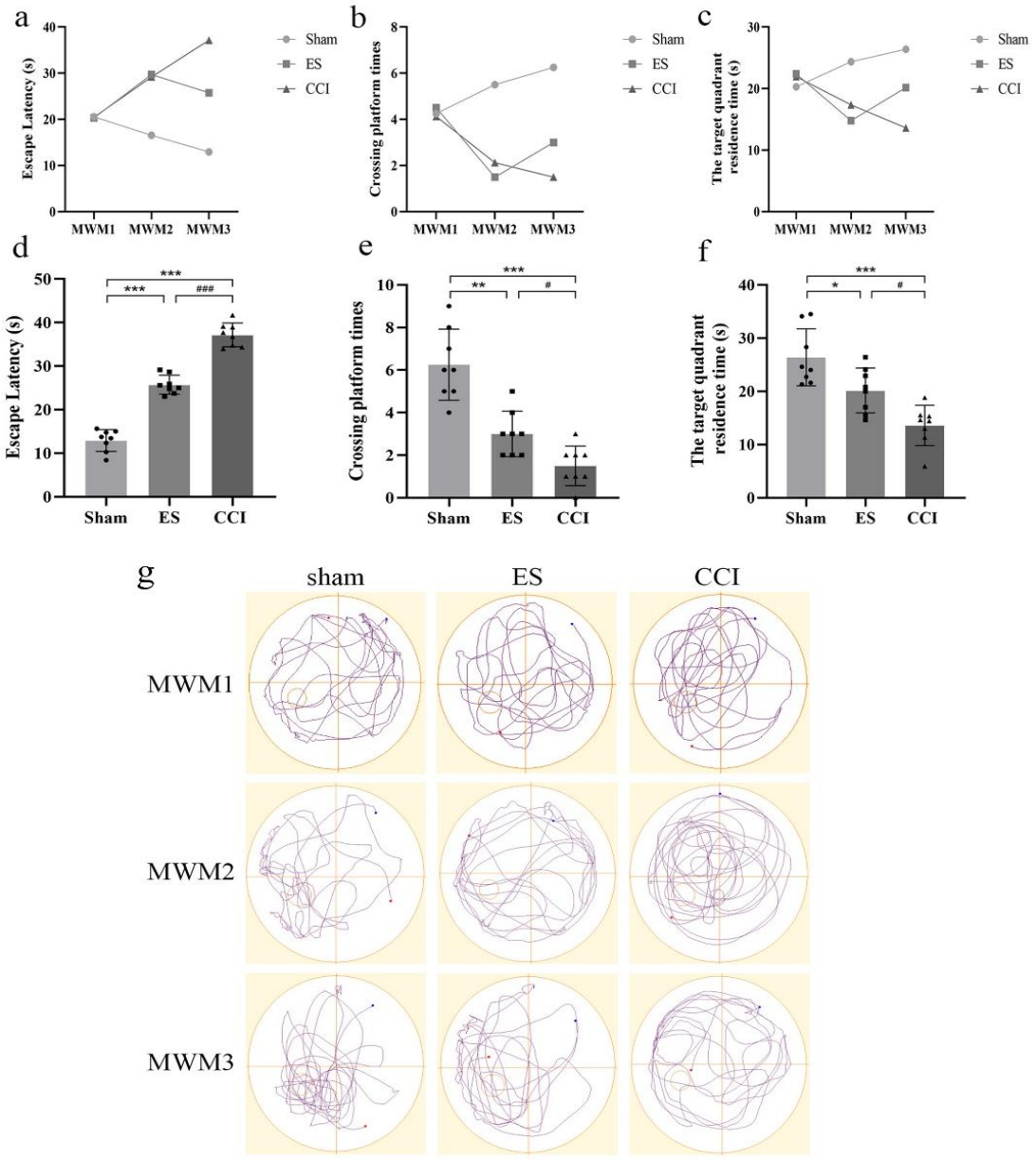


Fig. 2. Morris water maze results. **(a)** Escape latency during the navigation task at different experimental time points. **(b)** Number of platform crossings during the probe test. **(c)** Time spent in the target quadrant during the probe test. **(d-f)** Escape latency, platform crossings, and time spent in the target quadrant after intervention. **(g)** Representative swimming trajectories during the probe test. Data are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the sham group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. the CCI group.

3.2. Effects of sciatic nerve electrical stimulation on hippocampal neuronal morphology

Following four weeks of intervention, morphological changes in hippocampal neurons were assessed by HE staining (Fig. 3). In the sham group, hippocampal neurons were densely packed with

well-organized layers, clear cellular boundaries, distinct nuclei, and uniform cytoplasmic staining, indicating normal neuronal morphology (Fig. 3a). Compared with the CCI group, the ES group exhibited increased neuronal layers, a more orderly cellular arrangement, and a higher proportion of neurons with intact morphology, with fewer collapsed or damaged cells observed (Fig. 3b). In contrast, the CCI group showed marked neuronal damage, characterized by reduced cell layers, disorganized arrangement, indistinct cellular structures, uneven staining, and a large number of morphologically abnormal neurons (Fig. 3c). These findings indicate that ES of the sciatic nerve may alleviate hippocampal neuronal damage induced by CCI and contribute to the preservation of neuronal structural integrity.

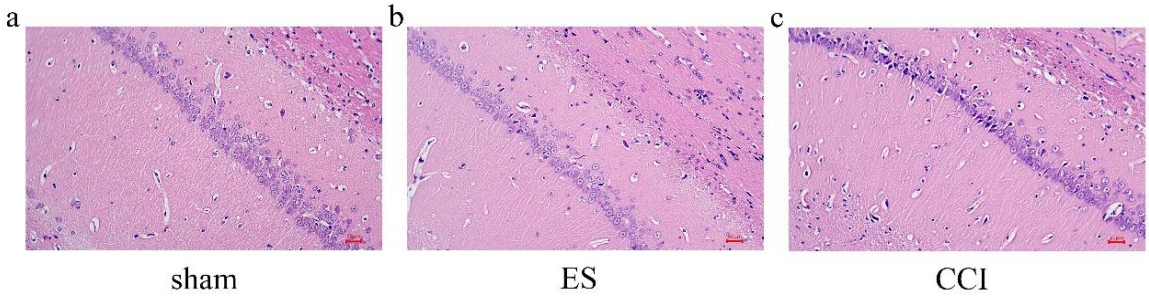


Fig. 3. HE staining of hippocampal neurons in different groups after four weeks of ES. Representative images from the **(a)** sham, **(b)** ES, and **(c)** CCI groups are shown. All images were acquired at 200× magnification.

3.3. Effects of sciatic nerve electrical stimulation on serum irisin levels

Following four weeks of intervention, serum irisin levels were quantified using ELISA assay, and the results are shown in Fig. 4. The CCI group exhibited a significantly lower serum irisin concentration compared with the sham group ($P < 0.05$). Compared with the CCI group, serum irisin levels were significantly increased in the ES group ($P < 0.05$), although they remained significantly lower than those observed in the sham group ($P < 0.05$). These findings indicate that CCI markedly reduces circulating irisin levels, and ES of sciatic nerve partially restores serum irisin expression.

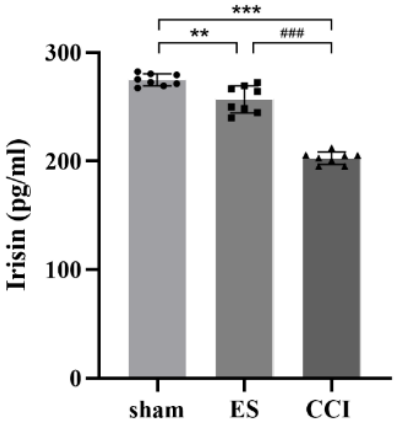


Fig. 4. Irisin levels in the serum of rats in each group after four weeks of ES. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the sham group. $^{\#\#\#}P < 0.001$ vs. the CCI group.

3.4. Effects of sciatic nerve electrical stimulation on PGC-1α, FNDC5, and BDNF mRNA expression in the brain and skeletal muscle

Following four weeks of intervention, the mRNA expression levels of PGC-1α, FNDC5, and BDNF in the brain and biceps femoris were quantified by RT-PCR, as shown in Fig. 5. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Compared with the sham group, the CCI group exhibited significantly reduced mRNA expression levels of PGC-1α, FNDC5, and BDNF in both the brain and biceps femoris ($P < 0.05$). In the ES group, the mRNA expression levels of FNDC5 and BDNF in both tissues were significantly increased compared with the CCI group ($P < 0.05$), although they remained significantly lower than those observed in the sham group ($P < 0.05$). These results suggest that CCI may suppress the transcription of PGC-1α, FNDC5, and BDNF related genes, whereas ES of sciatic nerve may partially reverse these alterations in both the brain and skeletal muscle.

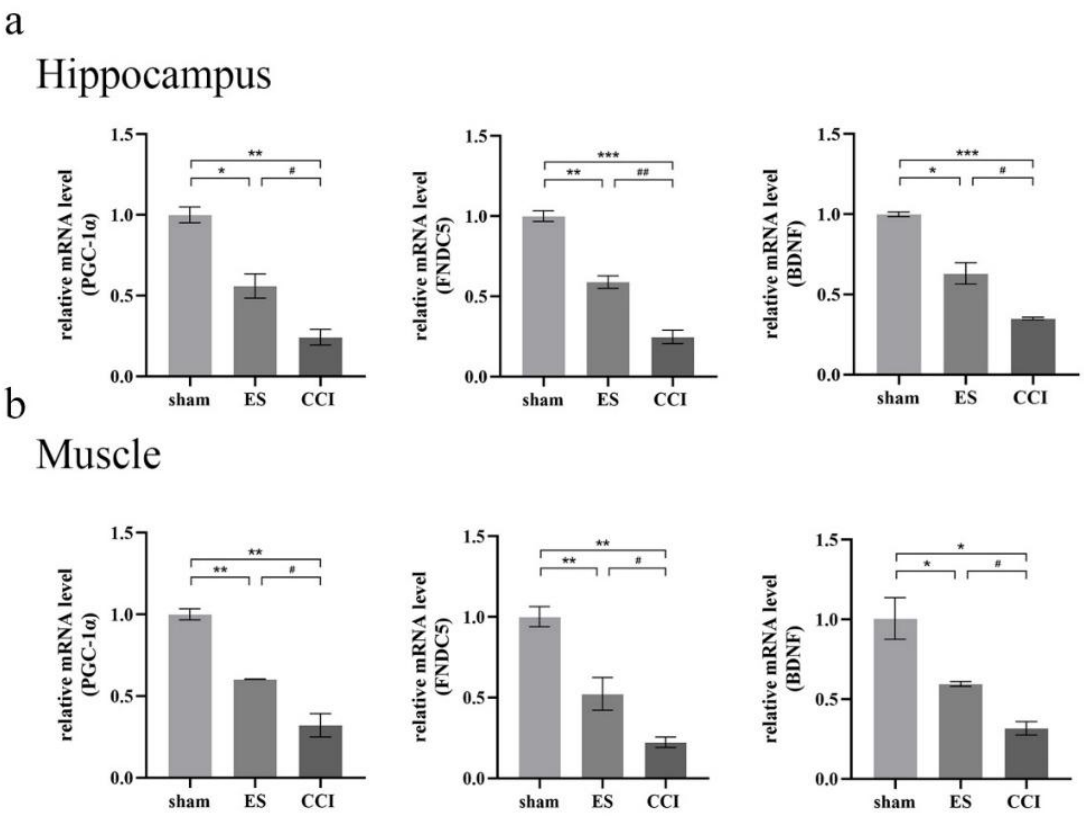


Fig. 5. Expression levels of PGC-1α, FNDC5, and BDNF in brain and skeletal muscle after four weeks of ES. **(a)** mRNA expression levels of PGC-1α, FNDC5, and BDNF in the hippocampus. **(b)** mRNA expression levels of PGC-1α, FNDC5, and BDNF in skeletal muscle. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the sham group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the CCI group.

3.5. Effects of sciatic nerve electrical stimulation on PGC-1α, FNDC5, and BDNF protein expression in the brain and skeletal muscle

Following four weeks of ES, the protein expression levels of PGC-1α, FNDC5, and BDNF in the brain and biceps femoris were analyzed by Western blot, as shown in Fig. 6. Relative protein expression levels were quantified using ImageJ software. Compared with the sham group, the CCI group exhibited significantly reduced protein expression levels of PGC-1α, FNDC5, and BDNF ($P < 0.05$). Although protein expression levels of these three proteins in the ES group remained significantly lower than those in the sham group ($P < 0.05$), they were significantly increased compared with the CCI group ($P < 0.05$). These findings indicate that CCI may markedly suppress the protein expression of PGC-1α, FNDC5, and BDNF, whereas ES of sciatic nerve partially restore their expression in both the brain and skeletal muscle.

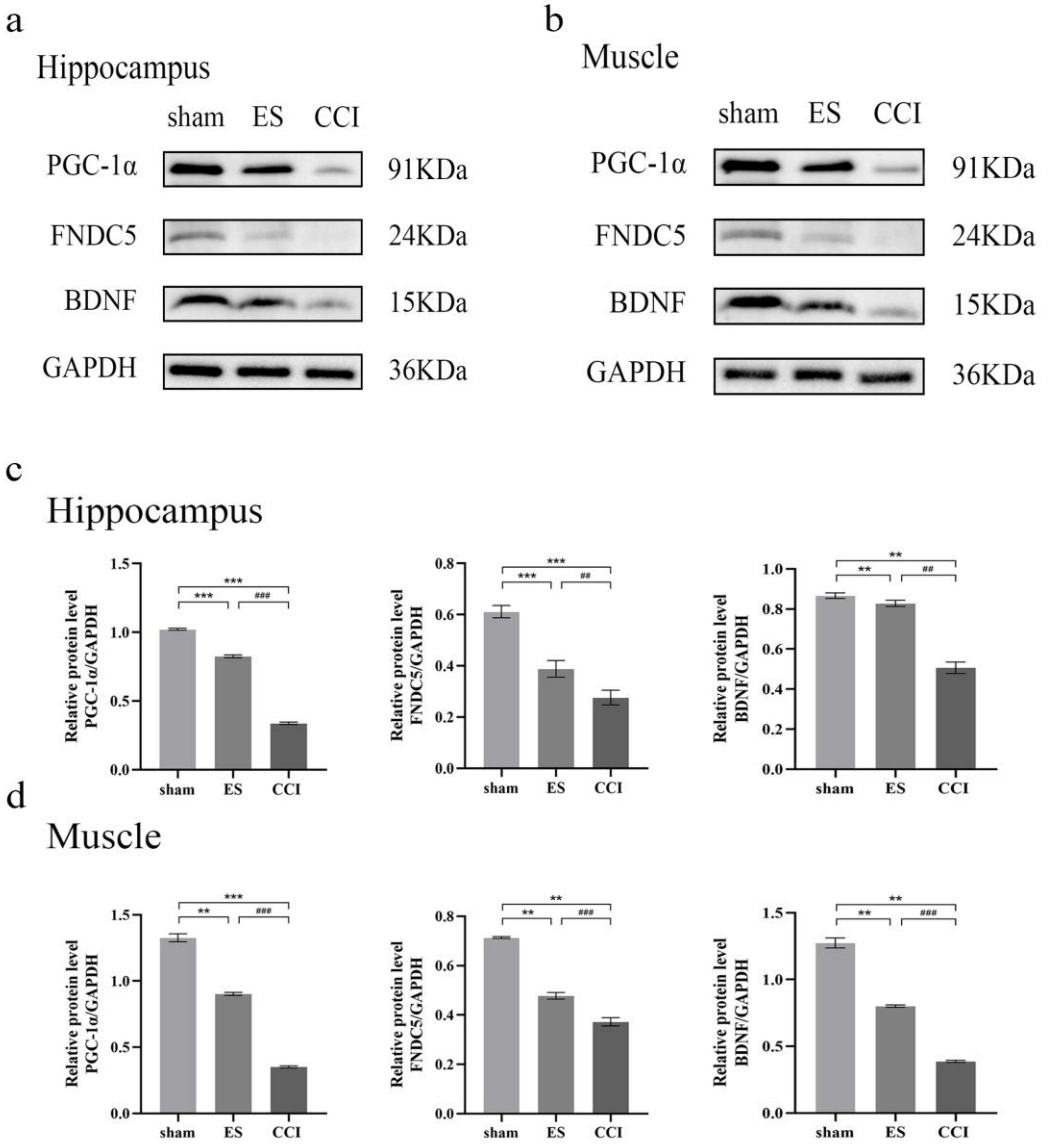


Fig. 6. Protein expression levels of PGC-1α, FNDC5, and BDNF in the brain and skeletal muscle after four weeks of ES, as determined by Western blot. (a, b) Representative Western blot bands of PGC-1α, FNDC5, and BDNF in the hippocampus (a) and skeletal muscle (b). (c, d) Quantitative analysis of relative protein expression levels of PGC-1α, FNDC5, and BDNF in the hippocampus (c) and skeletal muscle (d), normalized to GAPDH. Data are presented as mean ± SD from three independent biological replicates. **P < 0.01, ***P < 0.001 vs. the sham group; ##P < 0.01, ###P < 0.001 vs. the CCI group.

4. Discussion

CCI is a pathological condition characterized by a persistent decrease in cerebral blood flow secondary to cerebral small vessel disease. Among various brain structures, regions including the hippocampus, cerebral cortex, striatum, and cerebellum exhibit high sensitivity to ischemic insults [21]. Additionally, the hippocampus, comprising the hippocampal formation and the dentate gyrus, is embedded within the anterior temporal cortex and plays a pivotal role in higher cognitive functions, including learning and memory [22]. Prolonged cerebral hypoperfusion can induce global ischemia and hypoxia in brain tissues, resulting in progressive and irreversible neural damage as well as cognitive

impairment. Over the past few decades, multiple studies have demonstrated that cerebrovascular diseases are a major contributor to cognitive impairment [23,24]. A significant proportion of dementia cases are attributed to underlying vascular factors, with up to 70% of all dementia cases being attributed to these factors [25]. According to epidemiological statistics, vascular cognitive impairment is the second most prevalent form of cognitive impairment worldwide, due to its high incidence, disability, and mortality rates [26,27]. As one of the most pressing public health challenges, it poses a significant threat to human survival and quality of life, thereby severely impacting global health. This study used bilateral common carotid artery occlusion to establish a CCI model in rats. It utilized the MWM to determine the learning and memory capabilities of the subjects. The results revealed that, at 4 weeks post-surgery, the escape latency of the model group was significantly longer than that of the sham operation group ($P < 0.05$), with fewer platform crossings ($P < 0.05$), and a reduced time spent in the target quadrant ($P < 0.05$). Therefore, it is concluded that the learning and memory functions of the model group rats were significantly impaired, a finding consistent with those of other studies.

Electrical stimulation is an intervention technique that utilizes currents with specific frequencies, intensities, amplitudes, and durations to affect neuromuscular tissues. This technique delivers converted ionic currents into the body through electrodes, thereby increasing tissue excitability and achieving preventive and therapeutic effects. In 1790, the Italian physician Luigi Galvani performed the renowned frog leg experiment, in which he observed that electrical stimulation of isolated frog legs induced muscle contractions [28]. This discovery laid the foundation for the application of electrical stimulation therapy. With technological advancements and the rapid progress of medical science, various electrical stimulation techniques have been developed, including neuromuscular electrical stimulation, transcutaneous electrical nerve stimulation, functional electrical stimulation, spinal nerve stimulation, median nerve stimulation, vagus nerve stimulation, deep brain stimulation, and acupoint electrical stimulation [29].

The sciatic nerve, the largest peripheral nerve in the human body, innervates most of the muscle groups in the lower limbs, including the biceps femoris and triceps surae [30]. ES of the sciatic nerve can induce contractions in its innervated muscles, exhibiting extensive potential applications in nerve repair, increasing muscle strength, and improving motor function. Xing et al. [31] reported that electrical stimulation of the injured sciatic nerve significantly increased the number of glial cells in the gastrocnemius muscle, which could be associated with an elevation in muscle cell count and the cross-sectional area of muscle fibers. Li et al. [32] found that sciatic nerve electrical stimulation could promote the rehabilitation of peripheral nerve injuries. Additionally, other studies have demonstrated that sciatic nerve electrical stimulation can alleviate autonomic nervous system dysfunction and muscle tone disorders in patients with complex regional pain syndrome type I [33]. Recently, studies on the sciatic nerve have indicated that its electrical stimulation can regulate the homing process of endothelial progenitor cells, thereby contributing to neovascularization. Zheng et al. [34] confirmed that sciatic nerve electrical stimulation ameliorated cognitive deficits in rats with cranial injuries. This was demonstrated by an increased percentage of time spent in the target quadrant during the MWM test, along with elevated numbers of CD3 cells and von Willebrand factor in the injured hippocampal region. These physiological changes are associated with vascular remodeling in the body following brain injury. This indicates a correlation with the increased number of endothelial progenitor cells in peripheral blood induced by sciatic nerve electrical stimulation. Wang et al. [35] performed sciatic nerve-related acupoint electrical stimulation on a rat model of middle cerebral artery occlusion. They observed a decrease in cerebral infarct volume and an increase in the expression of vascular endothelial growth factor and BDNF in brain

tissue. Currently, the mechanisms underlying the physiological changes induced by sciatic nerve electrical stimulation remain unclear. These changes can be associated with alterations in physiological information within the body resulting from localized skeletal muscle contractions during electrical stimulation. Takahiro et al. [36] exposed the sciatic nerve in deeply anesthetized rats and applied localized electrical stimulation through an implanted stimulator to induce rapid maximal isometric contractions in the triceps surae. The stimulation parameters were set at 100 Hz, a pulse duration of 1 ms, and a stimulation period of 3 s. After 50 repetitions of maximal isometric contractions, compared with the sham operation group, the expression of BDNF protein and its receptor activation in the hippocampus significantly increased. However, the expressions of BDNF and FNDC5 in the stimulated muscle and peripheral blood exhibited no significant change, and serum concentrations remained unchanged [29]. These results indicate that localized electrical stimulation-induced muscle contractions in an unconscious state can directly upregulate BDNF expression in the hippocampus. Furthermore, Gu et al. [37] administered a 7-day electroacupuncture treatment to a focal middle cerebral artery occlusion model established using the suture method. The acupoints were selected at the affected Quchi and Zusanli points. The stimulation parameters were set to a sparse-dense wave frequency of 2 Hz/15 Hz, with a current intensity of 2-4 mA (adjusted to achieve a slight muscle twitch), administered for 20 min per session, once daily for 7 consecutive days. Concurrently, the expression levels of PGC-1α, irisin, and BDNF were determined in the perilesional cortex, hippocampus, and skeletal muscle at the acupoint site. The results indicated that electroacupuncture could activate the cortical PGC-1α/irisin/BDNF pathway, while the expression levels of PGC-1α, irisin, and BDNF in the local muscle remained unchanged. This suggests that the lack of significant changes in the expressions of PGC-1α, FNDC5, and BDNF in the muscle at the acupuncture site can be associated with the short treatment duration. In contrast, the upregulation of PGC-1α expression in muscle can be a more reliable indicator of adaptive changes following prolonged endurance training. Furthermore, Handschin et al. [38] indicated that PGC-1α gene expression exhibits fiber type specificity, with prolonged endurance training increasing the basal transcription levels of PGC-1α, while specific knockout of PGC-1α in animals results in decreased exercise endurance.

Irisin is a recently discovered exercise-induced myokine that regulates intercellular signaling across multiple organs [39]. The first description of irisin was published by Boström and colleagues in 2012, identifying it as a novel exercise-induced muscle factor derived from the proteolytic cleavage of FNDC5 [40]. PGC-1α is a key regulator of mitochondrial function and serves as an upstream transcriptional regulator of FNDC5, responsible for the synthesis of irisin. Consequently, PGC-1α-associated pathways modulate serum irisin and its expression in muscle, with irisin being a closely dependent factor on PGC-1α [41]. With advancements in medical technology, subsequent studies and experiments have confirmed that, in addition to being secreted by muscle, irisin is also present in adipose tissue and the brain [42]. Additionally, irisin is considered a bridge between exercise and metabolic homeostasis [43]. Common brain regions and cell populations containing irisin include Purkinje cells in the cerebellum, astrocytes in the hippocampus, neurons in the brain, the hypothalamus, and even cerebrospinal fluid [44]. Preliminary hypotheses regarding the physiological functions of irisin suggest the following mechanisms: exercise induces the upregulation of PGC-1α expression in skeletal muscle, resulting in an increase in FNDC5 expression on the cell membrane [45]. The extracellular portion of FNDC5 is cleaved to produce irisin, which is subsequently secreted into the bloodstream. Irisin binds to relevant receptors on white adipocytes, promoting the expression of uncoupling protein 1 and the browning of white adipose tissue [46].

With the gradual revelation of the multiple important roles of irisin in neural injury, it has become increasingly significant research focus. Currently, irisin has been reported to exhibit multiple biological effects, including anti-inflammatory, anti-apoptotic, and antioxidant activities [47]. Studies have found elevated irisin levels in the blood of rat models with pulmonary hypoxia-ischemia, suggesting that irisin can exert a protective effect against hypoxic-ischemic injury. The underlying mechanism can be associated with the inhibition of cell apoptosis [48]. Furthermore, recent evidence suggests that irisin can promote the differentiation of neural stem cells, enhance neuroplasticity, regulate brain function, and improve memory and cognitive abilities. A potential mechanism is that FNDC5 is highly expressed in the hippocampus, and irisin is released through the cleavage of the membrane-bound precursor protein FNDC5, resulting in increased irisin levels in the hippocampus [43]. Additionally, it has been observed that mice with knockout of the FNDC5/irisin genes exhibit significant cognitive impairments. In contrast, FNDC5/irisin overexpression increases synaptic plasticity and improves learning and memory functions, thereby exerting significant neuroprotective effects [49,50]. Multiple studies have demonstrated that central irisin is involved in regulating neural differentiation and proliferation, neurobehavior, energy expenditure, and cardiac function. Peripheral irisin levels are associated with increased neuroprotection and the upregulation of genes involved in learning and memory in the hippocampus [51]. Zhang et al. [52] compared serum irisin levels between patients with vascular cognitive impairment and healthy controls. They found that serum irisin levels in the vascular cognitive impairment group were significantly lower than those in the control group, suggesting that serum irisin can serve as a biomarker for vascular cognitive impairment. Other studies have indicated that irisin concentration can serve as a predictive indicator of functional outcomes, with decreased serum irisin levels being associated with poor prognosis [53]. Furthermore, Tu et al. [54,55] demonstrated that serum irisin levels are a strong independent biomarker for predicting the risk of post-stroke depression, and peripheral irisin can cross the blood-brain barrier to directly affect brain function. In this study, serum irisin levels in the rat model group were significantly lower than those in the sham operation group, indicating that CCI in rats is associated with impairments in cognitive learning and memory abilities as well as reduced serum irisin levels. This preliminary finding suggests that irisin can play a crucial role in the onset and progression of cognitive dysfunction associated with CCI. Possible explanations include the following: First, ischemia can reduce irisin levels through hypoxia-induced stress and subsequent systemic responses. Second, in animals with cerebral ischemia, reduced physical activity can result in decreased muscle metabolic levels, resulting in lower irisin levels in the body. Finally, alterations in neuromuscular control following cerebral ischemia can also contribute to reduced irisin secretion. To date, research on the neuroprotective effects of irisin in brain injury is still in its infancy. However, further exploration of its potential therapeutic applications has attracted significant attention.

This study investigates the impacts of bilateral sciatic nerve electrical stimulation on learning and memory abilities in a rat model of CCI. Western blot and RT-PCR were used to determine protein and mRNA expression levels of PGC-1α, FNDC5, and BDNF in the hippocampus and biceps femoris of rats in each group. The results revealed that the protein and mRNA expression levels of PGC-1α, FNDC5, and BDNF in the hippocampus and skeletal muscle of rats in the model group were significantly decreased. These findings suggest that CCI in rats is associated with cognitive dysfunction, accompanied by a concurrent reduction in the expression levels of PGC-1α, FNDC5, and BDNF in both brain and skeletal muscle tissues. This suggests that rats with cognitive dysfunction induced by CCI downregulate the expression of the PGC-1α/FNDC5 (irisin) pathway in both the brain and skeletal muscle. After 4 weeks of bilateral sciatic nerve electrical stimulation in rats, PGC-1α, FNDC5, and BDNF expression

levels in the hippocampus of the stimulation group were significantly downregulated compared to the sham operation group ($P < 0.05$), while being significantly upregulated compared to the model group ($P < 0.05$). Furthermore, HE staining demonstrated that the degree of neuronal damage in the stimulation group was less severe, with a significant reduction in the number of apoptotic cells. Integrating previous research, we conclude that 4 weeks of sciatic nerve electrical stimulation in rats with CCI-related cognitive dysfunction can improve their learning and memory abilities. The possible mechanisms are as follows: (1) Directly upregulating the levels of PGC-1α and FNDC5 (irisin) in the hippocampus, thereby further increasing the expression level of BDNF in the brain; (2) upregulating the expression of PGC-1α/FNDC5 (irisin) in locally stimulated muscle tissue, which in turn increases serum irisin levels. Irisin can cross the blood-brain barrier to stimulate the expression of BDNF in brain tissue, thereby alleviating brain damage and improving the learning and memory abilities of rats. This contradicts the mechanism described by Takahiro et al [36]. where short-term sciatic nerve electrical stimulation caused rapid muscle contractions that only elevated BDNF levels in the hippocampus.

5. Conclusion

The present study demonstrates that ES of sciatic nerve improves learning and memory function in rats with CCI through both central and peripheral mechanisms. These effects may be mediated by the upregulation of the PGC-1α/FNDC5/irisin/BDNF signaling pathway in the brain and skeletal muscle, which is associated with increased BDNF expression in brain tissue.

CRediT Authors Contribution Statement

Juan Song: Writing – original draft, Methodology, Data curation, Funding acquisition. **Zimu Hu:** Methodology, Investigation, Formal analysis. **Fajia Ling:** Investigation, Funding acquisition. **Xinyi Bian：** Software, Formal analysis. **Qingqin Xu:** Software, Formal analysis. **Wei Zhang:** Formal analysis, Data curation. **Qian Lei:** Methodology, Investigation. **Yanan Zheng:** Methodology, Data curation. **Jian Li:** Formal analysis. **Kai Zhao:** Validation, Project administration. **Hemu Chen:** Software, Resources, Funding acquisition. **Xiaoping Gao:** Writing – review & editing, Methodology, Funding acquisition，Resources, Supervision.

Ethics approval and consent to participate

The study design was approved by the Experimental Animal Ethics Committee of Anhui Medical University (Hefei, China; approval number: LLSC20252876).

This study was conducted as an animal experiment, not a human clinical trial. Therefore, Clinical trial number: not applicable.

Consent for publication

We obtained consent from all participants for publication.

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Declaration of competing interest

The authors declare no competing interests.

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Data availability

Data supporting the current study are available from the corresponding authors upon reasonable request.

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