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Effect of Electroacupuncture Stimulation on Proliferation and Differentiation of Endogenous Neural Stem Cells in Rats with Spinal Cord Injury

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Abstract

The aim of this work was to investigate the effects of electroacupuncture (EA) stimulation on the proliferation and differentiation of endogenous neural stem cells (NSCs) in rats with spinal cord injury (SCI). One hundred rats were included and randomly divided into the sham-operation (SO) group, model (MO) group, EA group, and preacupuncture stimulation (PAS) group, with 25 rats in each group. All the rats in the SO group had their spinal cord of thoracic segment T10 exposed but without SCI. In the remaining three groups, the modified Allen's weight dropping method was adopted to make SCI models. Those in the SO group and the MO group did not receive any treatment. Those in the EA group were treated with EA after the modelling was completed, which stopped when the samples were collected at each time point. The spinal cord tissue of rats was subjected to immunohistochemical staining and real-time quantitative polymerase chain reaction (PCR) to detect the expressions of neurofilament nestin and glial fibrillary acidic protein (GFAP). The Basso-Beattie-Bresnahan (BBB) score of the MO group was much lower than that of the SO group on the 3rd, 7th, and 14th days after surgery ($P<0.05$). The BBB scores of the EA group and PAS group were notably higher than that of the MO group ($P<0.05$). The number of nestin-, GFAP-, and MAP-2-positive cells was significantly increased in rat tissues after spinal cord injury. On the 3rd, 7th, and 14th days postoperatively, the numbers of nestin-positive cells in the EA and PAS groups were considerably higher than those in the MO group ($P<0.01$). However, the numbers of GFAP-positive cells in the EA and PAS groups were considerably decreased compared with those in the MO group ($P<0.01$). The positive rate of MAP-2 in the model group was significantly increased compared to that in the sham-operation group ($P<0.001$). The positive rates of MAP-2 in the EA group and PAS group were significantly higher than those in the MO group ($P<0.01$). After spinal cord injury, EA could activate the proliferation of endogenous NSCs and promote their differentiation into neuronal cells. Consequently, injuries were repaired, and functions were rehabilitated.

Keywords Electroacupuncture stimulation · Spinal cord injury · Neural stem cells · Proliferation · Differentiation

QiLong Deng, Lili Ma, and Yu Yang are all first author of this paper and make same contribution

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Introduction

Spinal cord injury (SCI) is an injury of the spinal cord caused by trauma, often accompanied by symptoms such as sensory disturbance of limbs and loss of motor function [1–3]. The annual incidence of SCI is approximately 13–46/1 million people in developed countries. Although there are currently no national statistics in China, it is roughly estimated that SCI occurs in approximately 10,000 people per year in China [4, 5]. SCI can be classified into 5 grades according to standards of the American Spinal Injury Association [6]. (1) For grade A complete injury, patients lose sensation and motor ability in sacral segments S4-5. (2) For grade B incomplete injury, the spinal cord below the neurological level, including segments S4-5, shows sensory function but loses motor ability. (3) For grade C incomplete injury, there is motor function below the neurological level, and the muscle strength of more than half of the key muscles below the level is less than grade 3 (grades 0–2). (4) For grade D incomplete injury, with motor function below the neurological level, muscle strength of at least half of the key muscles below the level is greater than or equal to grade 3. (5) For grade E (normal), both sensory function and motor function were normal. Such classification is of great help in diagnosing the severity of SCI, formulating treatment plans, and judging the recovery of patients. SCI will accumulatively damage multiple organs below the neurological level. This will result in decreased or disappearance of sensory and motor functions and dysfunctions of the respiratory system, urinary system, digestive system, and other systems, seriously affecting the normal life of patients [7, 8]. Patients with SCI often develop genitourinary infections, pressure ulcers, hypothermia, respiratory failure, lung infections, etc. Spinal cord concussion can be cured, incomplete SCI can be partially recovered, and the prognosis of complete SCI is poor [9, 10].

In recent years, the discipline of acupuncture and moxibustion has been developing continuously, and various advanced medical instruments are being combined with acupuncture and moxibustion to achieve better effects in treating diseases. In particular, "electroacupuncture (EA)," which is acupuncture combined with electric conduction, was first popularized [11]. EA stimulation is simpler and more accurate than traditional acupuncture and thus has a better therapeutic effect on patients. The treatment of EA stimulation is very valuable in traditional Chinese medicine (TCM). When patients receive electroacupuncture stimulation therapy, they can stimulate specific acupoints to make the human body Qi and blood unblocked and the meridians and collaterals unblocked. It has been widely used in clinical aspects, such as reducing patients' body pain, and has become a treatment method accepted by most doctors [12]. EA has the following advantages [13]. First, it can replace long-term continuous acupuncture, saves manpower without affecting the treatment effect, and is especially suitable for scalp acupuncture and acupuncture anesthesia. Second, in some area that should not be stimulated by lifting, thrusting, swirling, rotating, etc., such as acupoints in the eye area and some governor meridian points, electroacupuncture can be used instead. It can control the depth and amplitude of acupuncture and strengthen the stimulation of meridian points so that the treatment effect can be improved. Third, according to the syndrome differentiation, light, medium, and heavy stimulations are used, and different frequencies of low, medium, and high are also set. Thus, patients with ineffective acupuncture can regain the effect and even recover. Generally, the stimulation can range from light to heavy. Weak stimulation and low frequency should be used for the elderly and weaklings, the area of the head, face, chest, and back, and chronic diseases. Medium and strong stimulation with medium-high frequency should be utilized for young people, asthenic syndromes, the area of the limbs, hips, and abdomen, and acute diseases [6]. However, it cannot be the monotonous. For some stubborn and incurable diseases, it seems that high frequency is the best. Fourth, the amount of stimulation can be objectively grasped, which is conducive to formulating quantitative indicators.

Spinal cord injury (SCI) is a common and serious neurological disorder in clinical practice [14]. Its causes mostly include traffic accidents, work accidents, violent incidents, and sports. It often leads to severe physical disability, visceral dysfunction, and psychological disorder and causes great physical and mental harm and economic burden to the family and society. As early as 1999, Liu SM et al. in Sweden confirmed that EA could promote the proliferation and differentiation of endogenous neural stem cells (NSCs) in rats with SCI and improve the repair ability of nerve tissue [15, 16]. The method of combining acupoints with electrical stimulation has been widely used in clinical acupuncture and moxibustion and has achieved good treatment effects [17]. In addition, electrical stimulation can promote local blood circulation, accelerate the connection of bone defect areas, and promote the formation and reconstruction of the skeleton. Many studies have proven that electrical stimulation is an effective treatment for fractures, and it can be used with confidence in clinical practice. EA to the Yanglingquan acupoint can accelerate fracture healing and has a significant effect on the formation time of calluses and the number of calluses formed [18, 19].

SCI manifests not only as sensory and motor dysfunction but also as neurogenic bladder, neurogenic intestinal dysfunction, neuropathic pain, muscle spasms, respiratory diseases, deep vein thrombosis of the lower extremities, abnormal bone

metabolism, and other serious complications, causing great psychological and physical trauma as well as economic burden to patients and their families [20, 21]. Therefore, timely and effective intervention of complications to shorten the rehabilitation cycle of SCI patients is of great social significance [22]. EA combines traditional acupuncture with nerve electricity and inputs regular stimulation close to human bioelectricity into the needle handle by connecting an electroacupuncture instrument. It has made some progress in exploring the clinical effect and mechanism of treating spinal cord injury and has advantages of safety and stability compared with other modern TCM therapies [23]. However, at present, there is still a lack of research on the safety of electroacupuncture in clinical application and its effect on the proliferation and differentiation of endogenous neural stem cells. Therefore, the aim of this work was to investigate the effect of electroacupuncture stimulation on the proliferation and differentiation of endogenous neural stem cells in rats with spinal cord injury to provide a theoretical reference for the clinical diagnosis and treatment of SCI.

Materials and Methods

Experimental Animals

One hundred healthy and clean adult Wistar female rats, weighing 180–220 g, were provided by the Experimental Animal Center of Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The experimental animals conformed to the national second-level standards. During the experiment, the operations were standardized, and the relevant guidelines for the management and protection of experimental animals abided.

Grouping and Modelling

One hundred Wistar rats were randomly divided into four groups: the sham-operation (SO) group, model (MO) group, EA group, and preacupuncture stimulation (PAS) group. Twenty-five rats were included in each group. All the experimental rats were anesthetized with isoflurane (1% oxygen + 5% isoflurane) and were shaved at the T10 vertebral level where the surgery was performed. Then, the rats that had been anesthetized were fixed on the stereotaxic apparatus in a prone position, hair removal was performed for skin preparation, and routine disinfection was also performed. Under sterile conditions, the spinous processes of the 12th thoracic vertebra were taken as the lower edge, and an upward incision of 3 cm long was made to cut the skin and subcutaneous tissue layer by layer to the spine. The cone of the T9-T11 segments was fully exposed, and T9 as well as part of the pyramidal spinous processes and vertebral plates of T11 and T10 were removed using sharp-nosed bone forceps. The spinal cord of the T10 segment was fully exposed, and the spinal dura mater was exposed. With a W.M. Keck Center Impactor Model III (Rutgers, NJ, USA), a 10-g heavy metal rod was used to hit the T10 segment of the spinal cord at a height of 5 cm [24]. The tail-swinging reflex and the body retraction of both hind limbs were observed as successful modelling. After complete hemostasis, the muscle skin was sutured layer by layer. Penicillin was injected intraperitoneally to prevent infection after the operation, and the injection dose was 40,000 units/day/animal for 3 consecutive days. The bladder should be pressed regularly to promote urination 2 ~ 3 times a day until the recovery of voluntary urination. The specific modelling process is shown in Fig. 1 below.

Treatment Methods

Different treatment methods were adopted after the modelling of rats in groups. The specific treatment methods for the four groups of rats are listed in Table 1.

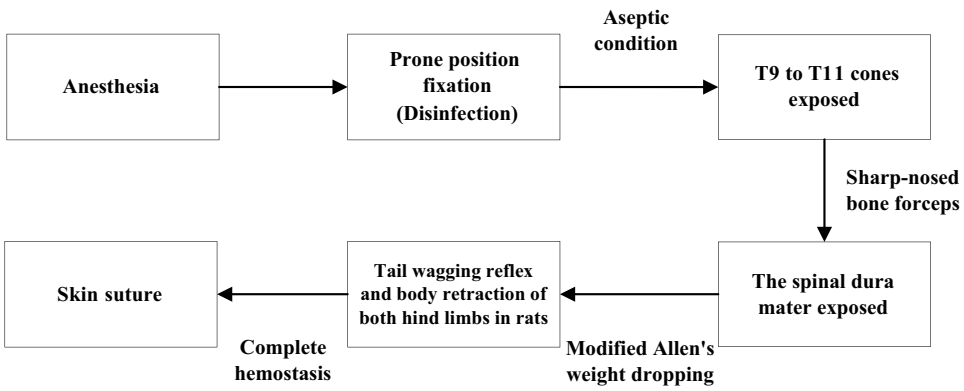

Fig. 1 The process of creating a rat model of SCI

Table 1 Treatment methods of each group

Groups	Treatments
SO group	The animals achieved physical stabilization 3 d after the SCI operation and were subjected to treatment(just observe)
MO group	The animals achieved physical stabilization 3 d after the SCI operation and were subjected to treatment(just observe)
EA group	The animals achieved physical stabilization 3 d after the SCI operation and were subjected to an EA stimulation which was performed on GV4 ("Mingmen," posterior midline underneath the spinous process of the 2nd lumbar vertebra) and GV14 ("Dazhui," posterior midline underneath the spinous process of the 7th cervical vertebra). Two acupuncture needles (HANS-200E, Jisheng Medical Instruments, China) (0.3*25 mm) were inserted to a depth of 5 ~ 7 mm at each acupoint, whereby in a continuous-wave of 2 Hz frequency, 0.4 mA intensity was produced. The EA were applied on a daily basis, 5 days per week for a total of 4 consecutive weeks, each time lasting for 20 min. All the above procedures were conducted after isoflurane (1% oxygen + 5% isoflurane) EA
PAS group	EA at GV4 and GV14 two acupoints for 1 week before modelling, once a day, 20 min each time; after successful modelling, the same as that in the EA group. All the above procedures were conducted after isoflurane (1% oxygen + 5% isoflurane)

Immunohistochemical Staining

Three days after surgery, treatment was initiated, and the number of days was calculated. On the 3rd, 7th, and 14th days, 4 rats with SCI were randomly selected from each group. The specific process of immunohistochemical staining is displayed in Fig. 2.

Sample Preparation of Real-Time Quantitative Polymerase Chain Reaction (PCR)

Three days after surgery, treatment was initiated, and the number of days was calculated. On the 3rd, 7th, and 14th days, the rats were killed by decapitation and placed on an ice box. The injured spinal cord tissue of rats was immediately removed, with a length of approximately 1 cm. It was quickly frozen in liquid nitrogen and then placed in a low-temperature refrigerator at − 80 ℃ for preservation [25, 26].

Total RNA Extraction and cDNA Synthesis

The injured spinal cord tissue of the rat was put in a mortar and ground with a pestle. During this process, liquid nitrogen was added, and it was stopped until the tissue became powdery. Then, RNAiso Plus was added, and grinding was continued until the lysate became transparent. Then, chloroform and isopropanol were added in sequence, and the obtained total RNA was dried and dissolved in RNase-free water. With a spectrophotometer, the concentration of total RNA was measured. The reaction conditions consisted of 37 ℃ for 15 min and 85 ℃ for 5 s.

The synthesized cDNA was stored in a − 80 ℃ low-temperature refrigerator for later use. The specific composition of the reverse transcription reaction system is shown in Table 2.

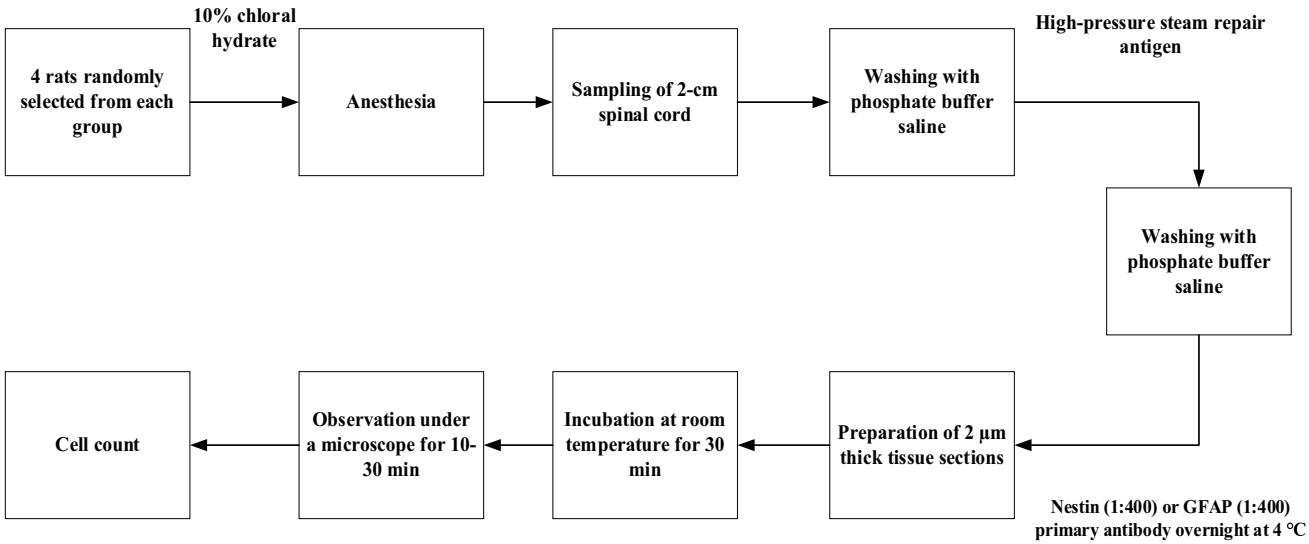

Fig. 2 Process of immunohistochemical staining

Table 2 Reverse transcription reaction system

Reagents	Dosage
5×PrimeSeript Buffer	2 μL
PrimeScript RT Enzyme Mix	0.5 μL
Oligo Dt Primer	0.5 μL
Random 6 mers	0.5 μL
Total RNA	4 μL
RNase-free water	2.5 μL

Table 3 PCR system

Reagents	Dosage
SYBR Premix Ex Taq	l2.5 μL
Upstream primer	0.5 μL
Downstream primer	0.5 μL
cDNA	l μL
Double distilled water	10.5 μL

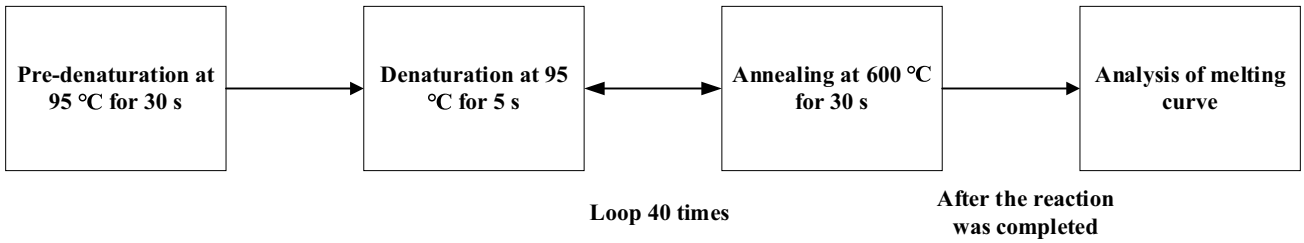

Fig. 3 Cycle process of the PCR system

Table 4 Primer sequences

Gene	Primers sequences (5′→3′)	Product size (bp)
β-actin	F: AGCTTGACATCCGTAAAGACCTC	82
	R: TAGGAGCCAGGGCAGTAATCT	
Nestin	F: TAGCTAGAGAGAGCGCGCTCGCTCGAT AGAT	123
	R: CTGAGACTCGCGATAGCTGCGATAGGC TAGC	
GFAP	F: CGGGAGTCGGCGAGTTA	285
	R: CACCGTCTTTACCACGATGTT	

Real-Time Quantitative PCR Amplification

Primers were designed and synthesized by Sangon Biotech. Rat reduced β-actin was the housekeeping gene. The relative quantification of gene expression was based on the comparative cycle of threshold (CT) method. The PCR system was 25 μL, and the specific composition and cycling process are shown in Table 3 and Fig. 3 below. Primer sequences are shown in Table 4.

Indicator Detections

(1) The hind limb motor function of rats was evaluated. Rats in each group were scored for hind limb motor function by a double-blind method on the 3rd, 7th, and 14th days after treatment (including no treatment groups). The scoring was performed according to the Basso-Beattie-Bresnahan (BBB) [27] scale. The minimum value was 0, indicating that the joints of the hind limbs were inactive. The maximum value was 21, indicating that the motor function of the hind limbs was completely normal.

(2) The calculation method of the CT value is shown in Eq. (1):

$$Ratio\left(\frac{A}{B}\right)=\frac{(Etarget)^{\Delta CT,target(control-sample)}}{(Eref)^{\Delta CT,ref(control-sample)}} \quad (1)$$

In the above equation, $\Delta CT, target(control-sample)=(CTtarget)D-(CTtarget)E$ and $\Delta CT, ref(control-sample)=(CTref)D-(CTref)E$. A represented the treatment group, B represented the model group, D referred to the mean of the model group, and E was the mean of the treatment group. Etarget represented the amplification efficiency of the target gene, and Eref represented the amplification efficiency of the housekeeping gene.

Statistical Processing

The data obtained by immunohistochemical detection were processed by SPSS 24.0. The Shapiro–Wilk test was adopted to test whether the data were normally distributed. A univariate multisample mean was utilized to compare the measurement data in accordance with the normal distribution between multiple groups, and an independent sample t test was adopted to compare the measurement data between two groups. The measurement data that did not conform

to a normal distribution were tested by the rank sum test. Enumeration data were examined. The statistical results with $P<0.05$ were considered statistically significant. The data obtained by real-time quantitative PCR (polymerase chain reaction) detection were processed by REST-2008 designed by Pfaffl et al. (2002) [28]. CT values were expressed as the mean ± standard deviation ($\bar{x} \pm s$), and the result calculated by the software was the ratio of the relative quantitative expression of the target gene in the treatment group and the model group.

Results

Comparison of Hindlimb BBB Scores of Rats in Each Group at Each Time Point

As shown in Fig. 4, 3 days after surgery, the BBB score was 21.00 ± 0.00 in the SO group and 1.38 ± 0.61 in the MO group. The score of the MO group was much lower than that of the SO group, and the difference was considered to be statistically significant ($P<0.05$). The score of the EA group was 1.91 ± 0.82, while that of the PAS group was 2.01 ± 0.86. The scores of the EA and PAS groups were significantly higher than those of the MO group ($P<0.05$). Seven days after surgery, the BBB score was 21.00 ± 0.00 in the SO group and 1.42 ± 0.64 in the MO group; the score in the MO group was significantly lower than that in the SO group ($P<0.05$). The score was 3.25 ± 0.71 for the EA group and 3.95 ± 0.95 for the PAS group, which were significantly higher than those for the MO group ($P<0.05$). At 14 days after surgery, the BBB score was 21.00 ± 0.00 in the SO group and 2.19 ± 0.79 in the MO group, which was significantly lower than that in the SO group ($P<0.05$). The score of the EA group was 6.65 ± 0.74, while it was 7.02 ± 0.83 in the PAS group, both of which were much higher than those in the MO group, with statistically significant differences ($P<0.05$).

Nestin-Positive Cell Count in the Injured Spinal Cord of Rats in Groups at Each Time Point

As shown in Fig. 5 below, 3 days after surgery, the count of nestin-positive cells in the injured spinal cord was 2.28±0.61 in the SO group, 6.34±0.95 in the MO group, 12.99±1.05 in the EA group, and 18.56±1.71 in the PAS group. The Nestin-positive cell counts in the EA and PAS groups were markedly higher than those in the MO group, with statistically significant differences ($P<0.05$). The Nestin-positive cell count in the PAS group was significantly higher than that in the EA group ($P<0.05$). Seven days after surgery, the Nestin-positive cell count in the injured spinal cord of rats was computed as 2.09±0.48 in the SO group, 10.81±1.32 in the MO group, 22.01±2.19 in the EA group, and 27.01±1.31 in the PAS group. The counts of Nestin-positive cells in the EA group and PAS group were statistically higher than those in the MO group ($P<0.05$), while those in the PAS group were statistically higher than those in the EA group ($P<0.05$). At 14 days after surgery, the counts of Nestin-positive cells were 2.12±0.49, 7.99±0.89, 24.65±1.21, and 30.89±3.01 in the SO, MO, EA, and PAS groups, respectively. The counts in the EA and PAS groups were remarkably higher than those in the MO group, with statistically significant differences ($P<0.05$). The count in the PAS group was higher than that in the EA group, showing a statistically

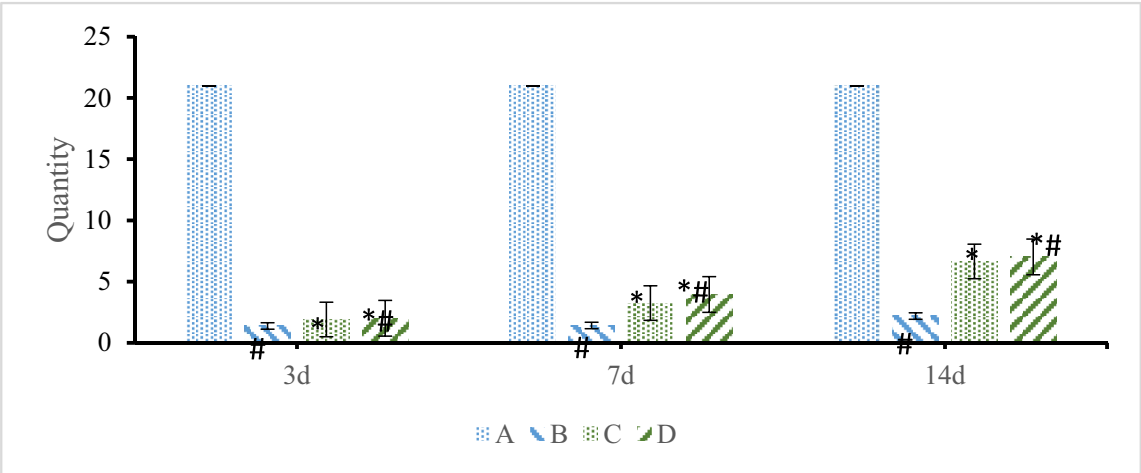

Fig. 4 Comparison of hindlimb BBB scores of rats in each group at each time point. A, B, C, and D represented the SO, MO, EA, and PAS groups, respectively. The asterisk (*) indicated a statistically significant difference compared to the MO group ($P<0.05$), while the number sign (#) indicated the same difference compared to the SO group ($P<0.05$)

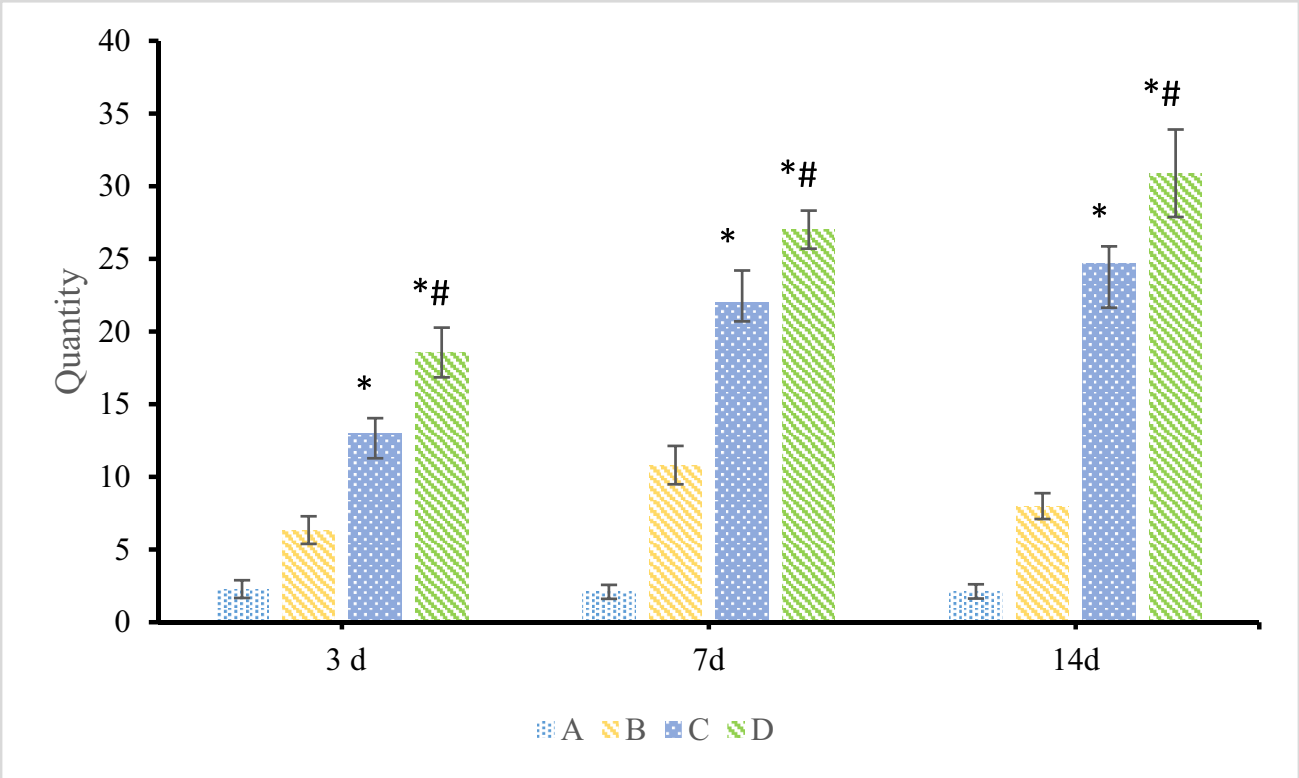

Fig. 5 Comparison of Nestin-positive cell counts in the injured spinal cord of rats in each group at different time points. A: SO group; B: MO group; C: EA group; and D: PAS group. The asterisk (*) and number sign (#) indicated statistically significant differences compared to the MO group and SO group, respectively ($P<0.05$)

significant difference ($P<0.05$). The details were compared as follows.

Number of GFAP-Positive Cells at Different Time Points in Each Group

Figure 6 below showed the comparison of the number of GFAP-positive cells in the injured spinal cord of the four groups of rats at different time points. The number of GFAP-positive cells in the injured spinal cord of rats in the Mo group was significantly higher than that in the SO group, and the difference between the two groups was extremely significant ($P<0.001$). The number of GFAP-positive cells in the injured spinal cord of rats in the EA and PAS groups was significantly lower than that in the MO group ($P<0.01$).

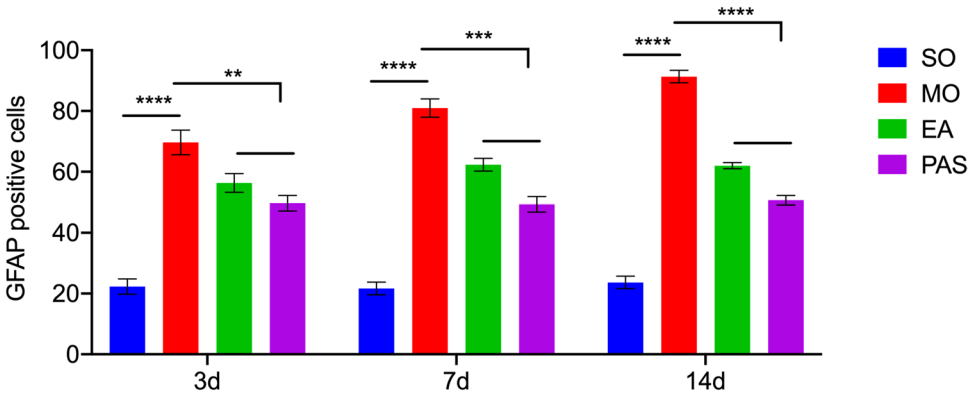

Fig. 6 Comparison of the counts of GFAP-positive cells in the injured spinal cord of rats in the groups at each time point. Four asterisks (****) indicated a significant difference compared with the SO group ($P<0.001$). Two asterisks (**) indicated a statistically significant difference compared with the model group ($P<0.01$)

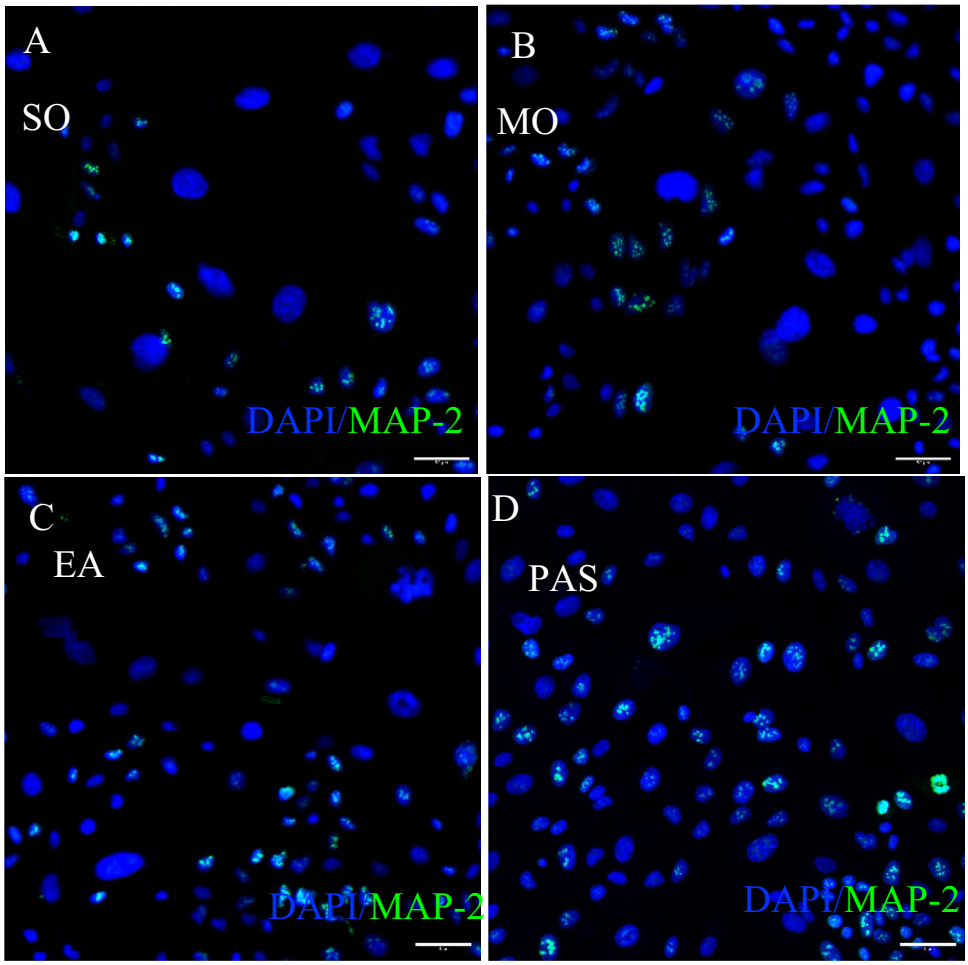

Fig. 7 The positive rate of MAP-2 in each group in the immunohistochemical experiment. **A** SO group. **B** MO group. **C** EA group. **D** PAS group

The Positive Rate of Microtubule-Associated Protein 2 (MAP-2) in Each Group

As shown in Fig. 7, microtubule-associated protein 2 (MAP-2) is a mature neuronal marker. The immunohistochemistry results showed that the positive rate of MAP-2 in the model group was significantly increased compared to that in the sham-operation group ($P < 0.05$). The positive rates of MAP-2 in the EA group and PAS group were significantly higher than those in the MO group ($P < 0.05$).

The Gene Expression of Nestin in the Injured Spinal Cord of Rats

As shown in Fig. 8, the relative expression levels of Nestin mRNA in spinal cord injury tissue were significantly

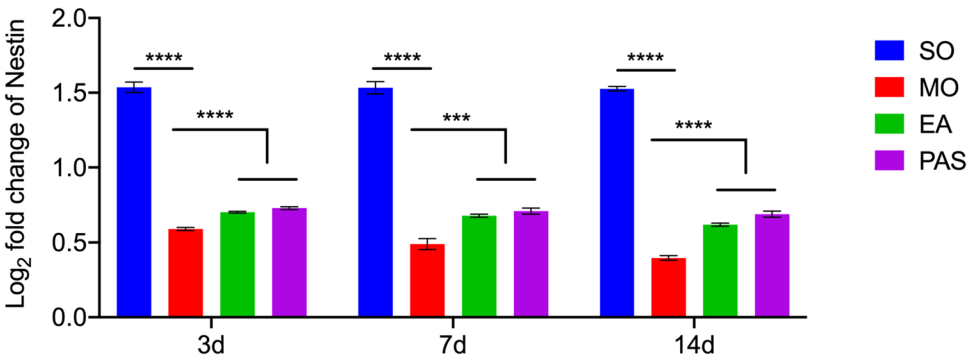

Fig. 8 The gene expression of Nestin in the injured spinal cord of rats. Four asterisks (****) indicated a significant difference compared with the SO group ($P < 0.001$). Three asterisks (***) indicated a statistically significant difference compared with the model group ($P < 0.01$)

increased compared with those in the sham operation group at each time point ($P < 0.001$). The expression of Nestin mRNA was higher in both the electroacupuncture stimulation group and the preacupuncture stimulation group than in the model group ($P < 0.001$).

The Gene Expression of GFAP in the Injured Spinal Cord of Rats

As shown in Fig. 9, the relative expression levels of GFAP mRNA in spinal cord injury tissue were significantly increased compared with the sham operation group, EAS, and PAS at each time point ($P < 0.001$).

Discussion

Acupuncture and moxibustion have been common ways of diagnosing and treating diseases in traditional Chinese medicine since ancient times. It occupies an important position in traditional Chinese medicine and is widely recognized in many other countries [29]. In recent years, with the progress of science and technology, EA stimulation has begun to appear in the public eye as an improved version of acupuncture. The principle is to set up an electroacupuncture instrument to produce a pulse current of different frequencies and intensities. When such electric current affects disease-related acupoints, it will stimulate the release of a variety of neuromediators and endogenous opioids to achieve sedation, analgesia, and regulation of physiological functions, among which pain relief is the most important purpose [30]. Since the 1950s, electroacupuncture stimulation therapy has been widely used in the clinical treatment of diseases, its efficacy has been confirmed by many studies and clinical practice, and acupuncture can indeed achieve the effect of reducing pain and inflammatory reactions [31]. Second, electroacupuncture stimulation therapy is more popular than other drug treatment methods because it is less difficult to perform and causes almost no harm to the body.

Nestin is a cytoskeletal protein in the central nervous system of all mammals. It is usually abundant in epithelial NSCs. The expression of neurofilament Nestin is often affected by the development and differentiation of NSCs. During the development of NSCs, the expression of neurofilament Nestin gradually increases [32]. This process will not continue indefinitely; when the differentiation of NSCs is completed, the expression of neurofilament Nestin will decrease [33]. In the process of human central nervous system development, Nestin is often regarded as one of the markers of NSCs or neural precursor cells. GFAP is essentially a skeletal protein and an important component of astrocytes. After the central nervous system is injured, the reactive proliferation of astrocytes and the excessive increase in protrusions and glial filaments lead to increased expression of GFAP. Therefore, GFAP can be utilized to label astrocytes specifically and is considered a marker of astrocyte activation. Microtubule-associated protein 2, a mature neuronal marker, is involved in neuronal development, structural stability, protrusion formation, and the regulation of synaptic plasticity. The results indicate that electrical stimulation can affect the differentiation and regression of endogenous neural stem cells, inhibit the differentiation of endogenous neural stem cells into astrocytes, promote the differentiation of endogenous neural stem cells into neurons, and facilitate the repair of spinal cord injury.

In this work, 100 Wistar rats were selected and randomly divided into four groups: SO group, MO group, EA group, and PAS group, with 25 rats in each group. For all the rats in the SO group, the thoracic T10 segment of the spinal cord was exposed, and they did not have SCI. In the remaining three groups, the modified Allen's weight dropping method was adopted to prepare the SCI models. The position of the T10 segment was hit to cause the injury. The rats in the SO and MO groups did not receive any treatment. The rats in the EA group

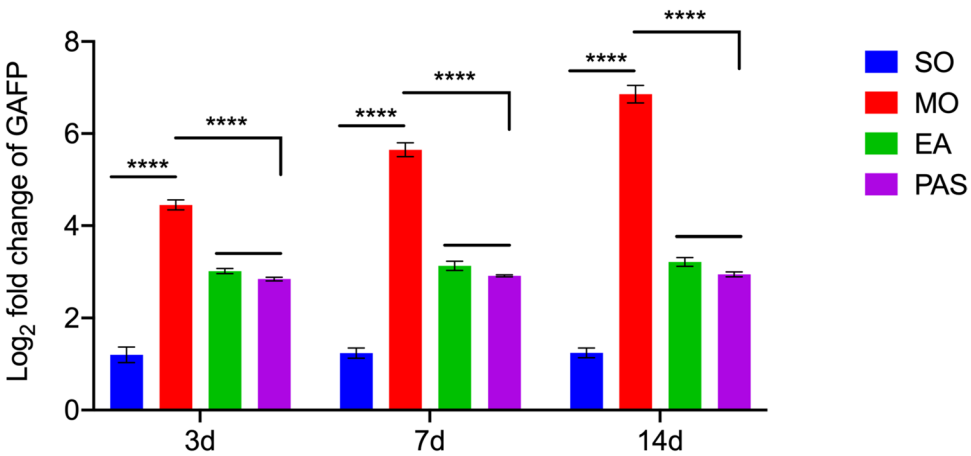

Fig. 9 The gene expression of GFAP in the injured spinal cord of rats. Four asterisks (****) indicated a significant difference between groups ($P < 0.001$)

were treated with EA after modelling, which stopped when the samples were collected at each time point. The spinal cord tissue of rats was collected for immunohistochemical staining and fluorescence real-time quantitative PCR to detect the expression of neurofilaments Nestin and GFAP. First, the BBB scores of the hind limbs of rats in the four groups were compared at each time point. The scores in the MO group were remarkably lower than those in the SO group 3, 7, and 14 days after SCI surgery; the differences were statistically significant. The scores in the EA group and PAS group were notably higher than those in the MO group; the differences were also statistically significant. Such results revealed that EA had a certain treatment effect on SCI, which was consistent with the research results of Giraldo et al. [34]. Then, the Nestin-positive and GFAP-positive cells in the injured spinal cord of rats were compared among the four groups at various time points. The counts of neurofilament Nestin-positive cells in the EA group and PAS group were observably higher than those in the MO group 3, 7, and 14 days postoperatively; the counts of GFAP-positive cells in the EA group and PAS group were observably lower than those in the MO group 3, 7, and 14 days postoperatively; the differences were statistically significant.

Conclusion

EA stimulation can activate the proliferation of endogenous NSCs, inhibit their differentiation into glial cells, promote the differentiation of endogenous neural stem cells into neurons, and alleviate pathological damage. In summary, this study has provided a theoretical basis and data reference for the application of TCM EA stimulation in spinal cord injury and the study of its effects on endogenous neural stem cells. However, this work only analyzed the effects of EA stimulation on the proliferation and differentiation of endogenous neural stem cells after SCI in animal models. Further studies and analysis are needed in the future to draw more accurate and reliable conclusions.

Author Contribution QD, LM, YY, TC, LZ, QH, YJ, and LM conceived and designed the study and drafted the manuscript. All authors contributed to data collection. Analysis of data and writing draft of the manuscript was by QD, LM, YY, TC, LZ, QH, YJ, and LM. All authors read and approved the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this article.

Declarations

Ethics Approval and Consent to Participate All experimental procedures using rats were conducted in accordance with the animal care and use guidelines approved by the institutional ethics committee at Taizhou Hospital of Zhejiang Province Affiliated to Wenzhou Medical University and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Also, the study was carried out in compliance with the ARRIVE guidelines.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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