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Effects of repetitive transcranial magnetic stimulation on learning and memory cognitive function in rats with vascular cognitive impairment and its neural induction mechanism

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Abstract

Background The treatment of vascular cognitive impairment (VCI) is challenging, and its neurological mechanisms are not yet fully understood. Repetitive transcranial magnetic stimulation (rTMS) offers a new non-invasive treatment approach.

Methods One hundred male SD rats were grouped: intervention group (IG), model group (MG), sham group (SG), and control group (CG), to prepare the rat model of VCI. The Morris water maze (MWM) test was conducted, and oxidative stress (OS) markers, neurotrophic factors, apoptosis factors, and the amplitude of postsynaptic potential (PSP) in the hippocampus of rats were measured.

Results Post-intervention, IG's escape latency was lower than MG but higher than SG and CG. IG's hippocampal malondialdehyde (MDA) content, Bax, and Caspase-3 (Cas-3) were lower than MG but higher than SG and CG, while the tendency was opposite for Bcl-2 expression and the content of glutathione (GSH) and superoxide dismutase (SOD). IG's brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and *N*-methyl-D-aspartate receptor 1 (NMDAR1) in the hippocampus were higher than MG but lower than SG and CG; The changes in the amplitude of PSP in the hippocampal region of IG at 10, 30, and 60 min were all higher than those in MG but lower than those in SG and CG ($P < 0.05$).

Conclusion Low-frequency rTMS visibly improved the learning and memory abilities of VCI rats and reduced OS levels.

Keywords VCI, rTMS, PSP, OS, Neural plasticity

Background

VCI is a type of cognitive impairment caused by cerebrovascular disease, characterized by a decline in learning and memory functions [1, 2]. This condition is closely related to factors such as stroke, arteriosclerosis, and hypertension. As these causes become more prevalent, the incidence of VCI is rising annually among the elderly population [3]. Studies have shown that VCI patients experience a visible decrease in abilities such as information processing, problem-solving, and adapting to new environments, severely affecting their quality of life and social functioning [4]. In addition to cognitive dysfunction, VCI can also lead to emotional problems such as anxiety and depression, exacerbating the suffering of patients. Therefore, it is of great clinical and social

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significance to delve into the pathogenesis of VCI and its impact on learning and memory [5].

Currently, the treatment methods for VCI mainly include pharmacotherapy, psychological intervention, and rehabilitation training. Pharmacotherapy often uses acetylcholinesterase inhibitors, antioxidants, etc., aiming to alleviate symptoms and improve patients' cognitive abilities [6–8]. However, these drugs have limitations in efficacy, especially for patients with more complex causes, often with poor results. In addition, rehabilitation training, through cognitive training and behavioral intervention, can improve patients' functions to some extent, but its effects vary from person to person and requires continuous input and support [9, 10]. In recent years, rTMS has attracted increasing attention as an emerging non-invasive neuromodulation technology. rTMS uses the principle of electromagnetic fields to stimulate specific areas of the cerebral cortex, modulating neuronal activity and synaptic plasticity, thereby having a positive impact on cognitive function [11]. Studies have shown that rTMS can effectively promote the recovery of learning and memory abilities and improve cognitive function, especially with visible effects on neural plasticity [12]. Further research has also pointed out that rTMS can increase the expression of BDNF, promote the growth and differentiation of neurons, alleviate neuroinflammatory responses, and thereby improve cerebral blood perfusion. This provides new treatment ideas for the rehabilitation of VCI patients [13].

The aim of this article is to explore the impact of rTMS on the learning and memory cognitive functions of VCI rats and to delve into its neural induction mechanisms. Through a series of systematic experimental designs, the specific improvement effects of rTMS on learning and memory abilities, including spatial learning ability and memory tests, are evaluated. The potential mechanisms of rTMS in neural plasticity, neurotrophic factor expression, and inflammatory responses are studied, with the hope of providing new theoretical basis and practical references for the clinical treatment of VCI. This article not only helps to improve the understanding of the pathogenesis of VCI but also lays the foundation for exploring the application of neuromodulation technology in other neurological diseases, which has important scientific and clinical significance. It is hoped that it can bring a help for the rehabilitation of VCI patients and provide new directions and ideas for future research in related fields.

Materials and methods

Experimental animals

One hundred male SD rats (Beijing Vital River Laboratory Animal Technology Co., Ltd.) of SPF grade, aged 1–2 months, weighing between 180–220 g, were prepared for experimental research. These rats were used for experimental studies to ensure that they were in a healthy state before the start of the experiment and met the physiological parameters required for the experimental design. All experiments followed the basic principles of animal experimentation, to minimize the use and suffering of animals and to raise them in a healthy and good environment. The experimental design was reviewed and obtained the approval by the ethics committee to ensure compliance with ethical standards and scientific rationality. These measures aimed to protect animal welfare and improve the scientific nature and effectiveness of the experiments.

Grouping and construction of vascular dementia rat model

A total of 100 rats were randomly assigned to four groups: intervention group (IG), model group (MG), sham surgery group (SG), and control group (CG), with 25 rats in each group. The IG rats, after the successful establishment of the vascular dementia model, received low-frequency transcranial magnetic stimulation (TMS) therapy with parameters set at 1 Hz frequency, 50% of maximum output intensity, 20-min duration, and a 2-s inter-pulse interval, applied to the prefrontal cortex area with precise site marking to ensure consistency. The MG rats only underwent the model construction without any treatment, serving as a baseline control to observe the effects of cerebral ischemia on cognitive function. The SG rats underwent carotid artery exposure surgery without occlusion to exclude the potential influence of surgery alone on the results. The CG rats did not receive any surgery or intervention, serving as a baseline comparison for behavioral and physiological parameters in a healthy state.

The rat model of vascular dementia was prepared by simulating intermittent cerebral ischemia through repeated intermittent clamping of the bilateral common carotid arteries with hypotension. Preoperative anesthesia for the IG and MG rats was achieved using sodium pentobarbital (Shanghai Harling Biotechnology Co., Ltd.). The rats were placed on the surgical table, and their body temperature was maintained at a constant level. An incision was made in the neck skin to expose the bilateral common carotid arteries, which were carefully dissected to avoid damaging surrounding tissues and nerves. A microvascular clamp (Beijing Meike Innovation Technology Co., Ltd.) was used to occlude the bilateral common carotid arteries for a duration of five minutes. During occlusion, tail artery blood pressure was monitored to maintain a hypotensive state and ensure that the rat's blood pressure remained within an appropriate range (typically 40–60 mmHg). After each occlusion, blood flow was restored and observed for 2–5 min to ensure

normal recovery. This process was repeated 3–5 times to ensure the repetitive nature of simulated cerebral ischemia. Although each occlusion was of short duration, the repeated procedure more accurately mimicked the pathological process of chronic cerebral ischemia and vascular dementia. Additionally, a pre-treatment CG was added, where rats underwent the same occlusion procedure (same duration and frequency) without hypotensive intervention. This group was included to eliminate the potential impact of hypotension on model outcomes and ensure that the effects of repeated occlusions on the rat's nervous system were fully considered.

After the occlusion, the clamps were slowly removed to prevent sudden changes in blood flow, and the neck incision was closed with sutures and disinfected. Postoperatively, the rats were returned to their cages and monitored for recovery, observing for any abnormal reactions. Suitable environmental conditions and diet were provided to ensure proper care during the recovery process.

Regarding the behavioral testing schedule: after establishing the surgical model, a minimum of 7 days was allowed for the rats to recover from the surgical trauma to avoid any artifacts caused by immediate behavioral testing. Behavioral testing for the vascular dementia model typically began on the 7th postoperative day to ensure the stability of the model and the reliability of the experimental data.

For the SG, only the neck skin was incised to expose the bilateral common carotid arteries, but no clamping operation was performed. After completion, the wound was cleaned and sutured to ensure that the surgical process was consistent with the IG and the MG, but without introducing blood flow interruption.

Intervention method

The rats in the IG were subjected to low-frequency TMS therapy. The TMS device was prepared, and the treatment parameters (frequency, stimulation intensity, and duration) were set, with a frequency of 1 Hz, stimulation intensity at 50% of maximum output, a treatment duration of 20 min, and an inter-pulse interval of 2 s. The application of 1 Hz low-frequency stimulation in the prefrontal cortex has been supported by relevant literature [14, 15]. Studies noted that this frequency can effectively modulate neural activity and may help improve cognitive function in vascular dementia models. The choice of this frequency is based on its potential to regulate brain function and reduce neural damage, particularly for patients with vascular dementia. 1 Hz low-frequency stimulation has been shown to be effective in multiple clinical and animal experiments.

The stimulation site was selected in the prefrontal cortex because it plays a crucial role in cognitive functions, decision-making, and memory, and is significantly affected by vascular dementia. The stimulation site was marked using a marker pen to ensure precise targeting. The stimulation coil was gently placed on the marked location, ensuring good contact without causing discomfort to the animal. The TMS device was then activated to begin low-frequency transcranial magnetic stimulation. During the stimulation, the rats' reactions were closely monitored to ensure no obvious stress responses, such as severe shaking or escape behavior. After the stimulation, the coil was slowly removed, and the rats' recovery was observed. The rats were then returned to their cages, and their behavior and health were monitored to ensure no adverse reactions. One treatment cycle consisted of five sessions, with each session performed every 5 days, for a total of 5 cycles.

MWM experiment

The MWM experiment was adopted to record the rats' escape latency and the number of times they crossed the platform within a fixed period in a constant-temperature pool. A round pool with a diameter of about 120 cm and a depth of 50 cm was prepared, and the water temperature was kept constant (22–25 °C). Non-toxic white pigment was applied to the pool to make the water opaque. A transparent diving platform with a diameter of about 10 cm was placed below the water surface, usually in one quadrant of the pool, with the platform height being 1–2 cm below the water surface. The day before the experiment, the rats were placed around the pool environment for 30 min to adapt and reduce stress reactions. Each day, 3–4 trials were conducted with an interval of 1 h for a total of 4 days of training. The rat was placed in any quadrant of the pool (head outward), and the timer was started to record the time from the rat entering the water to finding the platform (escape latency). If the rat failed to find the platform, 60 s later, it was manually guided to the platform, and the escape latency was recorded. In addition, the rat stayed on the platform for 15 s as a reward to help it remember the location of the platform. On the 5th day after the training, the test was conducted, the diving platform was removed, and the rat was allowed to swim freely in the pool, recording the number of times it crossed the original platform position within 60 s. The data of escape latency and the number of times crossing the platform were analyzed through a video recording system.

Preparation of rat hippocampal brain slices

The rats were anesthetized intraperitoneally with 1% PS, fixed on the operating table to ensure the stability of their heads, and the hair on the head was shaved with a razor to expose the skull. The skull was carefully cut open with

a surgical knife, usually at the midline of the top of the head, to form an appropriate opening. Then, the entire brain was gently removed with tweezers and placed in the pre-cooled physiological saline to maintain tissue activity. On the cold physiological saline, the brain was placed on the dissection board, and the brain was carefully cut open with a blade to expose the hippocampal region, and the hippocampal region was cut into brain slices with a thickness of about 300–400 μm using a freezing microtome (Nanjing Enhancer Biotechnology Co., Ltd.), and the cut brain slices were placed in the cooled physiological saline to maintain tissue activity. After the cutting was completed, the hippocampal brain slices were transferred to a culture dish containing culture medium for subsequent experiments (such as electrophysiological recording, drug treatment). If long-term storage is required, the hippocampal brain slices can be placed in frozen preservation solution at − 80 °C.

ELISA

ELISA was employed to detect the densities of MDA, GSH, and SOD in the rat hippocampus. The rat hippocampal tissue was quickly homogenized, and subjected to centrifugation, and the upper liquid was taken as the sample. Based on the kit guidance (Wuhan ELK Biotechnology Co., Ltd.), the standard and samples were accurately diluted and applied to the plate with 96 wells, with at least three replicate wells for each sample. Then, 5% non-fat milk was applied for blocking at 25 °C for 1 h to prevent non-specific binding. Next, the processed serum samples were put, mixing, gently shaking to avoid bubble formation. Then, the enzyme-labeled first antibodies (Ab) were put, reaction at 25 °C for 1 h, and the plate was washed 3–5 times to remove unbound Ab. Next, the fluorescent-labeled second Ab was applied, avoiding light, for 30 min, and the plate was rinsed 3–5 times. Then, TMB substrate solution was applied, avoiding light, at 25 °C for 30 min. After the reaction was completed, the reaction was terminated, gently mixing. The optical density (OD) values were read at 450 nm, and the standard curve was plotted. The densities of MDA, GSH, and SOD in the serum were computed based on the OD values of the samples and the standard curve.

Western blot (WB) detection

The use of hippocampal brain slices for Western blot analysis was chosen because brain slices preserve the tissue structure and cellular environment, which facilitates the study of protein expression in specific regions of the brain. This is particularly beneficial in neurobiological research, as it provides more physiologically relevant data. While slice preparation methods may affect the accuracy of some results, it is understood that direct sampling from the hippocampal region may provide more reliable protein expression data. Direct sampling avoids potential tissue damage during the slicing process and allows for more precise measurement of protein expression levels.

The WB method was adopted to detect Bax, Bcl-2, Cas-3 specific Ab, and BDNF, GDNF, neurotrophic factor (NGF), and NMDAR1 in hippocampal brain slices. The hippocampal brain slices were placed into cold cell lysis solution (RIPA lysis solution), and homogenized to obtain cell lysate, centrifugation at 12,000 rpm for 10 min at 4 °C. After collecting the upper liquid, the protein concentration was quantified using the Bradford method. Samples and protein standards were applied for SDS-PAGE gel electrophoresis, and the separated proteins were transferred to nitrocellulose membranes at 100 V and 4 °C. The membrane was placed into a blocking solution, incubation for 1 h at 25 °C to reduce non-specific binding. The membrane was immersed in the diluted first Ab solution against Bax, Bcl-2, and Cas-3, incubation overnight at 4 °C. The membrane was rinsed three times with TBST buffer (Beijing Solarbio Technology Co., Ltd.) for 5–10 min each, then immersed in the diluted HRP-labeled second Ab solution, incubation for 1 h at 25 °C, followed by three washes for 10 min each. Enhanced chemiluminescence (ECL) substrate was applied for color development. The membrane image was obtained using a relative system. The intensity of each protein band was recorded, which was quantified using ImageJ image analysis software to compute the relative expression.

Electrophysiological recordings of the rat hippocampus

The hippocampal brain slices were placed in a glass-bottomed culture dish in the recording chamber, and an appropriate amount of artificial cerebrospinal fluid was applied to maintain the temperature at 32–34 °C. Fine glass microelectrodes were prepared using an electrode puller, filling with 2 M KCL solution, ensuring the electrode impedance was between 2–5 MΩ. The electrodes were connected to electrophysiological equipment, and settings were adjusted to ensure proper control of current and voltage. The electrodes were slowly inserted into specific areas of the hippocampal brain slices (such as the CA1 or CA3 region) to record excitatory synaptic transmission or single-cell activity. During data recording, stimulation was applied using an electrical stimulator, and excitatory PSP (EPSP) was recorded. Data were processed using software (such as Clampfit or Matlab) to compute the characteristic parameters of excitatory and inhibitory synaptic transmission (such as amplitude, time constant, frequency). The average amplitude of the PSP recorded at six time points before stimulation for 30 min was taken as the baseline value. The relative values (%)

obtained by comparing the change in amplitude (MN value) at each time point after high-frequency stimulation with the baseline value were adopted as the PSP recording results.

Statistical processing

Data analysis was performed using *SPSS version 22.0*. For normally distributed continuous data, the results are expressed as mean ± standard deviation (x ± s), and comparisons between groups were made using one-way analysis of variance (ANOVA). For comparisons among the four experimental groups at different time points, two-way ANOVA was used to assess the interaction and main effects of time and group. For continuous data that did not follow a normal distribution, the Mann–Whitney U test was used for intergroup comparisons. Categorical data are presented as frequencies and percentages (%), and group comparisons were made using the chi-square test (Chi-square test). All tests were two-tailed, and a P value of <0.05 was considered statistically significant.

Results

Contrast of escape latency and target quadrant time in rats

After modeling, the escape latency was visibly higher, while the target quadrant time was visibly lower in the IG and the MG as against the SG and the CG ($P<0.05$). However, there was no statistically meaningful distinction in escape latency and target quadrant time between the IG and the MG ($P>0.05$). Following intervention, the escape latency of the IG was visibly lower as against the MG but remained above the SG and the CG; the target quadrant time was the opposite ($P<0.05$) (Fig. 1).

Contrast of the times of rats crossing the platform

The number of platform crossings in the IG and the MG was visibly lower as against the SG and the CG ($P<0.05$), but there was no statistically meaningful distinction between the IG and the MG ($P>0.05$). Following intervention, the count in the IG was visibly higher as against the MG but remained below the SG and the CG ($P<0.05$) (Fig. 2).

Contrast of MDA, GSH, and SOD contents in rat hippocampus

In Fig. 3, the hippocampal MDA content in the IG was visibly lower relative to the MG but remained above the SG and the CG; the hippocampal GSH and SOD contents in the IG were visibly higher relative to the MG but remained below the SG and the CG ($P<0.05$).

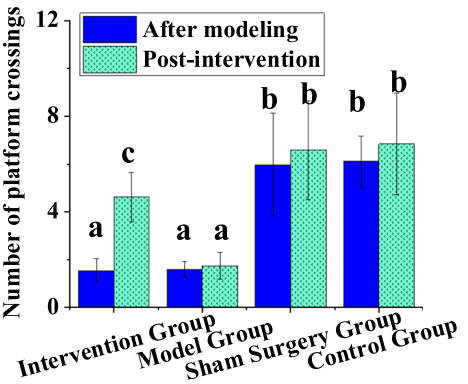

Fig. 2 Comparison of the number of platform crossings among the four groups of rats before and after intervention. a, b and c indicate statistically meaningful distinctions, $P<0.05$

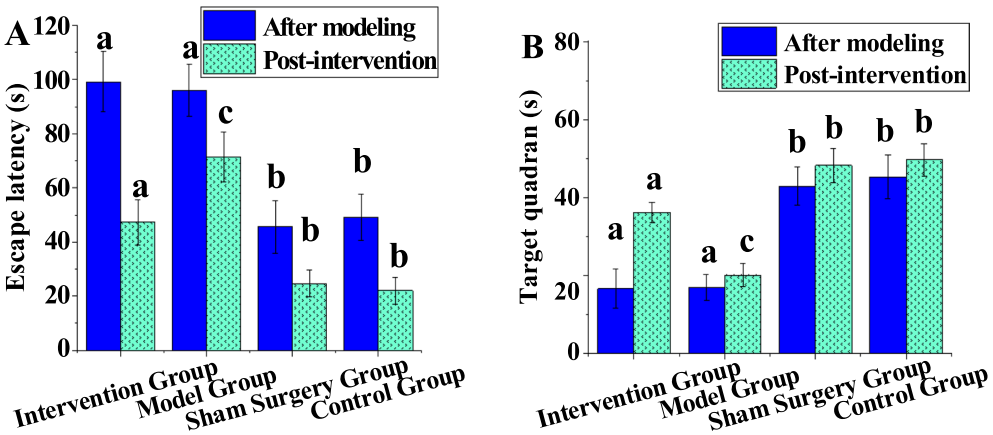

Fig. 1 Comparison of the escape latency and target quadrant time among the four groups of rats before and after intervention. (**A** is escape latency; **B** is target quadrant time). a, b, and c indicate statistically meaningful distinctions, $P<0.05$

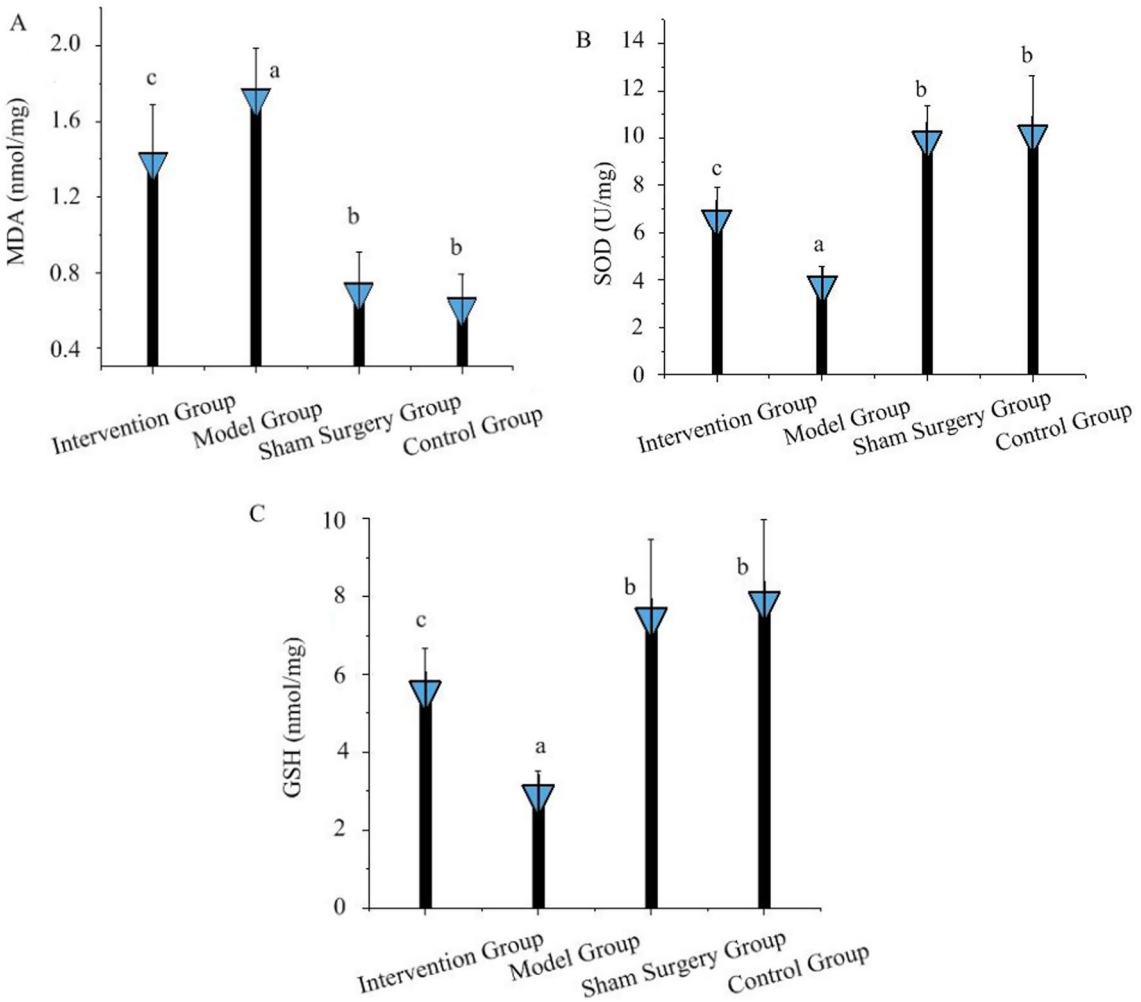

Fig. 3 Comparison of hippocampal MDA and SOD levels among the four groups of rats after intervention. (**A** is MDA; **B** is SOD; **C** for GSH). a, b and c indicate statistically meaningful distinctions, $P < 0.05$

Contrast of Bax, Bcl-2, and Cas-3 in rat hippocampus

In Fig. 4, hippocampal Bax and Cas-3 in the IG were visibly lower relative to the MG but remained above the SG and the CG; hippocampal Bcl-2 in the IG was markedly higher relative to the MG but remained below the SG and the CG ($P < 0.05$).

Contrast of protein expression of neural indicators in the hippocampus of rats

In Fig. 5, hippocampal BDNF, GDNF, and NMDAR1 in the IG were markedly higher relative to the MG but remained below the SG and the CG ($P < 0.05$).

Contrast of PSP amplitude changes in the hippocampus of rats

In Fig. 6, the changes in the hippocampal PSP amplitude in the IG at 10, 30, and 60 min post-stimulation were markedly higher relative to the MG but remained below the SG and the CG ($P < 0.05$).

Discussion

The treatment of VCI is challenging, primarily due to the unclear neuro-mechanisms underlying the condition. The etiology of this disease is associated with various factors, including chronic ischemia, microvascular changes, and neuroinflammation [16]. Effective

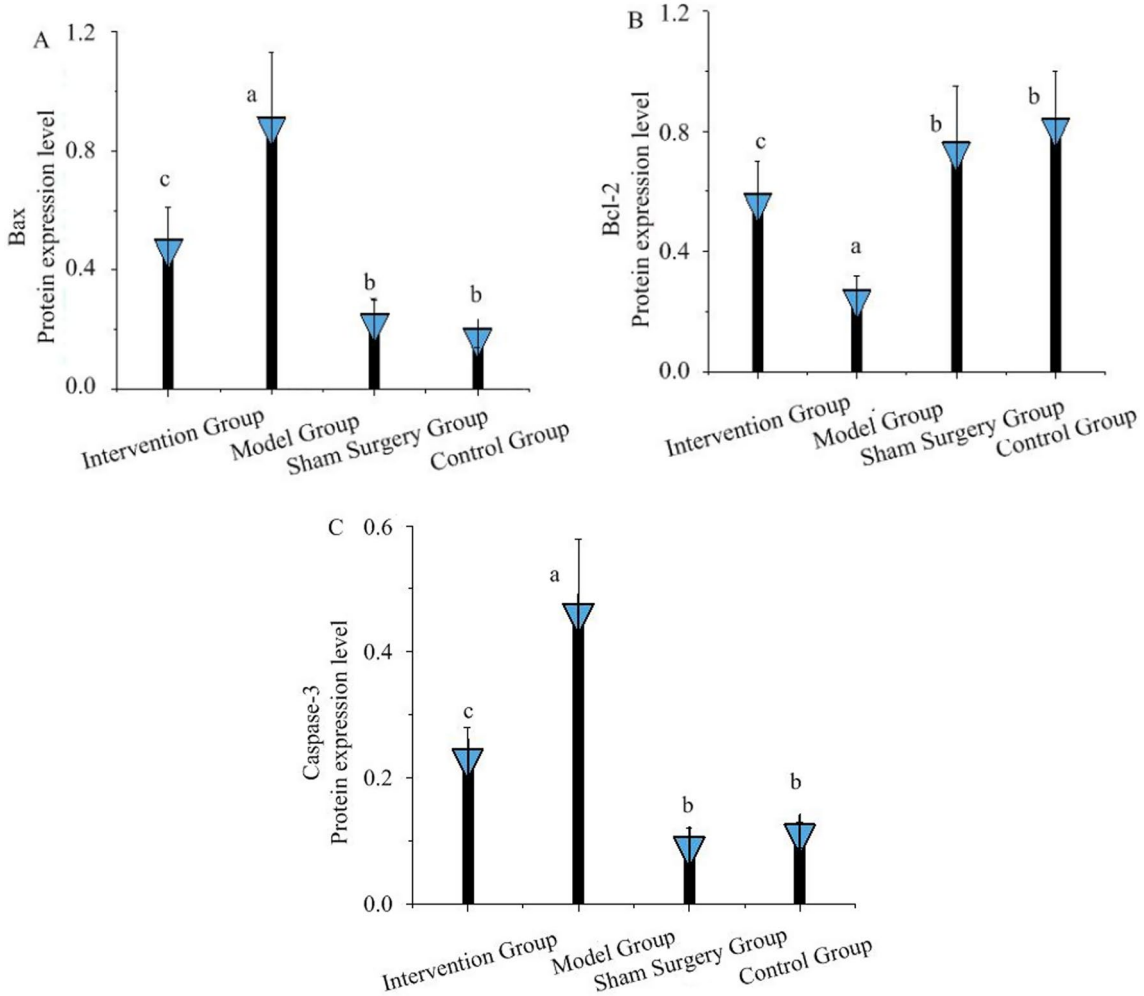

Fig. 4 Comparison of the hippocampal protein expression levels of Bax, Bcl-2, and Caspase-3 among the four groups of rats after intervention. (**A** is Bax; **B** is Bcl-2; **C** is Cas-3). a, b and c indicate statistically meaningful distinctions, $P < 0.05$

treatment modalities targeting specific mechanisms are lacking, and current therapies focus on managing risk factors such as hypertension, diabetes, and hypercholesterolemia. However, these measures have limited effects on improving cognitive function [17]. Additionally, individual differences and the presence of comorbidities further complicate the formulation of treatment plans [18]. Therefore, delving into the pathogenesis of VCI and exploring new therapeutic targets will be an important direction for future research. For this article, a total of 100 rats were randomly divided into four groups: IG, MG, SG, and CG, with 25 rats in each group, and the rat model of vascular dementia was prepared. The MWM test was first adopted to record the rats' escape latency in a constant-temperature pool and the number of platform crossings within a fixed period. It was found that after modeling, the escape latency was markedly higher, while the target quadrant time was markedly lower in the IG and the MG as against the SG and the CG. Following intervention, the escape latency of the IG was markedly lower as against the MG, but still higher in contrast to the SG and the CG, with the target quadrant time being the opposite. This is similar to the study [19], which adopted the MWM test to detect the effect of ligustrazine on vascular dementia rats, indicating that low-frequency TMS treatment has a certain effect on improving the cognitive function of rats with vascular dementia, but it can't completely restore normal cognitive function in VCI models. In addition, following intervention, the number of platform crossings in the IG was markedly higher as against the MG, but still lower in contrast to the SG and the CG, indicating that low-frequency rTMS treatment can improve the spatial learning and memory abilities of rats with vascular dementia. Although it has not fully returned to normal levels, its therapeutic

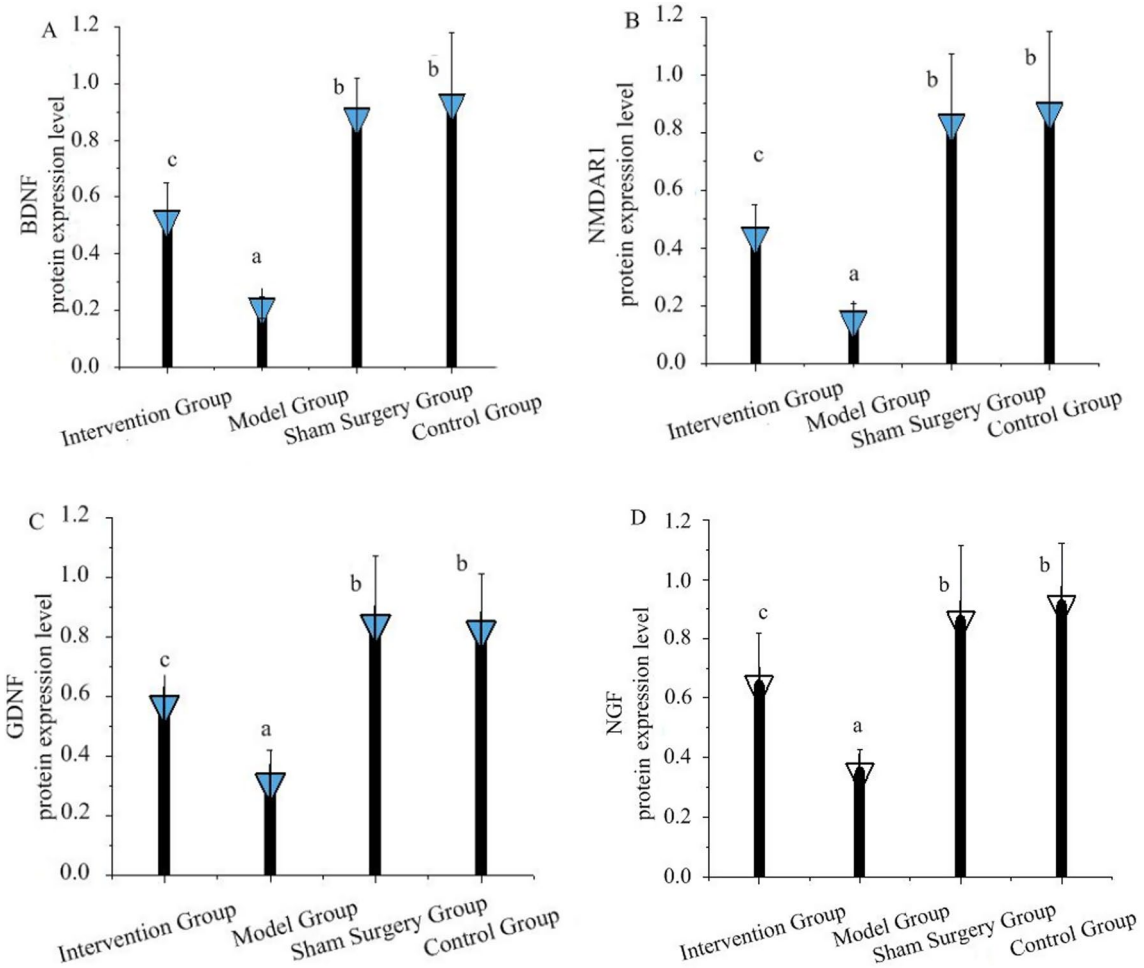

Fig. 5 Comparison of the hippocampal protein expression levels of BDNF, NT-3, NGF, and NMDA-R1 among the four groups of rats after intervention. (**A** is BDNF; **B** GDNF; **C** is NMDAR1; **D** for NGF). a, b, and c indicate statistically meaningful distinctions, $P < 0.05$

effect is visible. Future studies can further explore the optimization and mechanisms of this treatment method to improve the recovery of cognitive function.

MDA, GSH, and SOD are important indicators for assessing OS and antioxidant capacity. Monitoring these indicators can evaluate the OS status of the hippocampal region and understand the pathogenesis and targeted treatment mechanisms of neurological diseases (such as vascular dementia) [20]. The findings of this article are consistent with the argument [21], suggesting that low-frequency rTMS treatment may improve the cognitive function of rats with vascular dementia by reducing OS, enhancing antioxidant capacity, and neuroplasticity. The findings of this article suggest that low-frequency rTMS may reduce damage to the hippocampal region by regulating the cell apoptosis pathway. This finding provides a new idea for the treatment of vascular dementia, emphasizing that intervention targeting the cell apoptosis mechanism may help improve cognitive function.

BDNF and GDNF play key roles in neuronal survival, development, and plasticity. Their increased expression may help repair damaged neurons and enhance the connectivity of neural networks, which is crucial for the recovery of cognitive function. NMDAR play a major role in learning and memory processes and promote synaptic plasticity. The increased expression of NMDAR1 in the IG may be related to enhanced neural signal transmission, providing a biological basis for the improvement of cognitive function [22]. These results indicate that low-frequency rTMS treatment can promote the expression of NGF to a certain extent, which may help improve the neuroplasticity and cognitive function of the hippocampal region. The increase in PSP amplitude may indicate an increase in the excitability of hippocampal neurons, which is crucial for neural plasticity and learning and memory functions. In addition, as the stimulation time extended, the PSP amplitude of the mice continued to rise, which may reflect the gradual adaptation and

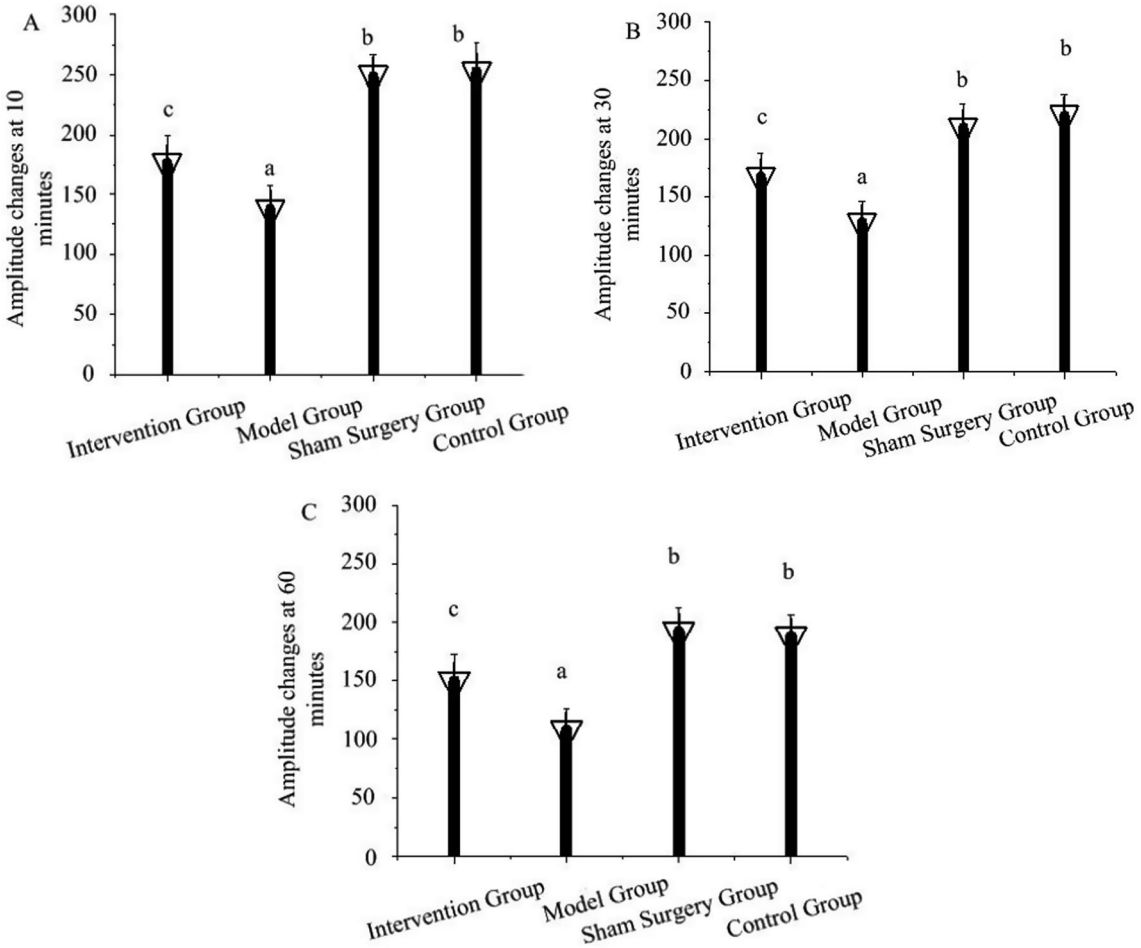

Fig. 6 Comparison of the changes in hippocampal PS amplitude among the four groups of rats after intervention. (**A** 10 min; **B** 30 min; **C** 60 min). a, b and c indicate statistically meaningful distinctions, $P < 0.05$

response of the nervous system to the treatment, providing a basis for the formulation of more optimized stimulation plans in the future [23]. These results also indicate that low-frequency rTMS can effectively increase the level of neural activity in the hippocampal region, and its neural impact mechanism may involve the expression of NGF, changes in synaptic plasticity, etc.

Conclusion

This article adopted low-frequency rTMS treatment to explore its effects on the cognitive function of rats with VCI and the underlying neural mechanisms. The results showed that the treatment markedly improved the learning and memory abilities of rats, reduced OS levels, regulated the expression of apoptosis-related proteins, and promoted the expression of NGF and NMDAR. These findings suggest that low-frequency TMS may enhance cognitive function by improving neural plasticity and reducing neural damage. However, this article has certain limitations, such as a limited sample size and a short observation period, which may affect the generalizability of the results. Future research should focus on the following aspects: increasing the sample size and extending the observation period to more comprehensively evaluate the therapeutic effects; exploring the impact of different frequencies and durations of stimulation on treatment outcomes to determine the optimal intervention plan; delving into the molecular mechanisms of this treatment to reveal its specific effects on the regulation of neural plasticity and apoptosis, providing a stronger theoretical basis for precision treatment of VCI.

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Author contributions
JW and HG participated the search and collection data, drafting of the manuscript; study concept and design, study supervision. All authors read and approved the final manuscript.

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Availability of data and materials
The original contributions presented in the study are included in the article.

Declarations

Ethics approval and consent to participate
All experimental protocols were approved by Yan'an University Xianyang Hospital. The experimental procedures were conducted following the National Institutes of Health (NIH) Guidelines for the Protection and Use of Laboratory Animals (NIH Publication No., as per regulations 85–23, updated in 1996. All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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