Enhancement of Cognitive Function in Rats with Vascular Dementia Through Modulation of the Nrf2/GPx4 Signaling Pathway by High-Frequency Repetitive Transcranial Magnetic Stimulation

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Summary

Repetitive transcranial magnetic stimulation (rTMS) represents a non-invasive therapeutic modality acknowledged for augmenting neurological function recovery following stroke. Nonetheless, uncertainties remain regarding its efficacy in promoting cognitive function recovery in patients diagnosed with vascular dementia (VD). In this study, VD was experimentally induced in a rat model utilizing the bilateral common carotid artery occlusion method. Following a recuperation period of seven days, rats were subjected to high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) at a frequency of 10 Hz. Cognitive function was assessed utilizing the Morris water maze test, and the levels of IL-6, TNF-α, SOD, GSH, MDA, and Fe^{2+} in cerebral tissue were quantitatively analyzed through enzyme-linked immunosorbent assay. Moreover, the gene and protein expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and glutathione peroxidase 4 (GPx4) were meticulously investigated *via* quantitative polymerase chain reaction (qPCR) and Western blotting techniques. The use of HF-rTMS notably augmented cognitive function in rats with VD, concomitantly reducing neuroinflammation, oxidative stress, and ferroptosis within the brain. The group subjected to HF-rTMS demonstrated an increase in the levels of both proteins and genes associated with Nrf2 and GPx4, in comparison to the VD group. These results highlight the potential of HF-rTMS treatment in enhancing cognitive function in rats diagnosed with VD through the modulation of the Nrf2/GPx4 signaling pathway. This modulation, in turn, mitigates processes linked with neuroinflammation, oxidative stress, and ferroptosis. Nevertheless, additional studies are essential to comprehensively elucidate the underlying mechanisms and clinical implications of HF-rTMS treatment in the treatment of VD.

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Introduction

Vascular dementia (VD) is widely recognized as a result of cerebrovascular disease, especially ischemic stroke, which precipitates cognitive and memory impairments [1,2]. Epidemiological evidence suggests a greater prevalence of VD relative to Alzheimer's disease, particularly among elderly populations, and it is more pronounced in developing countries as opposed to

developed nations [3]. Despite the implementation of rehabilitation and pharmacological interventions, dementia continues to pose a significant challenge, highlighting the urgent need for novel therapeutic approaches to improve treatment outcomes and prognoses [4]. Repetitive transcranial magnetic stimulation (rTMS), a potential therapy for neurological recovery after a stroke, is gaining popularity [5]. The significant contributions of the prefrontal cortex, temporal lobe, and hippocampus to cognitive impairment have been well-documented [6]. Notably, the dorsolateral prefrontal cortex (DLPFC) plays a crucial role in higher-order cognitive processes and executive functions, such as problem-solving, decision-making, planning, and working memory [7]. Employing high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) to target the DLPFC has demonstrated potential in improving working memory and executive function in patients with stroke [8,9]. Research on animal models suggests that rTMS has the potential to reverse ultrastructural alterations in ischemic areas of the brain. This effect is linked to an increased expression of endogenous brain-derived neurotrophic factor (BDNF), which supports neuronal preservation and regeneration [10]. Nonetheless, the specific mechanisms through which cognitive improvements occur following transcranial magnetic stimulation are still predominantly undefined.

The critical function of nuclear factor E2-related factor 2 (Nrf2) in mitigating oxidative stress and diminishing neuroinflammation *via* its signaling pathway is indispensable [11]. *In vitro* studies have demonstrated that antioxidant signaling regulated by Nrf2 effectively counteracts neuronal damage induced by Amyloid-beta (Aβ) [12]. Experimental findings have shown that Nrf2 improves cognitive function, decreases infarct volume, and ameliorates brain edema in rats experiencing stroke [13]. Glutathione peroxidase 4 (GPx4) maintains antioxidative activity by diminishing glutathione levels and converting lipid peroxides into lipocalciferol, thus obstructing cellular ferroptosis and safeguarding neurological functions [14]. The initiation of ferroptosis mechanisms in ischemic stroke is characterized by elevated extracellular glutamate levels, which in turn activate the N-methyl-D-aspartate receptor (NMDAR). This activation leads to an increase in cytoplasmic Fe^{2+} concentrations or hinders intracellular cystine transport, consequently impairing GPx4 functionality [15]. Recent research corroborates that the activation of the Nrf2/GPx4 signaling pathway attenuates ferroptosis, thus beneficially affecting cognitive deficits post-ischemic events and laying a theoretical groundwork for our study [16,17].

The aim of this study was to evaluate the potential effects and underlying mechanisms of HF-rTMS on cognitive dysfunction in a rat model of VD. The results offer substantial evidence endorsing HF-rTMS as a viable non-pharmacological intervention for VD.

Material and Methods

Animals

The Animal Ethics Committee of Jiaxing College, authorized under license number SYXK (Zhejiang) 2020-0007, ensured compliance with the guidelines and principles set forth in the NIH Guide for the Care and Use of Laboratory Animals for all animal experiments. The study was structured into three equally sized groups, each consisting of 30 male Sprague-Dawley rats: (1) the Sham group, which underwent sham surgery and received sham stimulation; (2) the VD group, consisting of rats with induced VD models who were subjected to sham stimulation; and (3) the HF-rTMS group, comprising rats with VD models that were treated with HF-rTMS. The ambient temperature was maintained at approximately 20 °C, with ad libitum access to food and water and a 12-hour light-dark cycle (lights activated at 7:00 am). To ensure the integrity of the study results, rats displaying any abnormal behavioral effects, such as seizures, were excluded. Rigorous measures were implemented to minimize both the suffering of the animals and the total number of animals used in the study.

Establishment of VD model

To create a rat model of VD, the two-vessel occlusion (2-VO) technique, which entails the permanent blockage of both common carotid arteries, was utilized following an approved protocol [18]. In summary, each rat was anesthetized with isoflurane (4 % for induction, 2 % for maintenance), followed by a midline incision in the neck to reveal the common carotid arteries. These arteries were then carefully dissected away from the vagus nerve. Subsequently, the carotid arteries were ligated using polyglycolic acid sutures. The ligation procedures were conducted one week apart, with the right carotid artery being ligated first, followed by the left carotid artery one week later. Conversely, rats subjected to sham surgery underwent identical procedures, with the exception of the actual ligation of the carotid arteries.

After the surgery, a one-week recovery period was allotted for the rats.

Morris water maze test

Cognitive function was evaluated using the Morris water maze (MWM) test 24 h following the completion of the last session of HF-rTMS treatment, in accordance with a protocol previously described [19]. The MWM pool was partitioned into four sections (1, 2, 3, and 4), with a platform of 12 cm diameter and 20 cm height positioned 2 cm beneath the surface of the water in section 3. Navigation trials were conducted over 5 consecutive days. The escape latency, representing the time taken for rats to locate the platform, was measured. Rats reaching the platform within 120 s were allowed a 20-second rest. In cases where rats failed to locate the platform within 120 s, they were gently guided toward it and given a 20-second opportunity to rest, with the escape time recorded as 120 s. Following the navigation trials, the platform was removed for a probe trial. Using a computer-based image analyzer, the time spent in the target quadrant and the frequency of rats traversing the platform site within 120 s were automatically recorded during the probe trial.

Repetitive transcranial magnetic stimulation

The HF-rTMS procedure involved the use of a high-focusing figure-of-eight coil (D70 P/N9925-00, with an outer wing diameter of 70 mm) in conjunction with a Magstim Rapid2 magnetic stimulator (Magstim, UK). During the HF-rTMS sessions, a fixator was used to gently immobilize rats in the HF-rTMS group. The HF-rTMS sessions were administered daily from 8:00 to 10:00 in the morning for 5 consecutive days. Following a 2-day interval, stimulation was conducted for an additional 5 days, resulting in a total of 10 days of rTMS therapy. Each HF-rTMS session involved a high-frequency stimulation protocol (10 Hz), comprising 60 standard trains. Each train comprised 20 pulses, with an interval of 8 s between each train. The stimulation intensity was set at 80 % of the maximum output of the coil. Rats in the Sham and VD groups underwent treatments analogous to those in the HF-rTMS group. The coil was positioned perpendicular to the head, allowing the rats to perceive the clicking noise without experiencing actual stimulation. Figure 1 illustrates the spatial relationship between the rat head and the coil during the rTMS procedure.
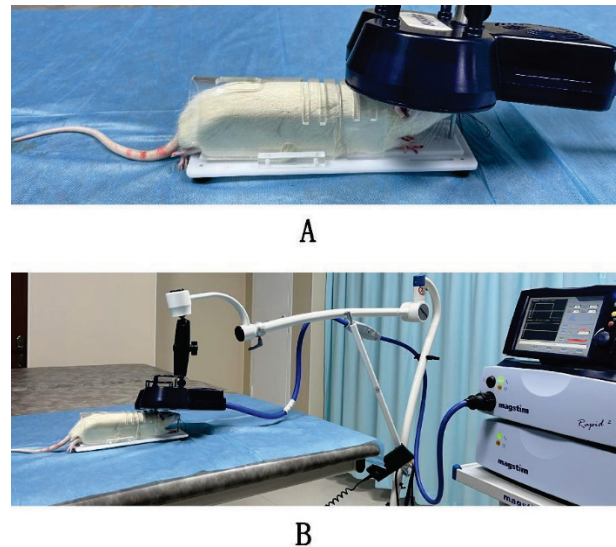

Fig. 1. A representation of the relationship between the coil and the head of a rat during stimulation.

Enzyme-Linked Immunosorbent Assay (ELISA)

Rat brain tissue was precisely weighed, and a volume of homogenizing medium, nine times that of the tissue weight (with a weight proportion of 1 mg tissue to 9 µl medium), was added. The brain tissue and homogenizing medium were mixed under ice-water bath conditions. Mechanical homogenization of the brain tissue and homogenizing medium was conducted at 2500-3000 RPM, resulting in a 10 % homogenizing solution. Subsequently, the homogenate was subjected to centrifugation for 10 min, after which, the supernatant was collected for further analysis. To assess neuro-inflammation markers, the concentrations of interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) in the rat brain tissue were determined. The levels of superoxide dismutase (SOD), malonic dialdehyde (MDA), and glutathione (GSH) were measured as oxidative stress markers. Additionally, the concentration of Fe^{2+} was quantified as a marker for ferroptosis. All necessary commercial kits for these analyses were provided by Nanjing Liboda Biotechnology Co., Ltd.

Quantitative Real-time PCR analyses for Nrf2 and GPx4

The total RNA was extracted using RNA extraction solution from Wuhan Servicebio Technology Co., Ltd., Wuhan, China, and its concentration was measured using a Nanodrop 2000 ultraviolet spectropho-tometer from Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA. Subsequently, reverse transcription (RT) was carried out using SweScript All-in-One First-

Strand cDNA Synthesis SuperMix for quantitative polymerase chain reaction (qPCR) (One-Step gDNA Remover) with random primers as RT primers. The resulting cDNA was employed for real-time PCR analysis to assess the mRNA expression of Nrf2 and GPx4, with GAPDH serving as the internal reference. Real-time PCR was conducted using 2× SYBR Green qPCR Master Mix (None ROX) from Wuhan Servicebio Technology Co., Ltd., Wuhan, China. The PCR protocol involved an initial denaturation phase at 94 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 15 s, primer annealing, and extension at 60 °C for 30 s. Fluorescence signals were collected at each 0.5 °C temperature increment within the range of 65-95 °C. The relative gene expression levels were determined using the $2^{-\triangle\triangle CT}$ technique method for data analysis. The primer sequences employed in the PCR were synthesized by Wuhan ServiceBio Technology Co., Ltd., located in Wuhan, China, and are detailed in Table 1.

Western blotting analysis for Nrf2 and GPx4

To remove blood, rat brain tissue blocks were subjected to 2 to 3 cycles of rinsing with pre-chilled PBS. The tissue was then sectioned into small pieces and lysate was added for homogenization. The resulting mixture was centrifuged at 12000 RPM for 10 min at 4 °C, resulting in the separation of the supernatant. Protein concentration in the supernatant was determined using a BCA assay kit to ensure consistent protein loading for subsequent analyses. Equal amounts of proteins were loaded onto a 10 % SDS-PAGE gel for electrophoresis. Following electrophoresis, proteins were transferred onto

a 0.45 µm PVDF membrane. The PVDF membranes were incubated overnight at 4 °C with primary antibodies: rabbit anti-Gpx4 (AB125066, diluted 1:1000, Abcam, Cambridge, UK), rabbit anti-Nrf2 (16396-1-AP, diluted 1:2000, Sanyin, Wuhan, China), and mouse anti-ACTIN (GB12001, diluted 1:2000, Servicebio, Wuhan, China). After three washes with TBST, membranes were incubated with secondary antibodies (GB23301, goat anti-mouse IgG HRP conjugated, diluted 1:5000; GB23303, goat anti-rabbit IgG HRP conjugated, diluted 1:5000; GB23303, goat anti-rabbit IgG HRP conjugated, diluted 1:5000; Servicebio, Wuhan, China). The strips were treated using a mixture of ECL luminescent solution, with actin serving as an internal reference for normalization. Raw data in TIFF format were analyzed using AIWBwellTM analysis software (Servicebio, Wuhan, China) to determine the relative expression of the target proteins.

Statistical analyses

Statistical analyses were conducted using SPSS 22.0 software, and GraphPad Prism (version 9.5.0, GraphPad Software, Inc.) was used for generating graphs. Multiple comparisons were assessed through one-way ANOVA, followed by *post hoc* Bonferroni testing. The data are presented as the mean ± standard deviation (SD), and statistical significance was considered at $p<0.05$.

Ethics approval

The study was approved by the Ethics Committee of Jiaxing College (approval No. SYXK (Zhejiang) 2020-0007).

Table 1. The sequence information of PCR primers.

Name	Sequence	Tm	Length of Amplification	Gene ID
Nrf2 forward	5'-GACATCCTTTGGAGGCAAGACAT-3'	60	268 bp	NM_031789.2
Nrf2 reverse	5'-TGGGAATGTGGGCAACCTG-3'			
GPx4 forward	5'-GACATCCTTTGGAGGCAAGACAT-3'	60	212 bp	NM_001039849.3
GPx4 reverse	5'-TGGGAATGTGGGCAACCTG-3'			
NADPH forward	5'-CTGGAGAAACCTGCCAAGTATG-3'	60	138 bp	NM_017008.4
NADPH reverse	5'-GGTGGAAGAATGGGAGTTGCT-3'			

Results

Effect of HF-rTMS on the learning memory ability of rats with VD

The spatial learning and memory abilities of rats

were evaluated. There were no significant differences in mean velocity observed among the Sham group, VD group, and HF-rTMS group (Fig. 2A). Noteworthy distinctions were observed in the MWM test: the VD group exhibited prolonged escape latencies and fewer platform crossings

from day 1 to day 4 compared to the sham group ($p<0.05$) (Fig. 2B, C). Conversely, over the initial four days, rats in the HF-rTMS group displayed reduced escape latencies and increased platform crossings compared to the VD group (Fig. 2B, C). Moreover, the swimming paths of the VD group demonstrated a higher level of disarray compared to the sham group, while the HF-rTMS group exhibited more organized swimming paths (Fig. 2D).
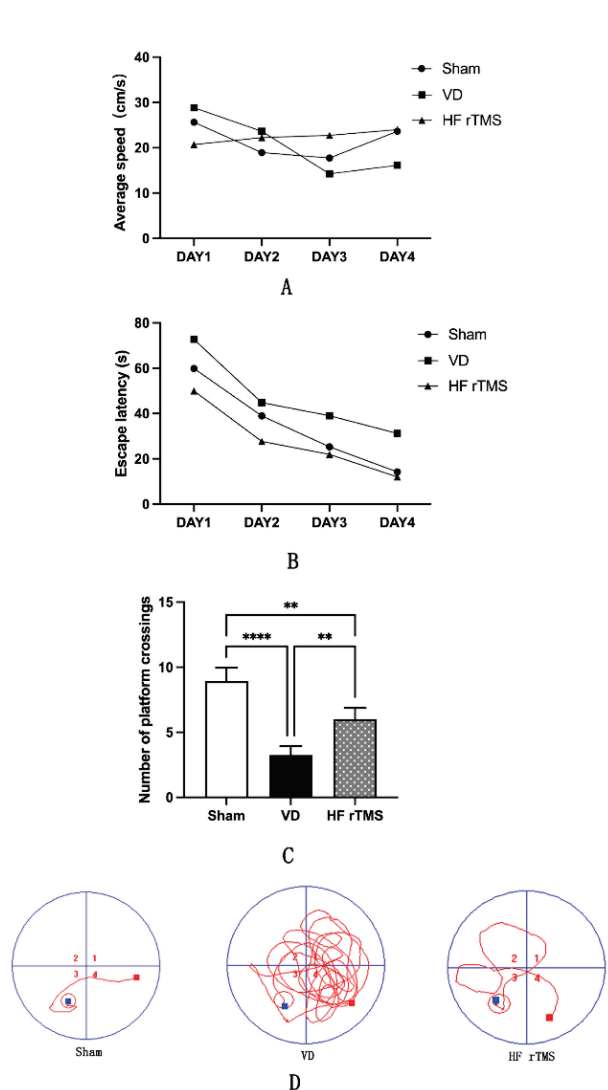
quantitative ELISA results ($p<0.05$). Following HF-rTMS treatment, the HF-rTMS group exhibited a significant reduction in IL-6 and TNF-α levels in brain tissue compared to the VD group ($p<0.05$). Notably, there were significant reductions in IL-6 and TNF-α concentrations in the brain tissue of the HF-rTMS group when compared to the sham group ($p<0.05$), as illustrated in Figure 3.
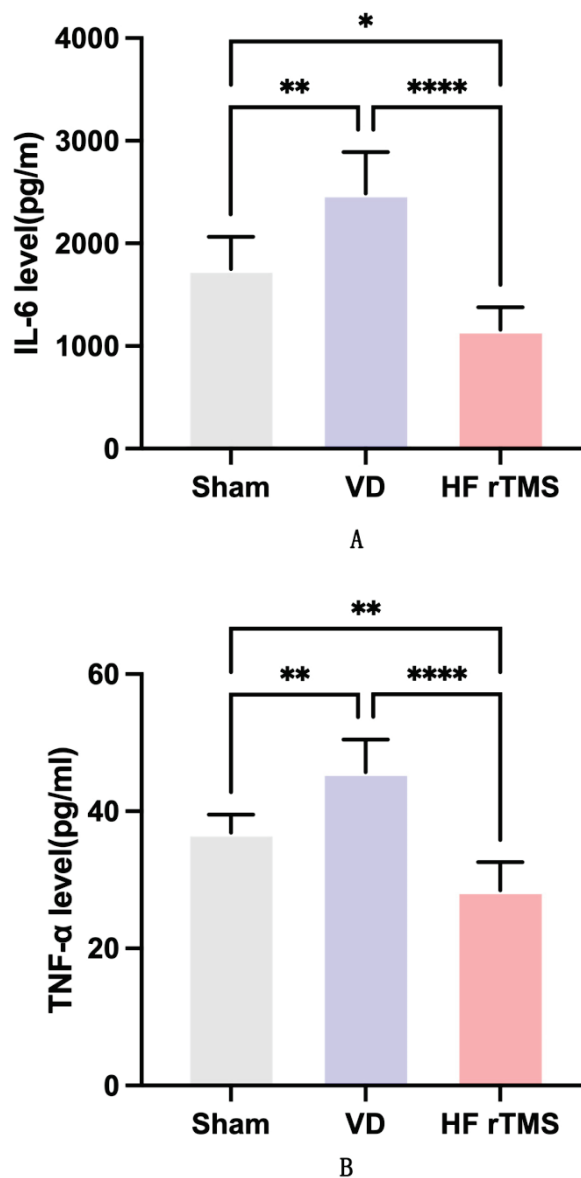

Fig. 2. Impact of HF-rTMS on the learning memory ability of rats with VD. (**A**) Mean velocity; (**B**) Escape latency; (**C**) Number of platform traversals; (**D**) Representative swimming trajectories for all groups in the MWM test (* $p<0.05$, ** $p<0.01$, **** $p<0.0001$). HF-rTMS, High-Frequency Repetitive Transcranial Magnetic Stimulation; VD, Vascular Dementia; MWM, Morris water maze.

Fig. 3. Impact of HF-rTMS on neuroinflammation in brain tissue of rats with VD. (**A**) IL-6 and (**B**) TNF-α levels (* $p<0.05$, ** $p<0.01$, **** $p<0.0001$).

Effect of HF-rTMS on neuroinflammation in brain tissue of rats with VD

       Compared to the sham group, rats in the VD group demonstrated significantly elevated levels of IL-6 and TNF-α inflammatory cytokines, as indicated by

Effect of HF-rTMS on oxidative stress in brain tissue of rats with VD

       In the brain tissues of rats with VD, significant decreases were observed in the levels of superoxide dismutase (SOD) and glutathione (GSH) ($p<0.05$),

whereas levels of malonic dialdehyde (MDA) experienced a significant increase ($p<0.05$), as determined using commercial kits. Following HF-rTMS therapy, there was a notable increase in the levels of SOD and GSH ($p<0.05$), and a decrease in the levels of MDA ($p<0.05$) compared to the VD group. However, there were no significant differences in SOD, GSH, and MDA levels between the sham group and the HF-rTMS group (p>0.05), as illustrated in Figure 4.
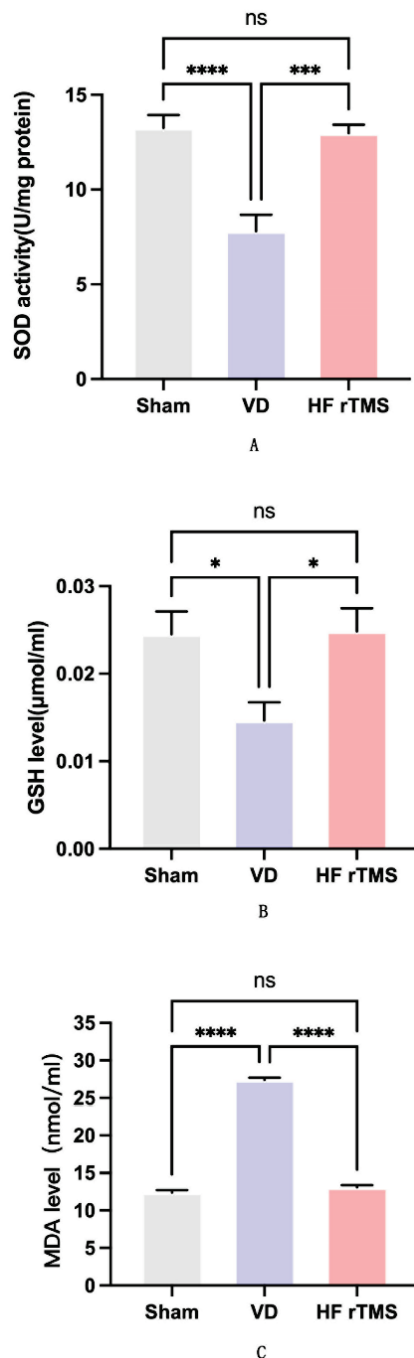

Fig. 4. Impact of HF-rTMS on oxidative stress in the brain tissue of rats with VD. (**A**) SOD activity; (**B**) GSH level; and (**C**) MDA level (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

Effect of HF-rTMS on ferroptosis in brain tissue of rats with VD

The quantitative ELISA analysis revealed a significant increase in Fe^{2+} levels in the rat brain tissue of the VD group compared to the sham group ($p<0.05$). However, the administration of HF-rTMS led to a significant decrease in Fe^{2+} concentrations in the brain tissue of rats with VD belonging to the HF-rTMS group ($p<0.05$) compared to the VD group. No notable disparities in Fe^{2+} concentrations were observed between the sham group and the HF-rTMS group (p>0.05), as illustrated in Figure 5.
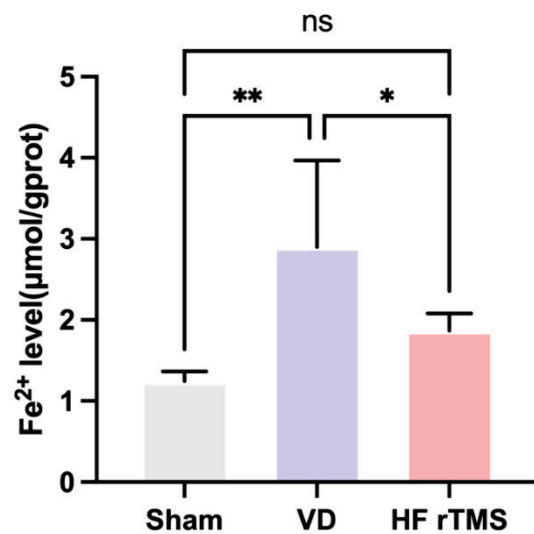

Fig. 5. Impact of HF-rTMS on ferroptosis in brain tissue of rats with VD. Fe^{2+} level (* $p<0.05$, ** $p<0.01$, ns $p>0.05$).

Effect of HF-rTMS on Nrf2 and GPx4 gene expression in brain tissue of rats with VD

Fluorescence PCR analysis demonstrated a significant decrease in the expression levels of both Nrf2 and GPx4 genes in the brain tissue of rats in the VD group compared to the sham group ($p<0.05$). Conversely, the application of HF-rTMS resulted in an increase in the levels of Nrf2 and GPx4 gene expression in the brain tissue of rats with VD ($p<0.05$). Importantly, there were no notable disparities in the expressions of Nrf2 and GPx4 genes between the sham group and the HF-rTMS group ($p>0.05$) (Fig. 6).

Effects of HF-rTMS on Nrf2 and GPx4 protein expression in brain tissue of rats with VD

Western blot analysis was used to assess the protein expressions of Nrf2 and GPx4 in rat brain tissues from the sham, VD, and HF-rTMS groups. In the brain tissues of rats from the VD group, the expressions of both

Nrf2 and GPx4 proteins were significantly lower compared to the sham group ($p<0.05$). Conversely, after receiving HF-rTMS therapy, there was a notable increase in the levels of Nrf2 and GPx4 proteins compared to the VD group ($p<0.05$). No significant differences were observed between the sham group and the HF-rTMS group ($p>0.05$), as depicted in Figure 7
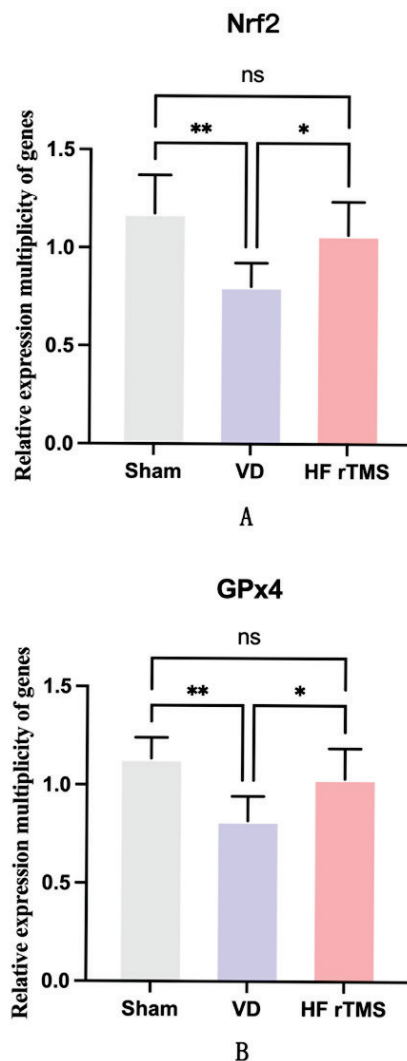

Fig. 6. Impact of HF-rTMS on the expression of Nrf2 and GPx4 genes in the brain tissue of rats with VD. (**A**) Relative expression multiplicity of the Nrf2 gene; (**B**) Relative expression multiplicity of the GPx4 gene (* $p<0.05$, ** $p<0.01$, ns $p>0.05$).

Discussion

Evaluating cognitive enhancement strategies for conditions like VD is considered essential due to its rising prevalence and significant impact on quality of life. The aim of this study was to explore the efficacy of HF-rTMS in modulating the Nrf2/GPx4 signaling pathway to alleviate cognitive impairments in VD rat models. Our results reveal a marked improvement in spatial learning and memory capabilities following HF-rTMS treatment in rats with VD. Moreover, in comparison to both the Sham stimulation and VD control groups, treatment with HF-rTMS resulted in a significant decrease in the levels of IL-6 and TNF-α in the brain tissues of rats. Subsequent evaluations post-treatment demonstrated notable elevations in the levels of SOD and GSH, along with a significant reduction in MDA levels, in rats with VD that underwent HF-rTMS treatment. Additionally, treatment with HF-rTMS led to a reduction in Fe^{2+} levels within the brain tissues of rats. Notably, both protein and gene expressions of Nrf2 and GPx4 were significantly upregulated in the HF-rTMS group in comparison to the VD group. This provides compelling evidence for the potential of HF-rTMS as an effective therapeutic strategy for VD.

Nonetheless, to fully ascertain the effectiveness and applicability of this intervention, further studies are required to uncover its underlying mechanisms. Over time, rTMS has demonstrated potential in improving cognitive functions in neurodegenerative diseases [20,21]. A recent meta-analysis underscored the significance of targeting the parietal and prefrontal cortex with TMS, revealing its neuro modulatory effects on cognitive processes such as number processing, magnitude processing, and arithmetic [22]. The DLPFC has emerged as a significant focus of therapeutic interventions, particularly for the elderly and individuals with mild cognitive impairment [23]. However, mechanistic studies elucidating the impact of TMS on VD are limited. Existing reports indicate that rTMS can modulate neurotransmitter levels such as acetylcholine, dopamine, and synaptophysin, leading to enhanced cognitive function [24,25]. In this study, we provide evidence of the positive impact of HF-rTMS on cognitive deficits in a VD rat model. Furthermore, we uncovered the anti-neuroinflammatory, antioxidant, and ferroptosis-inhibitory effects of HF-rTMS in the context of VD.

Given the pivotal roles of Nrf2 and GPx4 expression, along with established contributions of neuroinflammation, oxidative stress, and ferroptosis to cognition, a comprehensive assessment of their interplay becomes imperative [26-28]. Since the identification of Nrf2, exhaustive investigations have sought to elucidate its biological functions. A thorough understanding of its structural features, molecular mechanisms, functional attributes, activity regulation, downstream pathways, and
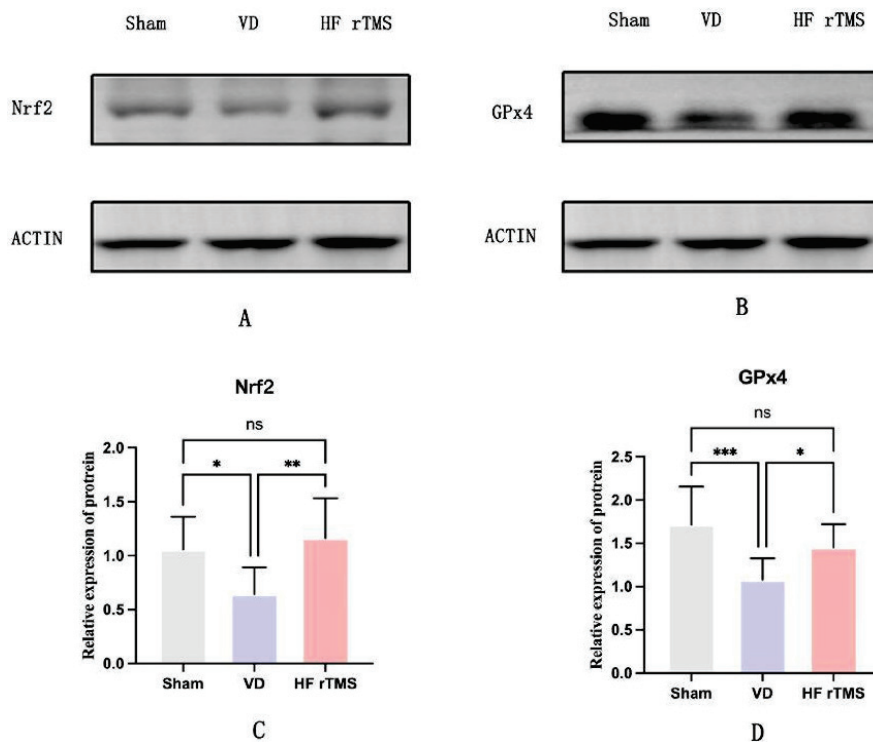
Fig. 7. Effects of HF-rTMS on Nrf2 and GPx4 protein expression in brain tissue of rats with VD. (**A**) Western blot analysis of protein expression for Nrf2 in rat brain tissue; (**B**) Western blot analysis of protein expression for GPx4 in rat brain tissue; (**C**) Relative expression of protein Nrf2; (**D**) Relative expression of protein GPx4 (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns $p>0.05$).

potential therapeutic applications for diverse ailments is essential [29,30]. Recent research affirms the engagement of Nrf2 in anti-neuroinflammatory processes [31]. In the context of cerebral ischemia/reperfusion injury, activation of the NF-κB pathway triggers the expression of inflammatory factors (IL-6, TNF-α, and IL-1β), thereby exacerbating brain damage and post-stroke dysfunction. Consistent with this, elevated levels of IL-6 and TNF-α, along with reduced Nrf2 expression, are observed in the brain tissue of rats with VD in our study. Furthermore, the HF-rTMS group demonstrates a significant increase in Nrf2 expression, accompanied by notable enhancements in spatial memory and cognitive abilities when compared to the VD group. While the precise mechanisms underlying Nrf2 upregulation by rTMS remain elusive, it offers a plausible explanation for cognitive enhancement post-transcranial magnetic stimulation, aligning with findings in an Alzheimer's disease (AD) rat model where lentiviral-transfected Nrf2 injection into hippocampal tissue significantly enhanced learning and memory capabilities [32]. However, further elucidation is required regarding the specific mechanism by which HF-rTMS modulates neuroinflammatory pathways.

The burgeoning evidence underscores the pivotal role of Nrf2 in orchestrating cellular defense mechanisms, particularly in governing antioxidant systems. The results of our study validate decreased SOD and GSH activity in rats with VD, effectively restored by

HF-rTMS-induced Nrf2/GPx4 activation. HF-rTMS also reduces MDA concentrations, showcasing its ability to attenuate oxidative stress from chronic cerebral hypoperfusion, providing neuronal protection. Yet, a comprehensive understanding of HF-rTMS-induced changes in the oxidation state warrants further assessment [33]. Dixon introduced ferroptosis, a distinct programmed cell death pattern induced by erastin disrupting cysteine transport, reducing glutathione, and inactivating GPx4. Ferroptosis, distinct from apoptosis and necrosis, is iron-dependent, marked by increased intracellular iron ions and lipid peroxide accumulation [34]. Multifaceted inducers of ferroptosis encompass the inhibition of system Xc-, inactivation of GPx4, elevation of ROS and iron levels, and the accumulation of lipid peroxides, constituting the participants of extensive investigation [35-37]. The investigation into neurological conditions is focused on the pivotal role of ferritin in iron storage [38,39]. GPx4, a selenoprotein glutathione peroxidase, decreases hydrogen peroxide, crucial in preventing ferroptosis by reducing phospholipid hydrogen peroxide and curbing lipoxygenase-mediated lipid peroxidation [40]. Our findings in VD-affected rat brains show significant reductions in Nrf2 and GPx4 expressions and increased Fe^{2+} levels. Notably, HF-rTMS activates Nrf2 and GPx4, decreases Fe^{2+} levels, and enhances cognitive function. Ferroptosis observed in the ischemic rat model corresponds with a decrease in GPx4 expression [41].

Heightened brain iron triggers pro-inflammatory factor release, contributing to iron accumulation. Despite increased research on ferroptosis and VD, further clarification on the connections between neuroinflammation, oxidative stress, and ferroptosis is essential [42,43].

Several limitations exist in our study. The challenge lies in the dimensions of the stimulation coil; despite opting for a 70 mm figure-of-eight coil to reduce overheating during high-frequency stimulation, even the most compact coil on the market remains excessively big for the head of a rat. This constraint prevents accurate single-brain region targeting using rTMS in rats. Thus, whole-brain stimulation was used to assess rTMS effects. Additionally, the small sample size may limit the depth of our results and the study of rTMS mechanisms. Furthermore, the complexity of cognition extends beyond neuroinflammation, oxidative stress, and iron-induced cell death; cognitive enhancement may result from a synergistic effect of multiple factors. Therefore, future research should consider the complexity of cognition and assess the interplay of these multiple factors.

Conclusions

In conclusion, our findings undeniably establish that the use of high-frequency transcranial magnetic stimulation significantly enhances cognitive capabilities in a rat model of VD. This enhancement appears to be intricately linked to the modulation of oxidative stress, neuroinflammation, and ferroptosis through the Nrf2/GPx4 signaling pathway. Overall, this study provides a conceptual framework supporting the potential use of HF-rTMS as a viable strategy to alleviate cognitive deficits associated with VD.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Abbreviations

ARE, Antioxidant Response Elements; GSH, glutathione; GSR, Glutathione; GSSH, Oxidized Glutathione; Reductase; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; γ-GCS, Gamma-Glutamylcysteine Synthetase; NF-κB, Nuclear Factor-κB; PUFAS, Polyunsaturated Fatty Acids

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