Repetitive Trans-Spinal Magnetic Stimulation Improves the Function of the Injured Spinal Cord in Rats

Wu Xiang

Shanghai Fourth People's Hospital Affiliated to Tongji University School of Medicine

Han Xiang

Army Medical University

Jiao-juan Huang

The General Hospital of Western Theater Command

Jun-yu Wang

Sichuan University

Rui-ling Tang

The General Hospital of Western Theater Command

Min Li

Shanghai Fourth People's Hospital Affiliated to Tongji University School of Medicine

Bing-jin Ji

Beibei Traditional Chinese Medical Hospital

Wen-chun Wang

The General Hospital of Western Theater Command

An-ren Zhang (✉ anren0124@163.com)

Shanghai Fourth People's Hospital Affiliated to Tongji University School of Medicine

Research Article

Keywords: trans-spinal magnetic stimulation, frequency, spinal cord injury, motor function

Posted Date: February 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1285709/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Studies have found that magnetic stimulation (MS) can induce the plasticity of the spinal cord and promote axon regeneration, resulting in improving motor dysfunction after SCI. However, In the clinical application of repetitive trans-spinal magnetic stimulation (rTSMS), there is no uniform standard for the formulation of treatment parameters, the most important of which is the treatment frequency. This progress has been compared in six groups of animals. After rTSMS, the BBB score and the inclined plate test score of SCI rats increased significantly. Besides, the frequency of 1 Hz significantly increased spinal cord function in the acute and subacute phases after SCI, and the frequency of 15 Hz increased spinal function in the intermediate and chronic phases after SCI. Western blot analysis showed that the expression of GAP-43 in the 15 Hz magnetic stimulation (MS) group increased significantly. Also, rTSMS induced the expression of GAP-43 which may promote axon growth and guided the formation of new nerve connections. This study showed that rTSMS improves spinal cord function in SCI rats. Furthermore, 1 Hz stimulus is more conducive to the recovery of motor function in the acute and subacute phases after SCI, and the frequency of 15 Hz is more conducive to the recovery of motor function in the intermediate and chronic phases after SCI. Besides, rTSMS increases the expression of GAP-43 in the injured spinal cord.

1. Introduction

Spinal cord injury (SCI) is a common central nervous system injury that can lead to permanent loss or reduction of the function of movement and sensation below the injury site ¹. The previous study has shown that, with the development of the world's economic level, the incidence of SCI is increased year by year ². Most patients suffering from SCI have a long-life expectancy and high cost of treatment and care, which has become a huge public health problem in the world.

Although a series of studies have been carried out to treat SCI, there is no exact and effective treatment method was found in clinics ³. After SCI, early surgical decompression and spinal fixation can reduce the secondary damage of injured spinal cord tissue and provide clinical benefits to patients, but it does not directly promote axon regeneration and repair. Based on the NASCIS (National Acute Spinal Cord Injury Study) II and III trials, methylprednisolone is recommended for the management of SCI ^{4,5}. However, some evidence shows that the methylprednisolone treatment did not improve patients' motor or neurological scores ⁶, and it is significantly related to patients with the increased risk of hyperglycemia and pneumonia ^{7,8}. At the same time, many drugs can effectively prevent the excitatory cascade of neuronal death under test conditions, such as erythropoietin, glibenclamide, but no drug has been proven to improve the prognosis of patients with SCI. Stem cell therapy is one of the emerging important methods for the treatment of SCI ⁹, however, the subsequent immune rejection or tumor formation problems have not been resolved, and the clinical treatment of SCI still needs further research. Multimodal neuromodulation technology combined with personalized rehabilitation strategies as a new means, such as, magnetic stimulation (MS), electrical stimulation, which can strengthen the

reconstruction of neural circuits, so as to achieve nerve regeneration and motor function recovery in patients with SCI ¹⁰.

Studies have found that MS can induce the plasticity of the spinal cord and promote axon regeneration, resulting in improving motor dysfunction after SCI ¹¹. For the treatment of SCI, trans-cranial magnetic stimulation (TMS) was mostly used in clinics, and there is evidence that repetitive trans-spinal magnetic stimulation (rTSMS) is better than TMS in improving motor function after SCI ¹². In the clinical application of rTSMS, there is no uniform standard for the formulation of treatment parameters, the most important of which is the treatment frequency. Previous studies have found that rTSMS treatment with frequencies at 0.5 Hz, 1 Hz, 5 Hz, 15 Hz can improve motor function after SCI ¹²⁻¹⁴. However, it is still unclear which frequency of rTSMS is best to improve motor function after SCI, and there is a lack of corresponding comparative studies, which severely limits the basic research and clinical application of this technology. Therefore, this study intended to use rTSMS with frequencies of 1 Hz, 5 Hz, 10 Hz and 15 Hz to act on the L2 spinal cord segment of rats. The central pattern generator (CPG) of rat is located at the L1-2 spinal cord segment., and previous studies have shown that MS of L2 spinal cord segment is more conducive to the recovery of motor function after SCI ¹³. Therefore, in this study, the L2 spinal cord segment was selected as the stimulation site, and through behavioral evaluation (BBB score and inclined plate test), observation of the gross morphology and structure of spinal cord tissue injury and detection of GAP-43 protein expression, to explore the effect of rTSMS with different frequencies on the motor function of SCI rats. The results showed that after rTSMS, the BBB score and the inclined plate test score of SCI rats increased significantly, the frequency of 1 Hz significantly increased the spinal cord function in the acute and subacute phases after SCI, and the frequency of 15 Hz increased the spinal function in the intermediate and chronic phases after SCI, and the expression of GAP-43 in the 15 Hz MS group increased significantly.

2. Materials And Methods

2.1 Animals

Ninety-six adult Sprague-Dawley (SD) female rats (260~310g, provided by Chengdu Dawley Biotechnology Co, Ltd). Rats were randomly divided into six groups (n = 16), including sham operation, control (acute SCI without stimulation), 1 Hz (1 Hz stimulation), 5 Hz (5 Hz stimulation), 10 Hz (10 Hz stimulation), and 15 Hz (15 Hz stimulation). All animals were maintained in the animal room under the controlled conditions with temperature of 23-25°C, relative humidity of 40-70%, and 12 hour light-dark cycle. The animals had free access to water and standard diet. All experiments were conducted in strict accordance with the recommendations of the "Regulations for the Administration of Affairs Concerning Experimental Animals," State Science and Technology Commission, China. All animal procedures were approved by the Ethics/Review Committee for Experimental Animal Use and Care of Chengdu General Hospital of the Military Region (2013YG-B005) and all experiments were performed in accordance with the ARRIVE guidelines 2.0.

2.2 Establishment of SCI model

Rats were anesthetized with the intraperitoneal injection of pentobarbital sodium (45 mg/kg), and then they were fixed on the surgery table. The surgical site was shaved and sterilized. Briefly, a longitudinal incision of 3 cm was made to expose the T9, T10, and T11 vertebral plates. Then, a laminectomy was performed at the T10 level to expose the dorsal epidural space of the spinal cord. A triangular needle was clamped with a needle-holding device so that the blunt end could completely pass through the space between the ventral dura and the vertebral body to avoid SCI and pulling. The aneurysm clip (Yasargil Titan Mini Clips, FT228T, Peter Lazic GmbH Tuttlingen, Germany, closing force 70 g) was fixed, and after opening, the clip was positioned at T10 through the channel to the opposite side to ensure that it completely crossed the spinal cord. Then, the clip was suddenly released, and the spinal cord was instantly compressed by the force, causing spasmodic tremor and oscillation in the rat body. When the clip was gently removed after 10 s, subdural congestion or hematoma was visible, with obvious clip marks outside of the dura¹⁵. The wound was then sutured in layers. The sham operation group only bites the lamina and don't damage the spinal cord. After operation, the rats were injected with penicillin (200,000 IU/mouse) for 7 consecutive days¹⁶. Artificial assisted urination was performed 3 times a day after the operation until the rats resumed urination spontaneously. During the experiment, 3 rats died of a ruptured bladder, 3 rats died of biting each other, and 9 rats died of unknown causes, which were not included in the experiments.

2.3 Trans-spinal magnetic stimulation

Rats in the 1 Hz, 5 Hz, 10 Hz and 15 Hz groups were treated with rTSMS at different frequencies from postoperative day 4 using a magnetic stimulator (Wuhan Iride Medical Equipment New Technology Co, Ltd of China.). The rat is fixed in a special box with a hole (the magnetic field can be shielded to target the magnetic field to the part requiring stimulation), the axis of the "8" shaped coil is perpendicular to the L2 spinal segment, and the center of the coil is tight with the spine. Treatment with a maximum output intensity of 35% (1.5 T), 5 seconds per sequence, 10 seconds intermittently, a total of 450 pulses, once a day, 5 days a week, a total of 8w. The rats in control group received no stimulation. During the stimulus in control rats, the magnetic coil was placed under the segments similar to the other groups, but MS was not transmitted. However, the magnetic coil vacuum cooling system still produced the typical auditory stimuli that were present during the actual stimulus.

2.4 Behavioral tests

2.4.1 Basso, Beattie and Bresnahan score

The movement function of the rats was evaluated by Basso, Beattie and Bresnahan (BBB) score at 1 day before operation, 1 week, 2 weeks, 4 weeks, 6 weeks and 8 weeks after operation, respectively. In the BBB score, the recovery of hindlimb movement function in each experimental group was evaluated, and the hind limb joint activity, weight bearing, gait, front and rear limb coordination, fine movement of the claw and tail position were evaluated. The BBB score ranged from 0 to 21, where 21 = normal and 0 =

complete paralysis¹⁷. The scoring was performed by two persons who were familiar with the scoring criteria but did not know the grouping, and the average of the scores were calculated.

2.4.2 Inclined plate test

The balance ability of the rats was evaluated by inclined plate test at 1 day before operation, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks after operation, respectively. Place the rat on an inclined rough plane, which can be adjusted to provide a different inclination angle. The longitudinal axis of the rat's body was perpendicular to the longitudinal axis of the inclined plate at 90°, starting from 0°, and at each time, the angle was raised at 5°. As the rat stopped steadily on the inclined plate for 5 seconds, angle of the inclined plate was increased until the rat did not stay for 5 seconds, and the maximum angle was recorded¹⁸. The scoring is performed by two persons who were familiar with the scoring criteria but did not know the grouping, and the average of the scores were calculated.

2.5 Hematoxylin-Eosin staining

After 8 weeks of intervention, rats were anesthetized and the thoracic cavities were exposed, the hearts and ascending aorta were inserted with the perfusion needles. Saline and paraformaldehyde (4% paraformaldehyde + PBS buffer) were successively infused to fix tissue. A spinal cord tissue specimen of about 1 cm in length was collected from the injured area, dehydrated by graded alcohol, and cut into 4 µm thick paraffin sections for Hematoxylin-Eosin staining and pathological diagnosis.

2.6 Western blotting

To investigate the mechanisms underlying the growth of nerve cells in the SCI, Western blotting was used to detect the expression of GAP-43 in the SCI. After 8 weeks of intervention, 3 rats in each group were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg), and a spinal cord tissue specimen with a length of about 1 cm was collected from the SCI site and placed in a refrigerator at -80°C. Briefly, after SDS polyacrylamide gel electrophoresis and PVDF membrane transferring, the PVDF membrane was putted in BSA solution for 2 h and washed with TBST for 3 times. And then the PVDF membrane was incubated with the primary antibody (primary antibody concentration: GAP43 1:50000; β-actin 1:5000) overnight at 4°C. On the next day, the PVDF membrane was washed with TBST for 3 times, and incubated with the secondary antibody at a concentration of 1:5000 for 2-3 h at room temperature. After that, the PVDF membrane was washed for 3 times with TBST. Finally, the protein on the PVDF membrane were displayed in ECL luminescent solution. The relative expression of the protein was detected as the integrated optical density (IOD) of the protein to be detected / the integrated optical density (IOD) of β-actin.

2.7 Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the data. Normally distributed data was described as the mean ± SD. Repeated measures analysis of variance (ANOVA) was

used to analyze behavioral data, and other data were compared using one-way ANOVA to compare the mean of multiple samples. In the case of uniform variance, LSD-t test was used. Differences were considered statistically significant at $P < 0.05$ and $P < 0.01$. All data were analyzed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA).

3. Results

3.1 rTSMS improved the BBB score of SCI rats

As shown in Figure 1, the BBB scores of the rats before modeling were 21 points. Repeated measurement analysis of variance showed that rTSMS improved SCI [$F(5,75) = 2159.402$, $P < 0.001$], and there was a significant time effect [$F(6,450) = 9309.712$, $P < 0.001$], and a significant interaction between rTSMS treatment and stimulation time [$F(30,450) = 381.997$, $P < 0.001$]. The LSD post-hoc test showed that compared with control rats, rTSMS at all frequencies improved BBB scores ($P < 0.001$), but there was no significant difference among the intervention groups. As shown in Figure 1, further analysis of the BBB scores in each week showed, that rTSMS at all frequencies did not improve BBB scores on the first day and the first week of stimulation ($P > 0.05$). The treatment started to be effective at the 2nd week, and the treatment effect became more obvious with the increase of time. Furthermore, the 1 Hz treatment showed a more significant effect at 2-4 weeks, and the 15 Hz showed a more significant effect at the 8th week. Therefore, we suggested the high-frequency stimulation showed better effective in the long-term treatment.

3.2 Rtsms Improved The Balance Function Of Sci Rats

As shown in Figure 2, repeated measures analysis of variance showed that rTSMS improved the balance function of rats [$F(5,75) = 406.906$, $P < 0.001$], and there is a significant time effect [$F(6,450) = 2069.847$, $P < 0.001$], and a significant interaction between rTSMS treatment and stimulation time [$F(30,450) = 97.967$, $P < 0.001$]. The LSD post-test showed that compared with the control rats, only 15 Hz treatment was significant ($P = 0.026$). Further analysis of the individual effects, that rTSMS at all frequencies did not improve the balance function on the first day and the first week of stimulation ($P > 0.05$). The treatment started to be effective at the 2nd week, and the treatment effect became more obvious with the increase of time, and the 1 Hz treatment showed a more significant effect at 2-4 weeks, and the 15 Hz treatment showed a more significant effect at the 8th week. Therefore, we suggested that we suggested the high-frequency stimulation showed better effective in rescuing the balance function in the long-term treatment.

3.3 rTSMS reduced the pathological damage of the spinal cord

In order to evaluate whether rTSMS may reduce SCI, HE staining was performed on the injured spinal cord tissue after 8 weeks of intervention to assess the degree of injury. As shown in Figure 3, the spinal cord tissue of in sham-operation group has a complete capsule and structure, and the neurons in the gray

matter have normal morphology, and the cytoplasm was stained more uniformly. The white matter contains myelinated nerve fibers and was covered with glial cells. The spinal cord tissue in other groups showed poor integrity, structurally fuzzy and unclear, neuronal necrosis or dissolution disappears, and nerve fiber tissue was arranged in a cord or necrosis, fracture or even dissolution disappeared, and some cavities were visible, and proliferation of fibrous tissue or microglia. It can be seen that rTSMS can reduce the pathological damage of the spinal cord.

3.4 GAP-43 protein expression was increased by 10 and 15 Hz stimuli

As shown in Figure 4, one-way analysis of variance and post-hoc test showed that the expression level of GAP-43 in control rats was significantly higher than that in sham-operation rats [$F(5, 17) = 16.231$, $P < 0.001$; post hoc test $p = 0.027$]; and the GAP-43 protein expression in the spinal cord tissue treated with 10 and 15 Hz stimuli was higher than that of control ones ($P = 0.002$ and $P < 0.001$, respectively). Furthermore, the GAP-43 protein expression in the spinal cord tissues treated with 10 and 15 Hz stimuli were significantly higher than that treated with 1 and 5 Hz stimuli ($P < 0.05$).

4. Discussion

This study showed that rTSMS induced an increase in GAP-43 expression at SCI tissues, thus improving motor function after SCI. First, we performed the BBB score and the inclined plate test score to determine that the treatment started to be effective at the 2nd week of MS, which indicated that the treatment effect becomes more obvious with time, and the 1 Hz treatment was more effective for 2-4 weeks, whereas the 15 Hz treatment was more effective at 8th week. These results preliminarily indicated that rTSM can improve SCI, but the choice of MS frequency at different time stages after SCI was different, and the mechanisms of which may be different. At the same time, HE staining showed that rTSMS could reduce the degree of SCI. Therefore, these results indicated that the rTSMS contributes to the recovery of SCI. We speculated that the rTSMS may activate the CPG of the spinal cord by inducing the expression of GAP-43 in the injured tissue of the spinal cord, thus improving the motor function of SCI rats. Therefore, after 8 weeks of MS intervention, quantitative western blot analysis was performed to observe the expression of GAP-43 in the spinal cord tissues. The results showed that the GAP-43 protein expression in the spinal cord tissues of the 10 and 15 Hz treated rats was higher than that of the controls, indicating that MS promotes axon growth by inducing the expression of GAP-43, activating CPG, and resulted in inducing spinal cord plasticity and improving SCI.

Our study was partly consistent with the results of previous studies^[14], which revealed that the 1 Hz treatment had a better effect than 15 Hz treatment within 4 weeks after SCI, but it did not further explore the choice of treatment frequency after 4 weeks. In this experiment, it was observed that the effect of high-frequency magnetic stimulation was more significant after 4 weeks of SCI, among them, the 15 Hz treatment was the most significant at the 8th week.

At the same time, this experiment comparatively studied the 4 frequencies of MS, which has a wider selection range and was more conducive to clinical application and promotion. We suggested that the physiological mechanisms of the acute phase, subacute phase, and recovery phase after SCI were not consistent, and CPG activation required a cumulative effect of time. Therefore, the frequency of treatment selected for different time periods after SCI was different. During the acute phase, hemorrhage and alteration of the blood-spinal cord barrier exposes the medullary cord to a rapid influx of inflammatory cells and cytokines¹⁹. Cell degeneration causes the release of adenosine triphosphate and potassium ions into the microenvironment, which activates the microglia to release further proinflammatory cytokines and recruit peripheral inflammatory cells. The phagocytes, activated for cellular debris elimination, release cytotoxic products, which in turn amplify cell damage²⁰. During the subacute phase, the edema causes further vascular alteration, meanwhile, the death of neurons causes the accumulation of glutamate and the excitotoxicity of nearby neurons²¹. Studies have shown that there are differences in the effects of MS frequency on the central nervous system, where high frequency (stimulation frequency > 1Hz) is excitation²², and low frequency (stimulation frequency $\leq$ 1Hz) is inhibition²³. We speculated that low-frequency MS may inhibit the inflammation of the SCI, thereby benefiting the repair of SCI, while high-frequency MS may overstimulate the spinal cord tissue that was already in the acute phase. This excessive excitability could increase the excitotoxicity of SCI tissues, which was not conducive to tissue repair.

During the recovery period of SCI, pathophysiological changes were mainly manifested as vascular remodeling, changes in extracellular matrix components and reorganization of distal neuronal circuits²⁴, and the decrease in cell number produces cystic vacuoles²⁵; at the same time, astrocytes, pericytes and ependymal cells produced dense chondroitin sulfate proteoglycans, forming scars to prevent neuron regeneration^{26,27}. In this stage, inflammation is no longer the main confounding factor, and high-frequency of rTSMS does not increase the excitotoxicity of SCI¹¹. At the same time, rTSMS repeatedly stimulates the CPG, increases the expression of GAP-43 in axons in the SCI tissue, produces cumulative biological effects, activates the CPG, thereby inducing the plasticity of the spinal cord and improving the motor function after SCI. Besides, compared with the 1 Hz treatment, the higher-frequencies treatment is more effective, which had stronger excitability to the CPG and had a more obvious improvement in motor function. The part stimulated in this experiment is the L2 spinal cord segment, which is located in the walking CPG of the rat's spinal cord²⁸, which can autonomously generate rhythmic movement²⁹. Studies have found that a variety of methods can induce the plasticity of the CPG, such as MS, walking training, which promote glutamate receptor content, gamma-aminobutyric acid type B receptor, glutamatergic neurons and GAP-43 expression, thereby inducing CPG plasticity and improving motor function after SCI¹². Therefore, this study shows that in the later stage of SCI, MS induces spinal cord plasticity by promoting the expression of GAP-43, thereby improving motor function after SCI.

At the same time, studies have shown that a single MS cannot activate the CPG, which requires repeated stimulation to start and maintain. The length of time required for activation depends on factors such as stimulation method, stimulation frequency, and stimulation intensity. The behavioral results of this

experiment showed that the behavioral scores of experimental rats in each group increased significantly after 6 weeks of SCI. It is speculated that CPG is activated at this stage, which promotes the formation of rhythmic movement, and in turn promotes the recovery of motor function, which is consistent with previous results¹³.

The stimulation frequency selected in this experiment was still relatively small, and the effect of MS in the acute phase on inflammatory factors had not been explored. The follow-up test could choose a higher frequency for further discussion under the premise of ensuring safety. At the same time, we can further explore the mechanism of MS to improve exercise in the acute phase after SCI.

5. Conclusions

This study showed that rTSMS improves spinal cord functions in SCI rats. Furthermore, 1 Hz stimulus is more conducive to the recovery of motor function in the acute and subacute phases after SCI, and the frequency of 15 Hz is more conducive to the recovery of motor function in the intermediate and chronic phases after SCI. Besides, rTSMS increases the expression of GAP-43 in the injured spinal cord.

Declarations

Acknowledgements

The present study was supported by the Program for The General Hospital of Western Theater Command of the Chinese People's Liberation Army (2013YG- B005) and the National Natural Science Foundation of China (81674044).

Author contributions

W.X., H.X., W.C.W., and A.R.Z. conceived and designed research; W.X., J.J.H., and J.Y.W. performed experiments; M.L. analyzed data; R.L.T. and B.J.J. interpreted results of experiments; H.X. prepared figures; W.X. drafted manuscript; W.X., H.X., W.C.W., and A.R.Z. edited and revised manuscript.

Data availability

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

References

1. Brian SA, Elizabeth ACL, Michael HF, Heidi F, Anthony C. Prevalence and Causes of Paralysis-United States, 2013. *Am. J. Public. Health.* **106**, 1855-1857 (2016).

2. Shalini S, et al. The burden of acute traumatic spinal cord injury among adults in the United States: an update. *J. Neurotrauma*. **31**, 228-238 (2014).
3. Leanne MR, Matt SR, Elizabeth JB. Restoring function after spinal cord injury: towards clinical translation of experimental strategies. *Lancet. Neurol.* **13**, 1241-1256 (2014).
4. Beverly CW, et al. Guidelines for the management of acute cervical spine and spinal cord injuries: 2013 update. *Neurosurgery*. **60**, 82-91 (2013).
5. Michael GF, Jefferson RW, Newton C. Methylprednisolone for the treatment of acute spinal cord injury: counterpoint. *Neurosurgery*. **61(suppl1)**, 36-42 (2014).
6. Ihtisham S, et al. The safety and efficacy of steroid treatment for acute spinal cord injury: a systematic review and meta-analysis. *Heliyon*. **6**, e03414 (2020).
7. Wang W, Zuo B, Liu H, Cui L. Intermittent injection of methylprednisolone sodium succinate in the treatment of cervical spinal cord injury complicated with incomplete paraplegia. *Pak. J. Med. Sci.* **35**, 141-145 (2019).
8. Nathan E, et al. Methylprednisolone for the treatment of patients with acute spinal cord injuries: a propensity score-matched cohort study from a Canadian multi-center spinal cord injury registry. *J. Neurotrauma*. **32**, 1674-1683 (2015).
9. Chen L, et al. A prospective randomized double-blind clinical trial using a combination of olfactory ensheathing cells and Schwann cells for the treatment of chronic complete spinal cord injuries. *Cell. Transplant.* **23(suppl 1)**, S35-S44 (2014).
10. Zheng Y, Mao YR, Yuan TF, Xu DS, Cheng LM. Multimodal treatment for spinal cord injury: a sword of neuroregeneration upon neuromodulation. *Neural. Regen. Res.* **15**, 1437-1450 (2020).
11. Quentin D, Chaima C, Nicolas G. Spinal cord injury: can we repair spinal cord non-invasively by using magnetic stimulation?. *Neural. Regen. Res.* **16**, 2429-2430 (2021).
12. Wang Q, Xu T. Effects of transcranial and transspinal low-frequency magnetic stimulation on the expression of growth-associated protein-43 after spinal cord injury. *Chinese Journal of Physical Medicine and Rehabilitation*. **34**, 565-569 (2012).
13. Liu H, et al. Effects of repetitive magnetic stimulation on motor function and GAP43 and 5-HT expression in rats with spinal cord injury. *J Int Med Res.* **48**, 300060520970765 (2020).
14. Zaghloul A, et al. Therapeutic effects of acrobatic exercise and magnetic field exposure on functional recovery after spinal cord injury in mice. *Bioelectromagnetics*. **32**, 49-57 (2011).
15. von Euler M, Seiger A, Sundström E. Clip compression injury in the spinal cord: a correlative study of neurological and morphological alterations. *Exp. Neurol.* **145(2 Pt 1)**, 502-510 (1997).
16. Ramón-Cueto A, Plant GW, Avila J, Bunge MB. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J. Neurosci.* **18**, 3803-3815 (1998).
17. Wang D, Fan YH, Zhang JJ. Transplantation of Nogo-66 receptor gene-silenced cells in a poly(D, L-lactic-co-glycolic acid) scaffold for the treatment of spinal cord injury. *Neural. Regen. Res.* **8**, 677–

685 (2013).

18. Wang D, Zhang JJ. Effects of hypothermia combined with neural stem cell transplantation on recovery of neurological function in rats with spinal cord injury. *Mol. Med. Report.* **11**, 1759-1767 (2015).
19. Isabelle P, Steve L. Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. *J. Comp. Neurol.* **500**, 267-285 (2007).
20. Hausmann ON. Post-traumatic inflammation following spinal cord injury. *Spinal. Cord.* **41**, 369-378 (2003).
21. Wang Y, et al. Necroptosis inhibitor necrostatin-1 promotes cell protection and physiological function in traumatic spinal cord injury. *Neuroscience.* **266**, 91-101 (2014).
22. Pascual-Leone A, Valls-Solé J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain.* **117 (Pt 4)**, 847-858 (1994).
23. Chen R, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology.* **48**, 1398-1403 (1997).
24. Brian KK, Wolfram T, Jonathan NG, John B, Alexander RV. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine.* **J4**, 451-464 (2004).
25. Michael DN, Jon S, Alex M. The pathology of human spinal cord injury: defining the problems. *J. Neurotrauma.* **21**, 429-440 (2004).
26. William BJC, Philip D, Eric H, Stephen MS. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. *J. Neurosci.* **30**, 6825-6837 (2010).
27. Nicole F, Michael GF. Rho-ROCK inhibition in the treatment of spinal cord injury. *World Neurosurg.* **82**, e535-e539 (2014).
28. Ian TG, Patrick JW. Deciphering the organization and modulation of spinal locomotor central pattern generators. *J. Exp. Biol.* **209(Pt 11)**, 2007-2014 (2006).
29. Edgerton VR, et al. Retraining the injured spinal cord. *J. Physio.* **533(Pt 1)**, 15-22 (2001).

Figures

Figure 1

Basso, Beattie, and Bresnahan (BBB) scores of rats in each group at different time points. (a–g). The BBB 22-point open-field locomotor rating scale was used to evaluate hind limb motor function in each group at different time points. ● $P < 0.05$, compared with sham group; ◻ $P < 0.05$, compared with control group; ■ $P < 0.05$, compared with 1 Hz group; ▨ $P < 0.05$, compared with 5 Hz group; □ $P < 0.05$, compared with 10 Hz group.

Figure 2

Inclined plate test of rats in each group at different time points. (a–g). The inclined plate test was used to evaluate the balance ability in each group at different time points. ● $P < 0.05$, compared with sham group; * $P < 0.05$, compared with control group; ■ $P < 0.05$, compared with 1 Hz group; □ $P < 0.05$, compared with 5 Hz group; ◻ $P < 0.05$, compared with 10 Hz group.

Figure 3

rTMS reduced the pathological damage of the spinal cord.

Figure 4

A western blot assay was performed to detect the GAP43 expression level at 8 weeks after SCI. ● $P < 0.05$, compared with sham group; ♂ $P < 0.05$, compared with control group; ■ $P < 0.05$, compared with 1Hz group; ▨ $P < 0.05$, compared with 5 Hz group; □ $P < 0.05$, compared with 10 Hz group.