Neuroendocrinology Letters Volume 46 No. 6 2025
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Repetitive transcranial magnetic stimulation promotes motor function recovery in mice after spinal cord injury through preserving mitochondrial integrity

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Submitted: 2025-11-27 *Accepted: 2025-12-12* *Published online: 2025-12-15*

Key words: **Repetitive transcranial magnetic stimulation; Mitochondrion; Spinal cord injury; TOM20; Motor function**

Neuroendocrinol Lett 2025; **46**(6):335–339 **PMID:** 41420881 46062505 © 2025 Neuroendocrinology Letters • **www.nel.edu**

Abstract

BACKGROUND: Despite the therapeutic potential of repetitive transcranial magnetic stimulation (rTMS) for spinal cord injury (SCI), little is known about its specific effects on mitochondria. This study therefore aimed to elucidate the effects of rTMS on mitochondrial structural integrity following SCI.
METHODS: Female wild-type C57BL/6J mice were randomly divided into four groups: normal group; spinal cord injury group; sham-rTMS group and rTMS group. Hindlimb motor function was evaluated using the Basso Mouse Scale (BMS). The mitochondrial integrity was assessed by transmission electron microscopy, and the expression of the outer membrane protein TOM20 was analyzed by Western blot and immunohistochemistry.
RESULTS: Compared to the SCI group, rTMS group induced a coordinated recovery, characterized by significantly improved BMS scores, enhanced mitochondrial structural integrity, and increased levels of the mitochondrial membrane protein TOM20 ($p < 0.05$).
CONCLUSION: Our findings demonstrate that rTMS promotes motor recovery after SCI via the preservation of mitochondrial structure.

INTRODUCTION

Spinal cord injury (SCI) is a devastating neurological condition with limited treatment options, conferring profound long-term socioeconomic burdens (Fehlings *et al.* 2025). SCI pathogenesis unfolds in two stages: the primary injury from the initial trauma, and a subsequent secondary phase that evolves over an extended period. This latter phase involves a self-perpetuating cascade of pathological mechanisms, notably oxidative stress and neuroinflammation (Gu & Liu 2025). Despite promising evidence that secondary injury might be modulated by stem cell or genetic approaches, major obstacles related to delivery, efficacy, and safety remain, limiting their clinical applicability (Yao *et al.* 2023; Michel-Flutot *et al.* 2024).

ORIGINAL ARTICLE

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique that induces electric currents via time-varying magnetic fields to alter cortical excitability (Dufor *et al.* 2023). Clinically, rTMS has been shown to improve walking speed and alleviate neuropathic pain in SCI patients (Fan *et al.* 2025), (Duan *et al.* 2021). Despite these promising functional benefits, its underlying mechanisms, particularly its impact on mitochondrial function—the central hub of cellular energy homeostasis—remain largely unelucidated.

Maintaining mitochondrial structural integrity is critical for functional recovery after SCI, owing to its essential role in oxidative metabolism and cellular redox homeostasis (Han & Xu 2021). This integrity is profoundly compromised following SCI, as evidenced by pathological manifestations such as mitochondrial swelling and disorganized cristae. Previous studies have shown that mesenchymal stem cells promote motor recovery by preserving mitochondrial integrity (Yao *et al.* 2023). However, the precise mechanism by which rTMS preserves mitochondrial ultrastructure—specifically through outer membrane translocase proteins like TOM20—remains poorly defined. This study was therefore designed to systematically investigate the effects of rTMS on mitochondrial morphology after SCI.

Mitochondrial integrity was evaluated at two complementary levels. At the ultrastructural level, morphological alterations were examined using transmission electron microscopy. At the molecular level, changes in the expression of TOM20—a key receptor protein of the mitochondrial outer membrane—were assessed by Western blot and immunohistochemistry.

MATERIALS AND METHODS

Animal and surgeries

All experiments complied with the animal care guidelines of the Suzhou Institute of Biomedical

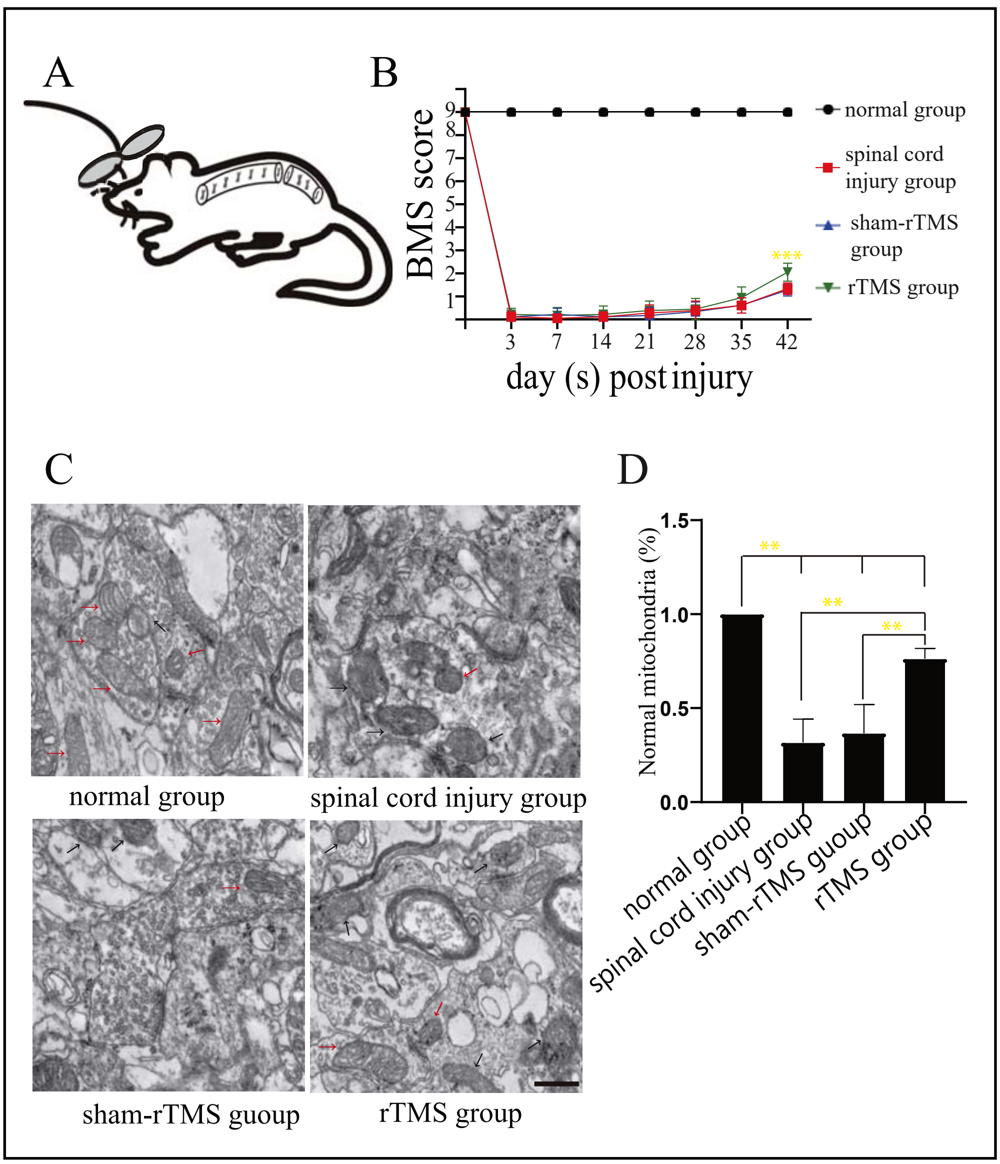

Fig. 1. rTMS promotes the recovery of motor function and protects mitochondria. A, Diagram showing the rTMS treatment mice after SCI. B, Time course of the change in BMS scores measured with the BMS rating scale after spinal cord complete transection. ***$p < 0.001$; two-way ANOVA followed with Dunnett's test. C, Representative TEM images of the morphology of mitochondria in four group, red and black arrows indicate normal and damaged mitochondria respectively. Scale bar, 500 nm. D, Quantification of normal mitochondria. **$p < 0.01$; one-way ANOVA followed with Dunnett's test. N = 9 mice per group.

Engineering and Technology. Female C57BL/6J mice were maintained in a specific pathogen–free facility with access to food and water. Mice were randomly assigned to four groups (n = 9/group): Normal, SCI, Sham-rTMS, and rTMS. At 8-10 weeks of age, mice were anesthetized with pentobarbital (40 mg/kg, i.p.) and subjected to a complete T10 transection. Post-operative care included manual bladder expression twice daily until the mice were sacrificed. The operator was blinded to the group allocation.

Repetitive Transcranial Magnetic Stimulation

Mice were treated using a China M-100 rTMS equipment with a 100-mm figure-of-eight coil. Awake mice were gently restrained in plastic containers post-surgery, and their heads were positioned at the coil center. Stimulation parameters were: 5 Hz, 0.5 Tesla, 10 pulses per train, 3 s inter-train interval, and 20 trains per session. Sessions were conducted once daily, five days per week, for six weeks. For sham stimulation, the coil was oriented perpendicular to the head. The mode diagram is presented as Fig 1 A.

Transmission electron microscope

The spinal lesion was fixed with electron microscope fixative solution. And Tissues dehydration in different concentrations of alcohol. After that, embedding, slice and dye are finished successively. Ultrastructure was examined using a Hitachi HT7800 transmission electron microscope, and images were captured and analyzed using ImageJ software.

Immunohistochemistry

Following perfusion with 4% PFA at the sixth-week endpoint, spinal cord segments encompassing the lesion were harvested. Tissues were post-fixed in the same fixative overnight at 4°C, then tissues was transferred to a graded sucrose series (20% for 8 h, followed by 30% for 18 h). Prior to sectioning, samples were embedded in OCT compound and serially section on a cryostat. For immunolabeling, sections were incubated overnight at 4°C with primary antibodies (TOM20; merck, mabt166; NeuN, abcam, ab104225; Dapi, Sigma, D9542) and then with Alexa 488– or 555–conjugated secondary antibodies (abcam, ab150081; ab150110) overnight at 4°C.

Western blotting

Protein samples were extracted from a 1-mm-long segment of the spinal cord centered on the lesion scar. The lysates were then subjected to SDS-PAGE and electrophoretically transferred to PVDF membranes. Blots were incubated with primary antibodies and secondary antibodies overnight at 4°C. Finally, blots were visualized with the ImageLabTM software system.

Behavioral analysis

The Basso mouse scale (BMS) open field locomotion rating scale was assessed from pre-operation to 6 weeks post-injury. This process is evaluated by two researchers who were blinded to the group allocation. To ensure objectivity, all BMS scoring was conducted by two researchers who were blinded to the experimental groups.

Statistical analysis

All data are expressed as the mean ± SEM. Statistical analyses were performed with GraphPad Prism 8.01. BMS scores were evaluated by two-way ANOVA with Dunnett's test, while other data were assessed by one-way ANOVA with Dunnett's test. A $p < 0.05$ was considered statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

RESULTS

rTMS significantly improves locomotor recovery after SCI

Hindlimb motor function was evaluated weekly using the BMS. Motor function in all injured mice was severely impaired during the first week post-injury and showed only slight gradual improvement thereafter. At day 42, rTMS achieved BMS 2.056 ± 0.391 vs. sham-rTMS 1.278 ± 0.264 ($p < 0.001$; Tab 1; Fig 1 B) — 8.6-percentage-point functional advantage (22.840 % vs. 14.196 % of normal recovery). This advancement, from complete paralysis to the ability to perform knee joint flexion and extension movements, demonstrates clinically meaningful but incomplete motor recovery, as normal locomotion (BMS 9) was not restored.

Tab. 1. Quantitative motor recovery

Group	Day 1 BMS	Day 42 BMS	Recovery (%)	*p*-value vs. Sham
Normal group	9	9	100%	<0.001
Spinal cord injury group	0	1.333 ± 0.25	14.815%	>0.999
Sham-rTMS group	0	1.278 ± 0.264	14.196%	—
rTMS group	0	2.056 ± 0.391	22.840%	<0.001

rTMS achieves 22.840 % functional recovery vs. 14.196 % sham (8.6-percentage-point improvement approximately). Recovery represents advancement to knee joint flexion and extension movements, but complete locomotion not restored. Two-way ANOVA followed with Dunnett's test. N = 9 mice per group.

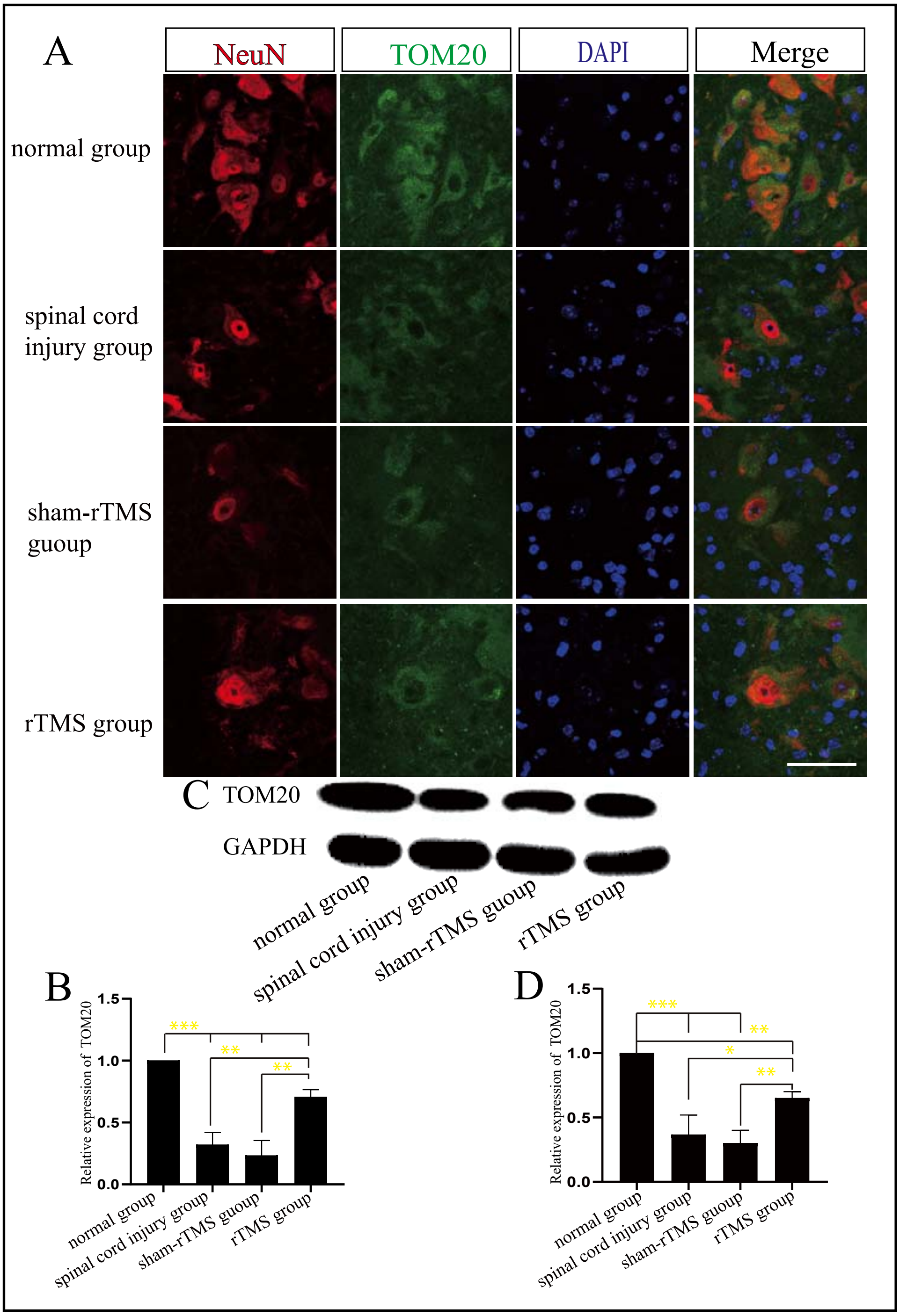

Fig. 2. rTMS upregulates the level of TOM20 after SCI. .A, Representative images of coronal sections showing NeuN (red), TOM20 (green) and DAPI (blue), respectively. Scale bars, 50 µm. B, Quantification of fluorescence intensity of TOM20. $^{**}p < 0.01$ and $^{***}p < 0.001$; one-way ANOVA followed with Dunnett's test. The intensities of more than 60 neurons from three mice in each group were quantified. C-D, Western blot analysis and quantification the level of TOM20 in the lesion area at the 42 days post-injury. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; one-way ANOVA followed with Dunnett's test. N = 9 mice per group.

rTMS preserves mitochondrial structural integrity after SCI

Transmission electron microscopy revealed that SCI induced severe mitochondrial damage, including membrane disruption and cristae disorganization. In contrast, rTMS treatment significantly preserved mitochondrial ultrastructure, maintaining cristae organization and membrane continuity ($p < 0.01$; fig 1 C-D). Based on the observed preservation of mitochondrial ultrastructure, we hypothesized that rTMS confers protection by stabilizing critical membrane protein complexes. We focused on TOM20, a key receptor essential for mitochondrial protein import and outer membrane integrity. Supporting our hypothesis, rTMS treatment effectively counteracted the downregulation of TOM20 following SCI, as demonstrated by complementary immunohistochemical and Western blot analyses. This finding suggests a molecular mechanism through which rTMS stabilizes mitochondrial structure and function ($p < 0.05$, Fig. 2).

DISCUSSION

Our study establishes that rTMS significantly promotes motor recovery after SCI. This functional improvement appears to be mediated by the critical role of rTMS in preserving mitochondrial structural integrity.

Mitochondrial dysfunction serves as a pivotal mechanism that integrates multiple pathways of secondary neural injury after SCI. It exacerbates local inflammation, impairs axonal regeneration (Wang *et al.* 2021). And mediates Ca^{2+}-induced mPTP opening, thereby triggering cytochrome C release and apoptotic cascades (Zhu *et al.* 2022). These damaging processes are further amplified by the concurrent accumulation of excitotoxins and reactive oxygen species, creating a vicious self-reinforcing cycle that ultimately culminates in neuronal death. Mitochondria-targeting strategies represent a promising therapeutic direction SCI. Previous studies have demonstrated that interventions such as mesenchymal stem cell transplantation or modulation of the JAK2/STAT3 signaling pathway with compounds like alpinetin can alleviate SCI by preserving mitochondrial integrity (Xiao *et al.* 2023; Yao *et al.* 2023). Furthermore, direct transplantation of functional mitochondria has been shown to promote functional recovery after SCI (Wang *et al.* 2024). Despite these advances, the precise molecular mechanism by which repetitive rTMS improves motor function—particularly through mitochondrial pathways—remains to be elucidated.

TOM20 serves as a core receptor subunit of the mitochondrial translocase of the outer membrane complex(Araiso & Endo 2022). It is responsible for recognizing and binding the signal sequences of precursor proteins to initiate mitochondrial import. In cooperation with subunits such as TOM40, it facilitates the assembly of a protein-conducting channel for precursor translocation (Busch *et al.* 2023). Beyond its canonical role in import, TOM20 is essential for maintaining broader mitochondrial integrity, supporting processes including oxidative phosphorylation and organelle biogenesis (Pitt & Buchanan 2021). Given TOM20's essential role in protein import and membrane stability, its upregulation by rTMS suggests a potential upstream mechanism for the observed preservation of mitochondrial structure.

We conclude that rTMS upregulates key mitochondrial membrane proteins to maintain organellar integrity, thus establishing a mechanistic basis for its efficacy in promoting functional recovery after SCI.

CONFLICT OF INTEREST

The authors declared that there was no conflict of interest associated with the manuscript.

ACKNOWLEDGEMENTS AND FUNDING

We would like to thank the associate editor and the anonymous reviewers for their helpful remarks. And this study was funded by the Jiangsu Geriatrics Society (grant number JGS2025MS0014), Suzhou Applied Basic Research (Medical and Health) Technology Innovation Project (grant number SYW2024115) and Nanjing Medical University Science and Technology Development Fund (grant number NMUB20240228).

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