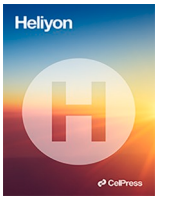
Heliyon 10 (2024) e37388

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Neuron-derived Netrin-1 deficiency aggravates spinal cord injury through activating the NF-κB signaling pathway

Xiaojian Qin [a,1], Xiaolan Zhang [b,1], Xiaodong He [b,1], Hui Xu [b], Qiannan Yao [b], Zifeng Li [b], Yayun Feng [b], Yichen Zhong [b], Ziyang Li [a], Gang Lv [a,**], Yanfeng Wang [a,*]

[a] Department of Orthopaedics. The First Affiliated Hospital, China Medical University, Shenyang, 110001, Liaoning Province, China
[b] Department of Pharmacology, Life Science and Biopharmaceutical Institution, Shenyang Pharmaceutical University, Shenyang, 110016, Liaoning Province, China

ARTICLE INFO

Keywords:
Netrin-1
NF-κB
Spinal cord injury
Behavior

ABSTRACT

Netrin-1 (NTN1) is involved in psychological alterations caused by central nerve system diseases. The primary objective of this research was to investigate whether a deficiency of neuron-derived NTN1 in the remote brain regions affects SCI outcomes. To examine the roles and mechanisms of neuron-derived NTN1 during SCI, Western blots, Nissl staining, immunochemical technique, RNA-sequence, and related behavioral tests were conducted in the study. Our study revealed that mice lacking NTN1 exhibited normal morphological structure of the spinal cords, hippocampus, and neurological function. While neuron-derived NTN1deletion mechanistically disrupted neuronal regeneration and aggregates neuronal apoptosis and ferroptosis in the intermediate phase following SCI. Additionally, neuroinflammation was significantly enhanced in the early phase, which could be related to activation of the NF-κB signaling pathway. Overall, our findings indicate that the deletion of neuron-derived NTN1 leads to the activation of the NF-κB pathway, contributing to the promotion of neuronal apoptosis and ferroptosis, and the pathological progression of SCI.

1. Introduction

Spinal cord injury (SCI), a kind of severe central nervous system damage with high incidence, may lead to a permanent paralysis due to a motor and sensory dysfunction [1]. The occurrence of SCI can trigger synaptic reorganization and remodeling in the brain [2], subsequently impacting the function of both the spinal cord and brain regions associated with memory, learning, and emotion regulation [3,4]. Additionally, SCI impairs hinders the process of neurogenesis and results in reactive gliosis within the hippocampus [5,6]. However, there is insufficient research exploring the impact of changes in remote brain structures on the functional recovery in mice with SCI.

* Corresponding author. The First Affiliated Hospital, China Medical University Department: Orthopaedics, BeiEr Road No. 92, Shenyang, Liaoning Province, 110001, China.
** Corresponding author. The First Affiliated Hospital, China Medical University Department: Orthopaedics, BeiEr Road No. 92, Shenyang, Liaoning Province, 110001, China.
E-mail addresses: 20171256@cmu.edu.cn (G. Lv), wyf_doctor2023@163.com, 20041096@cmu.edu.cn (Y. Wang).
[1] The authors contributed equally.

https://doi.org/10.1016/j.heliyon.2024.e37388
Received 16 November 2023; Received in revised form 24 August 2024; Accepted 2 September 2024
Available online 3 September 2024

Research has indicated that SCI results in permanent damage to the cortical circuits of the central nervous system, leading to sleep disturbances, anxiety, depression, and cognitive impairment [7]. The hippocampus, responsible for encoding spatial and environmental information relevant to learning and memory, plays a crucial role. The presence of newly generated neurons within the hippocampus enhances learning abilities that rely on this region by facilitating neuroplasticity [8]. At the early stage of SCI, astrocytes in the hippocampus were active, and the number of microglia increased [5]. Based on these findings, it is possible that a close functional connection exists between the spinal cord and the hippocampus.

Netrin-1(NTN1), a kind of secretory protein related to laminin, guides the direction of nerve growth by binding with its receptor, which can promote early synapse formation in addition to axon guidance function. NTN1 and its receptors (Unc5A-D) are well known to be involved in inflammation, tumor growth and axon regeneration [9,10]. Additionally, NTN1 expression has been observed in diverse cell types within the central nervous system, indicating its contribution to both acute and chronic inflammation [11,12] and have confirmed that the decrease of NTN1 level is related to cognitive impairment after SCI [13], and it was found that exogenous administration of NTN1 could enhance the functional restoration and recovery of motor function following spinal cord injury [14–17]. In addition, our studies found a significant increase in the expression levels of NTN1 protein in hippocampus and cortex following SCI. However, the impact of neuron-derived NTN1 on SCI recovery remain uncertain.

In the present study, we investigated the effects of NTN1 deletion in neurons on the outcomes of SCI. Interestingly, our findings demonstrate that NTN1 deletion in neurons triggers more aggressive neuroinflammation during the early stage of SCI through activation of the NF-κB signaling pathway. Additionally, it promotes the accumulation of neuronal apoptosis and ferroptosis in the intermediate stage of SCI.

2. Materials and methods

2.1. Animals

$Ntn1^{fl/fl}$ mice on C57Bl/6J background were generated by Cyagen Company (Suzhou, China). $Ntn1^{fl/fl}$ mice were mated with Emx1-Cre transgenic mice (JAX, Strain# 005628) to obtain $Ntn1^{fl/fl}$; Emx1-Cre mice (NTN1 ^{Emx1}-CKO). Ntn1fl/fl (NTN1$^{f/f}$) mice were used as control. Mice (6–8 weeks, 20–23 g) with a genetic deletion for NTN1 were used in the study. All animal experiments in this study strictly followed the guidelines established by the National Institutes of Health. Furthermore, the ethical treatment of animals was authorized by the Animal Care and Use Committee at Shenyang Pharmaceutical University (No.SYPU-1ACUC-GZR2020R-04.16-201).

2.2. Establishment of spinal cord injury model in mice

The NTN1 ^{Emx1}-CKO and NTN1$^{f/f}$ male mice (8–10 weeks old, 20–25 g) were initially anesthetized using 5 % isoflurane. Subsequently, the animals were placed under anesthesia using a combination of 1.5–2% isoflurane and air to remain unconscious and pain-free throughout the procedure. The spinal cord was carefully exposed at the T10 level. To produce a consistent and controlled impact, the clamp spacing was maintained for a duration of 5 s.

2.3. Behavioral analysis

2.3.1. Sucrose preference test

Each mouse was provided with two bottles of water on the initial day. After 24 h, the two bottles of water were replaced with two bottles sugar water (2 % glucose solution). After another 24 h, each mouse was given a bottle of water and a bottle of sugar water. Before the implantation, the volume and weight of both water and sugar water were measured. The intake of water and sugar water over a 24-h period was determined by weighing and measuring the subjects twice.

2.3.2. Forced swimming test

Each mouse was placed individually in a water-filled cylinder measuring 10 cm in diameter and 25 cm in height. The water depth was adjusted to prevent the mice from touching the bottom with their tails or hindlimbs. The swimming activity of each mouse was recorded for 5 min, along with the duration of swimming, climbing, and immobility behaviors. Immobility time referred to the period during which mice remained motionless or floated to keep their heads above water, representing a state of minimal activity or idleness excluding essential survival movements.

2.3.3. Y-maze spontaneous alternation

The Y-maze spontaneous alternation behavior test was performed after 28 days of SCI in accordance with the protocol described by Gu et al. (2022) [18]. This test is commonly used to evaluate the working memory function. For the purpose of the experiment, a group of mice were carefully positioned at the central location of the Y-maze. These mice freely explore the maze for a total of 10 min. Video recording equipment was used to record the behavior of mice throughout the experiment.

2.3.4. Light-dark box test

The serene chamber served as the placement for the light-dark box apparatus. The duration of the mice's stay in both the light and dark compartments was carefully observed, and the number of transitions they made between these contrasting zones was

meticulously recorded. All of this data was diligently documented within a 5-min time frame.

2.3.5. Open field test

The open field test was conducted 28 days after spinal cord injury to evaluate the behavior of mice, following the methodology outlined by Tucker et al. [19]. During this experiment, the mice were placed in one corner of the open field chamber, which measured 40 × 40 cm. They were positioned facing the wall and given 10 min to freely explore the chamber. The software enabled the quantification of the number of entries into the central zone and the distance covered in that area.

2.3.5.1. Basso Mouse Scale (BMS) scoring analysis. The Basso Mouse Scale (BMS) has been extensively used to assess the recovery of hind limb function after SCI, as described by Basso et al. [20]. The scale assesses multiple criteria including ankle range of motion, foot placement and instep landing, locomotor gait, trunk stability, and tail posture. Scoring is conducted on a scale from 0 to 9, encompassing both primary and ancillary grading systems. The primary scoring system of the BMS emphasizes hind limb ankle movement, sole-to-instep contact, trunk stability, and tail posture. To better capture hind limb function recovery, we have introduced a subscore scoring system. This system complements the primary scoring by evaluating hind limb landing coordination, paw touching, and trunk stability.

2.4. Western blot

BCA procedures were employed to quantify the supernatant solution. The protein samples were then deposited onto PVDF membranes after being separated using SDS-PAGE. The membranes were subsequently obstructed for a duration of 2 h at room temperature by using 5 % skimmed milk. Following this, the membranes underwent three consecutive TBST washes before being subjected to primary antibodies incubation at 4 °C. The primary antibody included: NTN1 (Protintech,1:1000), BAX (Protintech,1:5000), Bcl-2 (Protintech,1:6000), p65 (CST,1:1000), p-p65 (CST,1:1000) and β-actin (Protintech,1:10000). Finally, the protein bands were detected and analyzed by Chemiluminescence Imager.

2.5. Nissl staining

Three days post spinal cord injury, mice were anesthetized and underwent cardiac perfusion. Tissues from 1 cm above and below the injury site were then collected. These tissues were then fixed in a solution containing 4 % paraformaldehyde for a duration of more than 24 h. Subsequently, a series of sequential steps including gradient dehydration, tissue embedding, and sectioning were carried out. Finally, the sections were subjected to baking and gradient rehydration. The tissue sections were stained with reagent A from the Nissl staining kit, placed in a moist chamber, and incubated at 57 °C for 1 h. Subsequently, the sections were rinsed with distilled water until colorless, followed by applying reagent B to distinguish the tissue sections until the background turned colorless or light blue. After dehydration, the sections were sealed with neutral resin for stability. The acquired data was then statistically analyzed using Image J software.

2.6. Immunohistochemistry (IHC)

The paraffin sections were first placed in the oven for waxing. Subsequently, different purity levels of xylene and alcohol were used to rehydrate the mixture. After the samples were cooled to room temperature, they were incubated overnight at 4 °C with the primary antibody. Additionally, endogenous peroxidase blocking agents and non-specific stain blocking agents were also included in the incubation process. The primary antibodies used were NTN1 (Protintech, 1:500), Iba1 (ABclonal, 1:500), GFAP (Thermo Fisher, 1:500), and NeuN (CST, 1:500). Following three rounds of PBS washing, IgG polymer and peroxidase were added. Afterward, PBS was employed to thoroughly cleanse the cells before they were subjected to a subsequent step of staining with hematoxylin. Finally, dehydration, transparency, and sealing procedures were carried out.

2.7. Immunofluorescence

The tissue samples were subjected to immunofluorescence staining. They were first exposed to PBST at room temperature for 8–10 min, followed by 0.3 % Triton in 1xPBS for 15 min. Next, the samples were treated with a 5 % blocking serum for 30 min at 37 °C. Subsequently, the samples were incubated with the primary antibody (anti-NeuN antibody, 1:500). On the following day, the fluorescent secondary antibody (Alexan FluorTM 594 goat anti-rabbit lgG (H + L) 1:1000) was applied at a dilution ratio of 1:1000. Before incubation, the secondary antibody was appropriately diluted with antibody diluent. The incubation lasted for 1 h at room temperature, with the samples shielded from light.

2.8. RNA sequencing and functional enrichment

The spinal cord tissues were promptly collected and placed on ice for preservation. Three samples were obtained from each experimental group. The Trizol technique, known for its effectiveness in isolating RNA, was used to extract the RNA from the samples. All samples underwent mRNA-seq analysis. Statistical techniques were used to identify differentially expressed genes and assess their

biological significance between the two groups. The mRNA-seq analysis and quality control were carried out by Shanghai Novelbio Corporation (Shanghai, China).

2.9. Statistical analysis

All the data presented in this study are expressed as the mean ± SD. Statistical analysis was performed using either Student's t-test between two groups or ANOVA with Bonferroni post-tests among groups. A significance level of $p < 0.05$ was utilized to determine the statistical significance of the findings.

3. Results

3.1. Normal development of brain and long-term neurological function in NTN1^{Emx1}-CKO mice

As demonstrated in Fig. 1, mouse genotypes were identified through agarose gel electrophoresis. And the expression of NTN1 in the cortex and hippocampus of NTN1^{Emx1}-CKO mice was significantly reduced compared to control mice. In order to investigate the impact of neurons with conditional deletion of NTN1 on hippocampal structure and long-term brain function, we carried out further analysis. Fig. 2 shows that there was no notable difference in terms of neuronal count, distribution, and neuroinflammation level in the hippocampus between NTN1$^{f/f}$ and NTN1^{Emx1}-CKO mice. Additionally, our findings from behavior tests, including assessments of cognitive function, anxiety-related behavior and depression-like behavior in Fig. 3, indicate that the NTN1 deletion in neurons did not have an impact on the long-term brain function of mice. These collective results strongly suggest that the NTN1 deletion in neurons does not influence normal brain development or long-term brain function.

3.2. The effect NTN1^{Emx1}-CKO on normal development of spinal cords and motor function in mice

The development of the spinal cord and motor function is a complex and crucial process in mammals. In this study, we investigate

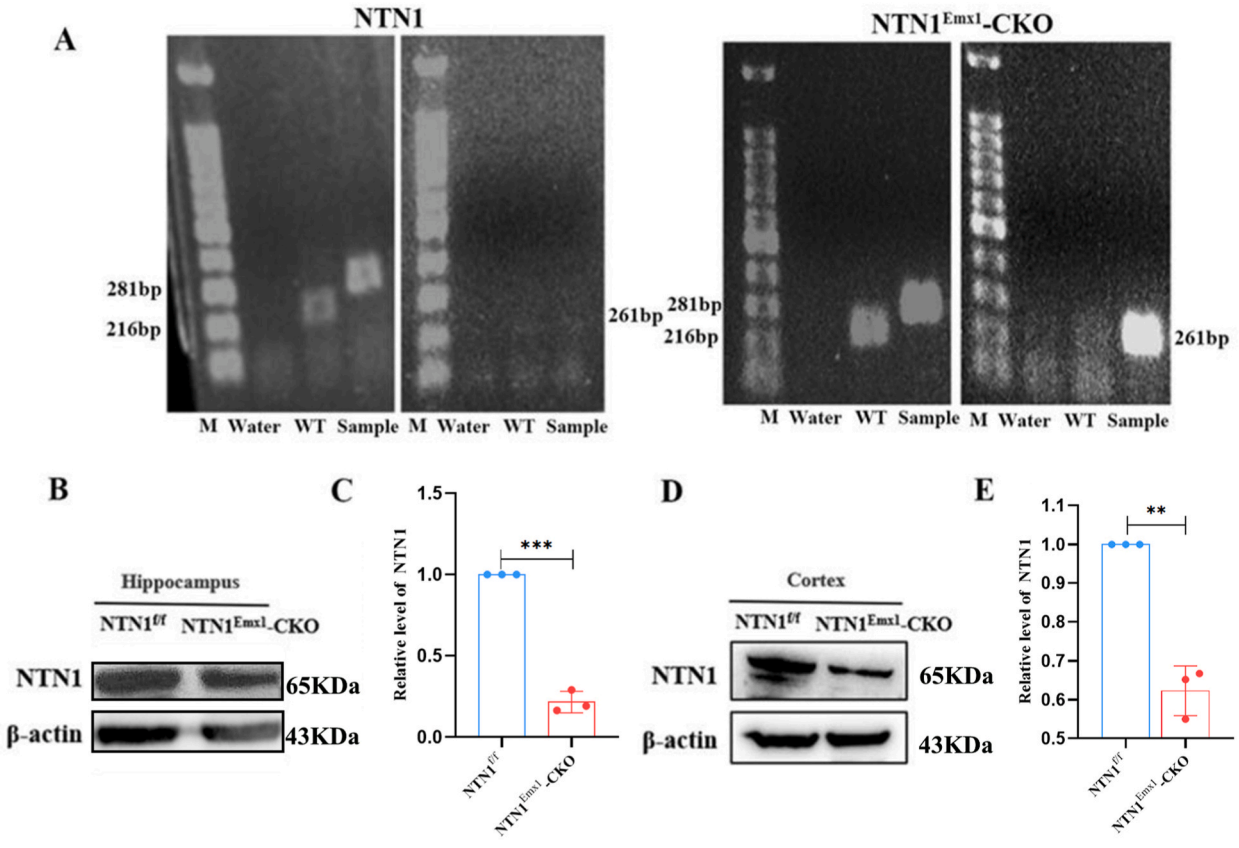


Fig. 1. Genotyping of NTN1 mice. (A) Mouse genotypes were determined through agarose gel electrophoresis. (B) The knockdown efficiency of NTN1 in the hippocampus was assessed using Western blot analysis. (C) The integrity density values (IDVs) of NTN1 normalized to β-actin. (D) The knockdown efficiency of NTN1 in the cortex was determined using Western blot analysis. (E) The IDVs of NTN1 normalized to β-actin. Data were presented as mean ± S.D. ($n = 3$), $^{**}p < 0.01$, $^{***}p < 0.001$.

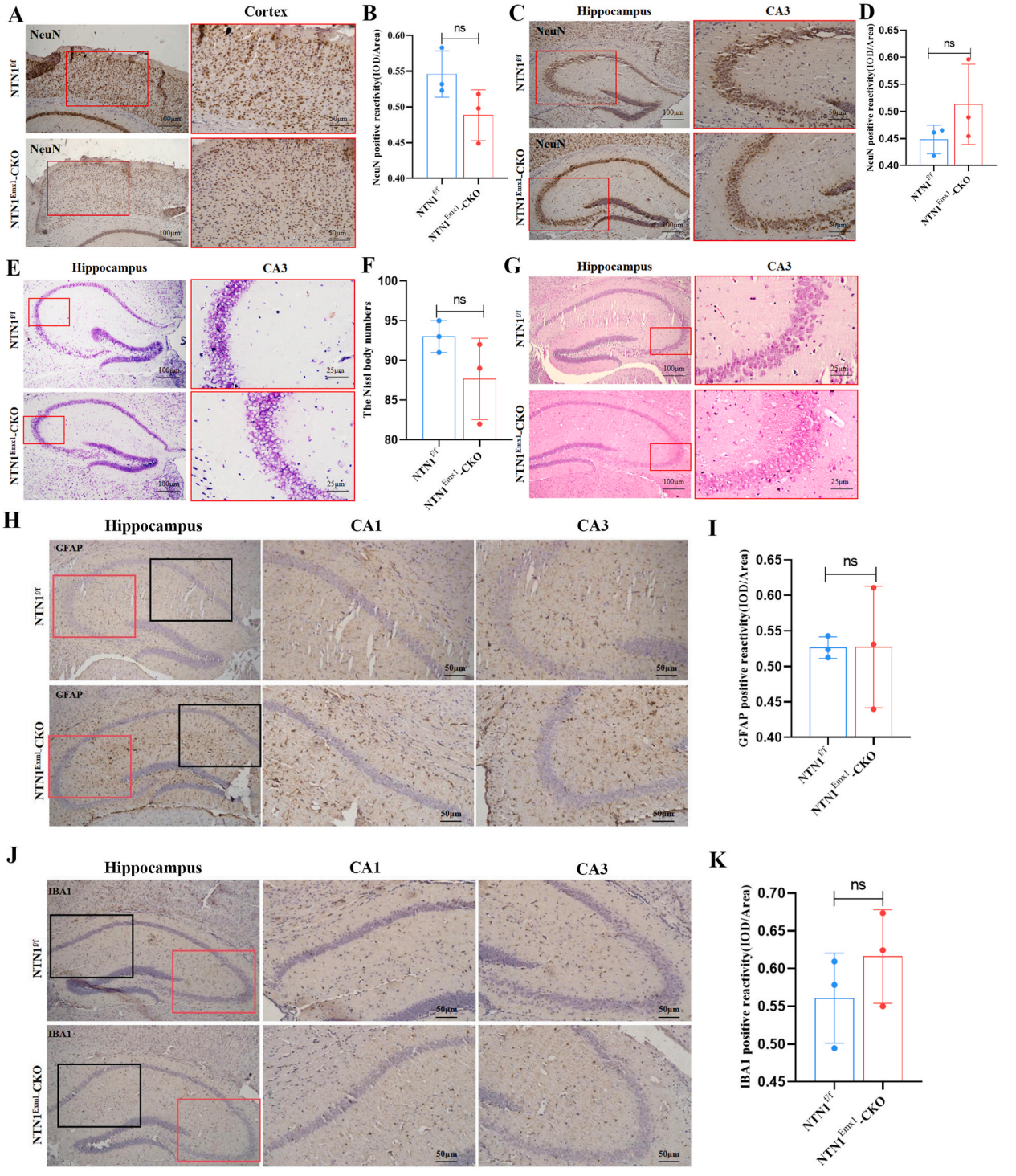


Fig. 2. Effect of NTN1 Knockout on Cortex and Hippocampal Neurons, and Neuroinflammation. (A) Representative images of immunohistochemical (IHC) staining showing NeuN + neurons in the cortex, and (C) hippocampus. (B, D) Quantification of NeuN + neurons. (E) Nissl staining in the hippocampus. (F) Quantification of Nissl bodies in the hippocampus. (G) Hematoxylin and eosin (HE) staining of the hippocampus. (H) Representative images of IHC staining showing GFAP-labeled astrocytes in the hippocampus. (I) Quantification of GFAP + astrocytes. (J) Representative images of IHC staining showing Iba1^{+} labeled microglia in the hippocampus. (K) Statistical analysis of Iba1^{+} cells. Data were presented as mean ± S. D. ($n = 3$), No significant differences (ns) were observed ($p > 0.05$).

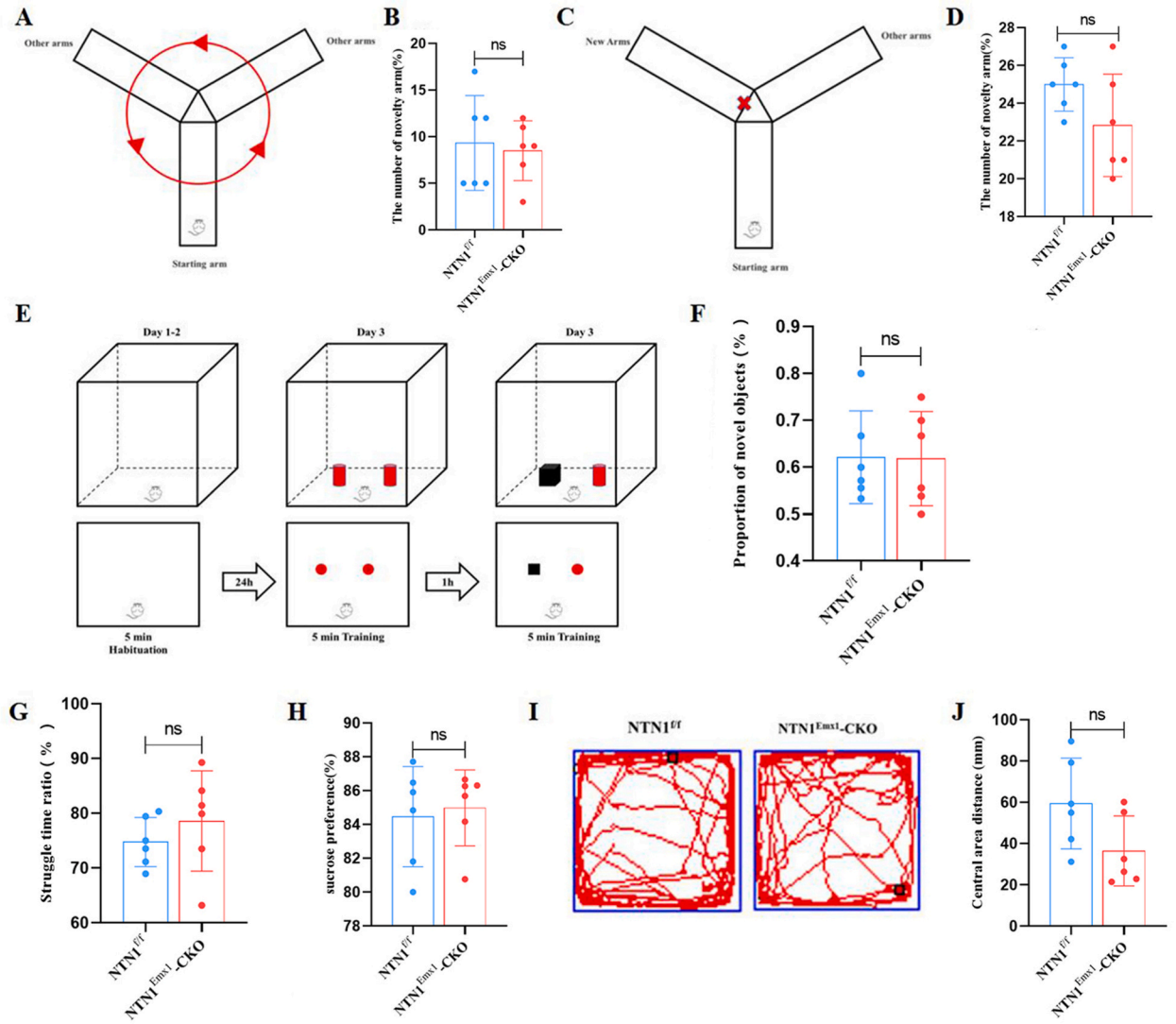


Fig. 3. Effect of NTN1 knockout on long-term behavior in mice. (A) The Y-maze without partitions. (B) Statistical representation of the Y-maze without partitions. (C) The Y-maze configuration with partitions. (D) Statistical diagram of C. (E) The diagram of new object recognition experiment. (F) New Object Recognition Experiment Statistical Chart. (G) Statistical chart of hanging tail experiment. (H) Statistical chart of sugar water experiment. (I) Diagram of the trajectory of the open field experiment. (J) Central distance statistical chart of open field experiments. Data were presented as mean ± S.D. ($n = 6$), ns $p > 0.05$.

the normal development of spinal cords and motor function in NTN1^{Emx1}-CKO mice, where the Netrin-1 gene was specifically deleted in the developing forebrain cortex and hippocampus. Our aim was to determine whether the NTN1 deletion in neurons had any impact on the development of spinal cords and motor functions.

We initially conducted a comparison of the weight, neuronal number, and neuronal distribution between the NTN1f/f control mice and the NTN1Emx1-CKO mice. Surprisingly, our analysis showed no significant differences in these parameters (Fig. 4A–D). This indicates that the conditional deletion of NTN1 in neurons does not visibly affect the overall development of the spinal cords in these mice. Furthermore, we assessed the motor function of these mice by conducting various motor tests. And we found that the conditional deletion did not impair the motor function of the NTN1^{Emx1}-CKO mice (Fig. 4E–I). These results suggest that the loss of NTN1 specifically in neurons does not lead to any noticeable deficits in motor function. In summary, our findings demonstrate that the NTN1 deletion in neurons does not significantly affect the normal development of spinal cords and motor functions in mice. Further studies are warranted to fully elucidate the precise functions of NTN1 in the context of SCI and its potential therapeutic implications.

3.3. NTN1^{Emx1}-CKO destroyed the functional recovery of motor functions after SCI

Our results demonstrated that the levels of NTN1 were found to be significantly increased in the hippocampus and cortex following

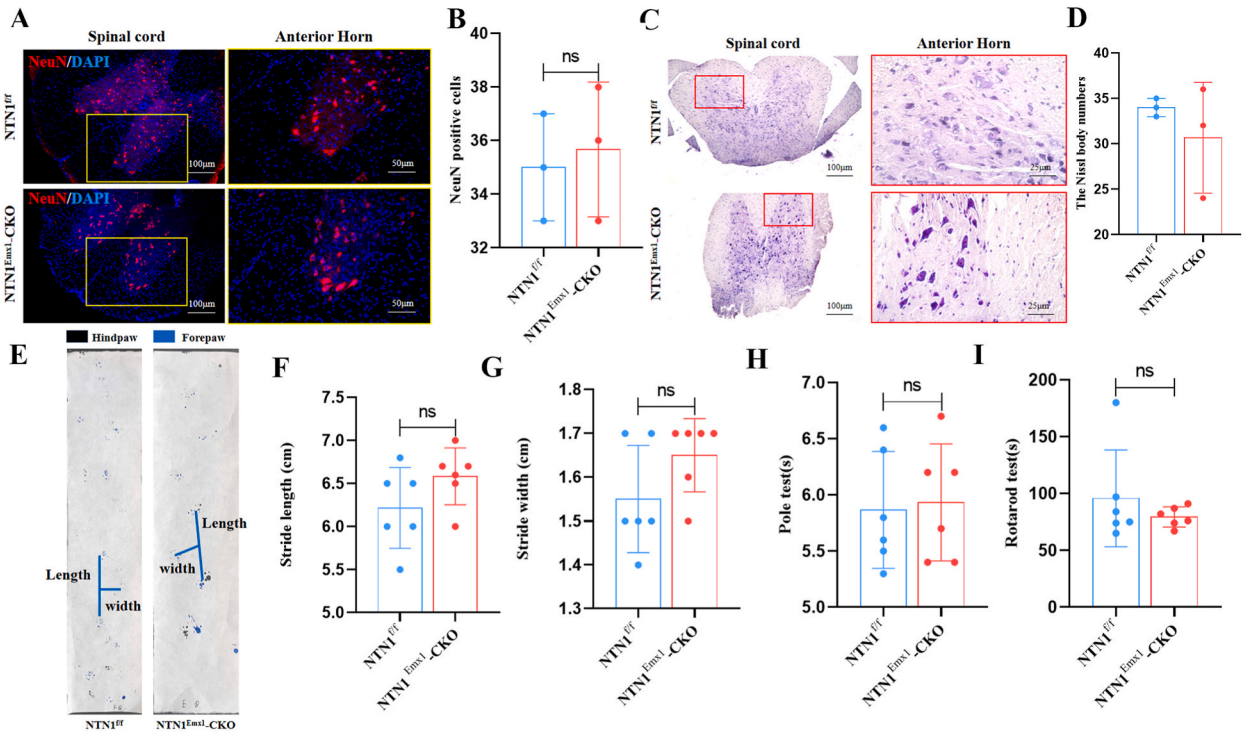


Fig. 4. Effect of NTN1 knockout on the neurons in spinal anterior horn and motor function of mice. (A) Immunofluorescent labeling of NeuN^{+} cells in the anterior horn of the spinal cord. (B) Quantification of NeuN^{+} neurons in spinal anterior horn. (C) Nissl staining in the anterior horn of the spinal cord. (D) Quantification of nissl bodies in spinal anterior horn. Data were presented as mean ± S.D. ($n = 3$), ns $p > 0.05$. (E) Gait analysis trajectory diagram. (F) Step length bar and (G) Step width chart. (H) Quantitative analysis of the time before landing on four limbs in the pole test. (I) Quantitative analysis of the time before falling in rotarod performance test. Data were presented as mean ± S.D. (n = 6), ns $p > 0.05$.

SCI (Fig. 5A–B). To examine the effect of NTN1 deletion on motor function following SCI, we conducted a study focusing on neurons in the hippocampus and cortex. Through the implementation of the Basso Mouse Scale (BMS) scoring analysis, we discovered a significant deterioration in gross voluntary movement among NTN1^{Emx1}-CKO mice compared to NTN1 f/f mice on days 14, 21, and 28 post SCI (Fig. 5C–E). Additionally, the footprint behavioral assays (Fig. 5F–H) displayed poorer performance in stride length and stride width by NTN1^{Emx1}-CKO mice at day 28 after SCI. These findings collectively indicate that the NTN1 deletion in neurons disrupts the recovery of motor functions following SCI.

3.4. *NTN1^{Emx1}-CKO increased the neuronal degeneration at intermediate stage after SCI*

To examine the impact of NTN1 deletion on the recovery of motor functions following SCI, we conducted a series of experiments. Our research findings, as illustrated in Fig. 6A–B, indicate that there was a substantial increase in both the area of injury site and the loss of neurons in NTN1^{Emx1}-CKO mice compared to NTN1$^{f/f}$ mice following SCI. These results suggest that the absence of NTN1 expression specifically in Emx1-CKO mice exacerbates the severity of damage and neuronal loss induced by SCI. To further investigate the effects of NTN1 deletion, we evaluated the level of MAP2, a marker for axonal regeneration after SCI. The results clearly show that NTN1Emx1-CKO mice exhibited significantly lower levels of MAP2 compared to NTN1f/f mice (Fig. 6C–D). This suggests that the lack of NTN1 in neurons impedes axonal regeneration after SCI.

Additionally, we performed immunostaining for MBP, a protein related to myelin, to evaluate neural regeneration at 28 days after SCI. Our analysis revealed a significant reduction in MBP intensity in NTN1^{Emx1}-CKO mice, as shown in Fig. 6E–F. Furthermore, an increase in the presence of glial scar were observed positive astrocytes, in NTN1^{Emx1}-CKO mice (Fig. 6G–H). During the intermediate phase following SCI, the absence of NTN1 in neurons appears to worsen the progression of neuronal loss and axonal degeneration. In summary, our results demonstrate that the NTN1 deletion in neurons impairs axonal regeneration following SCI. These findings shed light on the importance of NTN1 in promoting neural repair and recovery of motor functions after SCI.

3.5. *NTN1^{Emx1}-CKO induced neuroinflammation at early phase following SCI*

According to a study conducted by Boneschansker [21], it has been established that NTN1 plays an important role in modulating the intrinsic immune response of neurons to axonal injury. Building upon this knowledge, our research aimed to investigate whether NTN1^{Emx1}-CKO promotes neuroinflammation following SCI. We collected spinal cords from NTN1$^{f/f}$ and NTN1^{Emx1}-CKO mice on day 3

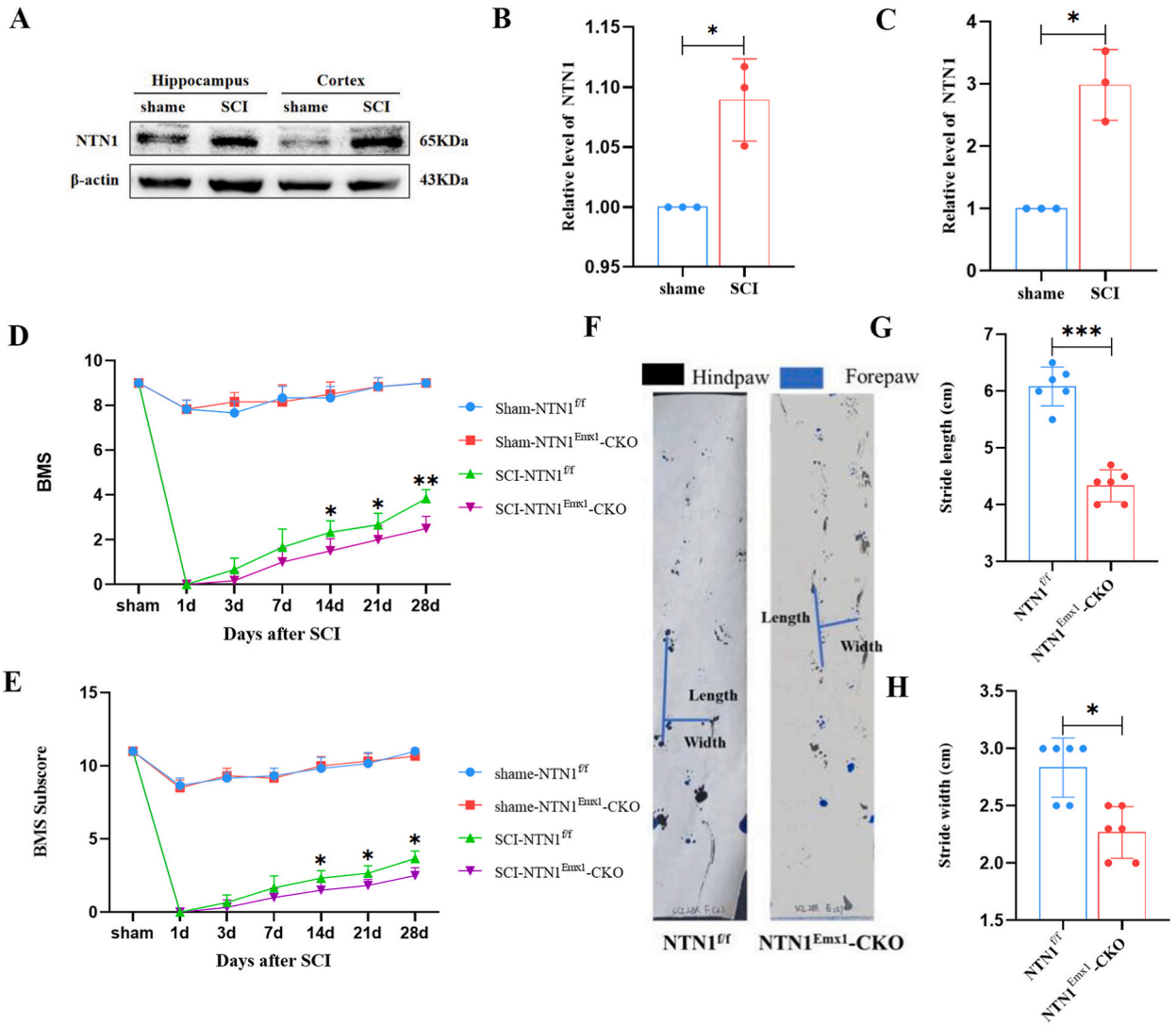


Fig. 5. Effect of NTN1 knockout on motor function and gait analysis in mice after spinal cord injury. (A) The expression of NTN1 in the hippocampus and cortex at day 3 post-SCI was assessed using Western blot analysis. (B,C) The IDVs of NTN1 normalized to β-actin. Data were presented as mean ± S. D (n = 3). *$p < 0.05$. (D) BMS primary score. (E) BMS subscore score. (F) Gait analysis trajectory diagram at day 28 after SCI. (G) Statistical diagram of step length after 28 days of SCI. (H) Statistical diagram of step width after 28 days of SCI. Data were presented as mean ± S.D. ($n = 6$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

after SCI. Our findings, as depicted in Fig. 7A–C, revealed that the area of hematoma was significantly greater in NTN1^{Emx1}-CKO mice on day 3 after SCI compared to the control group. Furthermore, Nissl staining and HE staining demonstrated a significant increase in neuron loss at the site of injury in NTN1^{Emx1}-CKO mice (Fig. 7D–G). To further elucidate the extent of inflammation, we conducted immunostaining using several cell markers to identify inflammatory cells. Interestingly, a significant increase in the number of inflammatory cells, such as Iba1^{+} microglia and GFAP^{+} astrocytes, was observed in NTN1^{Emx1}-CKO mice on day 3 after SCI (Fig. 7H–K). Collectively, The results strongly indicate that the deletion of NTN1 in neurons triggers early-phase neuroinflammation in the context of SCI, potentially hindering neural regeneration.

3.6. NTN1^{Emx1}-CKO induced the neuroinflammation after SCI through activation of NF-κB signaling pathway

To further investigate the underlying mechanism, we employed mRNA sequencing to analyze differential gene expression in the spinal cords of NTN1f/f and NTN1Emx1-CKO mice. The results demonstrate the overall pattern of gene expression (Fig. 8A). Moreover, the KEGG pathway enrichment analysis revealed that the differential genes were enriched in the NF-κB pathway, as shown in Fig. 8B–C. In a previous study, it was found that NTN-1 can mitigate early brain injury after SAH through the activation of the UNC5B PPARγ/NF-κB signaling pathway [22]. Interestingly, a significant increase in NF-κB activation were identified in the spinal cords of

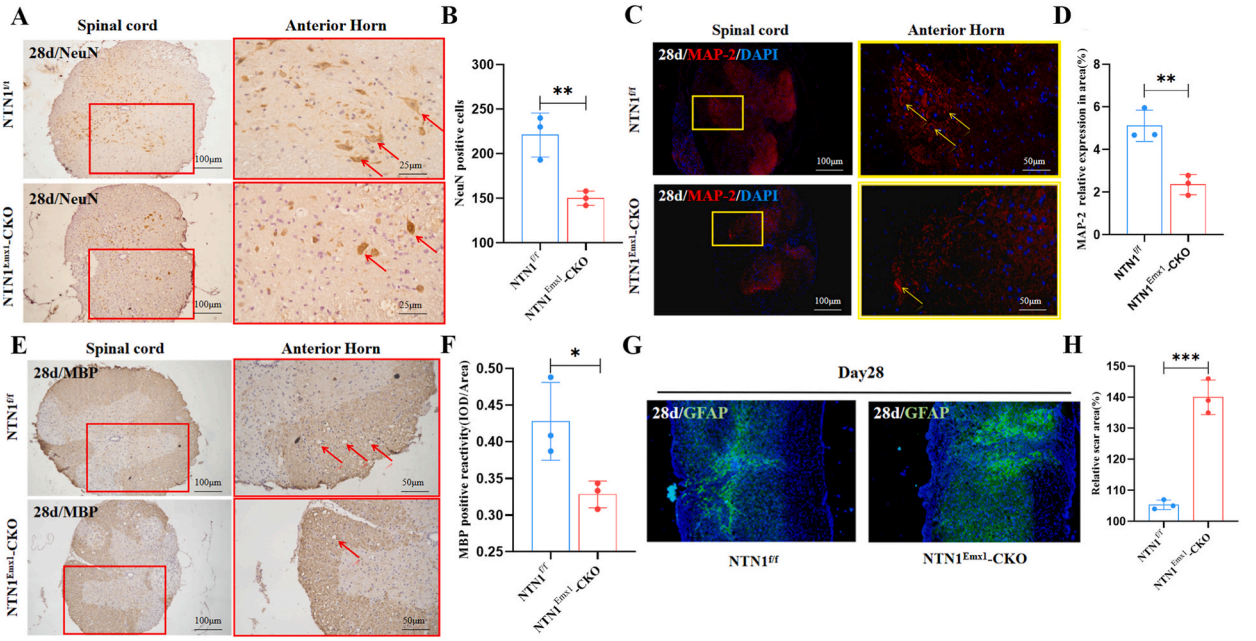


Fig. 6. Effect of NTN1 knockout on neuronal degeneration after SCI. Immunohistochemistry for NeuN (A)NeuN, MAP2 (C) and MBP (E) at day 28 after SCI. Statistical plot of NeuN (B), MAP2 (D) and MBP (F) analyzed using Image J. (G) Measurement of glial scar area 28 days after SCI (H) Statistical plot of glial scar analysis. Data were presented as mean ± S.D. ($n = 3$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

$NTN1^{f/f}$ mice at day 3 after SCI. Furthermore, the level of p-p65 proteins was higher in $NTN1^{Emx1}$-CKO mice, as shown in Fig. 8D–E. These findings suggest that NTN1 knockout leads to the activation of the NF-κB signaling pathway in the spinal cords following SCI.

Additionally, we evaluated the expression levels of IL-1β and TNF-α, known inflammatory mediators, in the spinal cords of both $NTN1^{f/f}$ and $NTN1^{Emx1}$-CKO mice at day 3 after SCI. Interestingly, the protein levels of IL-1β and TNF-α were greatly upregulated in $NTN1^{Emx1}$-CKO mice compared to $NTN1^{f/f}$ mice, as depicted in Fig. 8F–I. These results indicate that NTN1 knockout exacerbates inflammation in the spinal cords after SCI, possibly through the activation of the NF-κB signaling pathway.

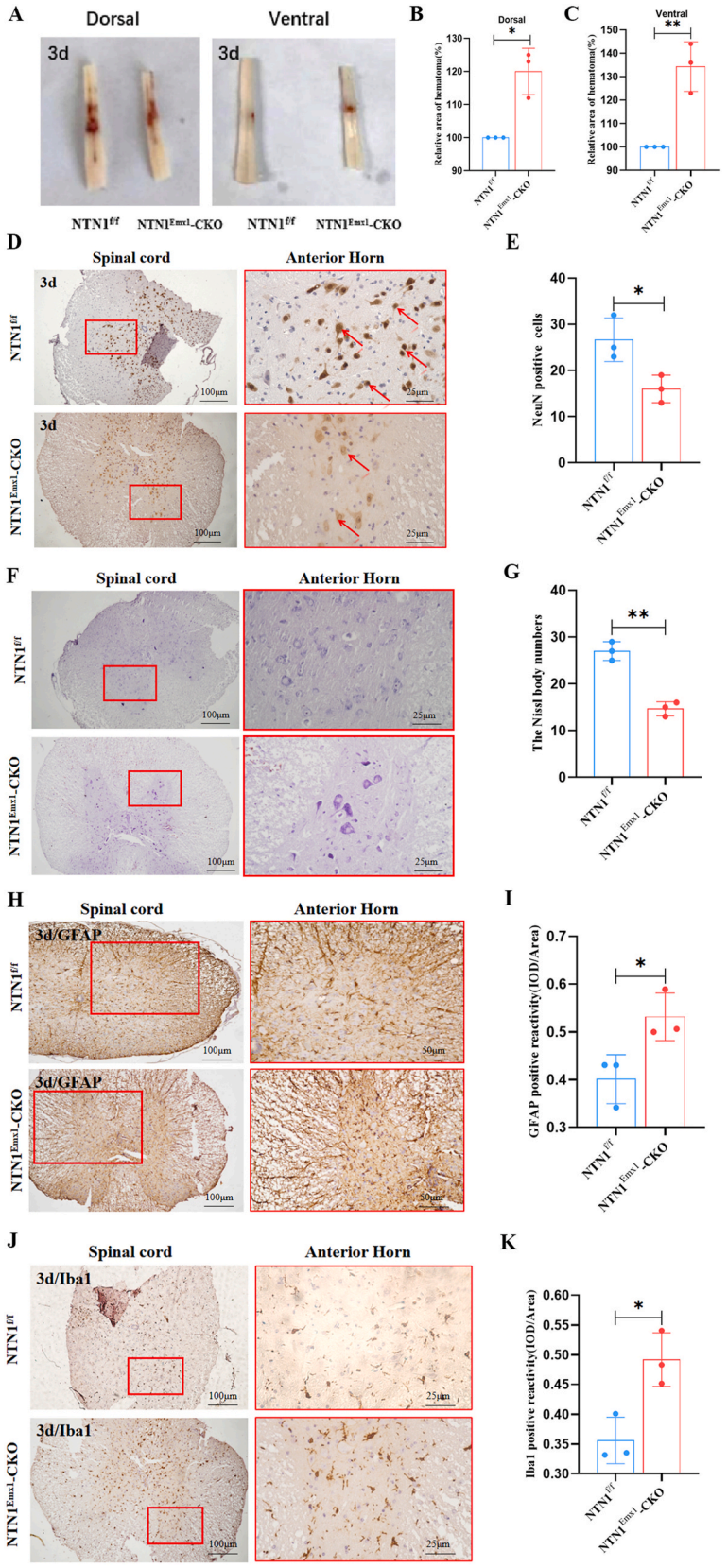
3.7. $NTN1^{Emx1}$-CKO induced the expression apoptosis and autophagy-related proteins after SCI

Previous research has established that apoptosis and autophagy-related proteins play a crucial role in the survival of neurons. As a result, this study was to examine how the knockout of the NTN1 gene in mice affected neuronal apoptosis and autophagy post-SCI. Compared to the $NTN1^{f/f}$ group, a significant increase in the levels of the apoptosis-related proteins, BAX in $NTN1^{Emx1}$-CKO group after SCI. Conversely, there was a considerable decrease in the protein levels of Bcl-2 (Fig. 9A–B). Furthermore, the immunohistochemical analysis demonstrated a noteworthy upregulation of Bax expression and a notable downregulation of p62 expression observed in the neurons of the anterior horn of the spinal cord (Fig. 9C–F). These findings suggest that NTN1 could regulate the survival of spinal cord neurons by modulating the expression of apoptosis and autophagy-related proteins.

Next, the mRNA levels of SAT1 and ALOX15 (genes involved in ferroptosis) were examined in the spinal cords of mice in groups at three days after SCI by real-time quantitative PCR. We found that compared to mice in $NTN1^{f/f}$ group, mice in group had significantly upregulated mRNA levels of ALOX15 and SAT1 in $NTN1^{Emx1}$-CKO group. Additionally, mitochondrial membrane rupture and significant mitochondrial shrinkage are important features of ferroptosis. Using transmission electron microscopy, compared to mice in $NTN1^{f/f}$ group, mice in $NTN1^{Emx1}$-CKO group had more pronounced changes in mitochondrial morphology and structure in neurons at three days after spinal cord injury (Fig. 9G–I). These data suggest that mice in $NTN1^{Emx1}$-CKO experienced more severe ferroptosis than mice in $NTN1^{f/f}$ group at three days after SCI.

4. Discussion

Recent evidence has accumulated to demonstrate that inflammatory and degenerative changes occur not only at the injured site, but also in various areas of the brain, including the hippocampus and prefrontal cortex after SCI [23]. Therefore, addressing cognitive complications caused by SCI from different perspectives can contribute to treatment approaches. This study aimed to explore the impact of neuron-derived NTN1 deficiency in the hippocampus and prefrontal cortex on the development and function of the spinal cord, as well as hippocampal and neurological functions following SCI. Our findings indicate that NTN1 plays a crucial role in limiting damage caused by SCI. Interestingly, the conditional deletion of NTN1 hinders neuronal regeneration during the intermediate phase following SCI. Additionally, we observed a significant enhancement of neuroinflammation in the early phase due to the activation of



(caption on next page)

Fig. 7. Effects of NTN1 deficiency on neuron regeneration after SCI. (A) Damage area at day 3 after SCI. (B, C) Damage area statistic plot in dorsal and ventral after SCI 3 days. (D) Immunohistochemical analysis of astrocyte expression in NeuN + cells in spinal cord at 3 days after SCI. (E) Quantification of NeuN + cell expression. (F) Nissl staining in spinal cord at 3 days after SCI. (G) Quantification of Nissl staining. (H, J) Immunohistochemical analysis of astrocyte expression in GFAP+ and Iba1+ cells in the spinal cord at 3 days after SCI. (I, K) Quantification of GFAP$^+$ and Iba1$^+$ cell expression using IHC staining. Data are presented as mean ± S.D. ($n = 3$), *$p < 0.05$, **$p < 0.01$.

the NF-κB signaling pathway. Hence, our findings indicate that the absence of NTN1 may exacerbate neuroinflammation through the NF-κB pathway, thereby promoting the pathological progression of SCI.

NTN1, an axon guidance factor, has demonstrated active involvement in promoting peripheral nerve regeneration [24]. These conditions target the structure and function of the hippocampus, leading to cognitive deficits [25]. However, the impact of neuronal knockout of NTN1 on the structure and function of the hippocampus has yet to be determined. In order to explore this, a study was conducted to analyze the arrangement of hippocampal neurons in a neuronal NTN1 knockout group as compared to a control group. The findings revealed that the hippocampal neurons were observed to be closely arranged without any significant dispersion in the neuronal NTN1 knockout group. This suggests that neuronal NTN1 knockout may not affect the number of hippocampal neurons. Furthermore, we investigated whether neuronal NTN1 knockout affects the function of hippocampus with involvement of inflammatory reactions. The results showed no significant changes in the number and distribution of inflammatory cells, indicating that neuronal NTN1 knockout does not cause neuroinflammation in the hippocampus of mice without SCI treatment. Interestingly, the organizational structure of the CA3 region is loosely rearranged, with neurons dispersed and significantly reduced in number for SCI mice (data not shown). The reduction of neurons in the CA3 region suggests that neuronal NTN1 expression plays an important role in maintaining the structural and functional integrity of hippocampal CA3 neurons after SCI [26,27]. The precise role of NTN1 in modulating the structure and function of hippocampal neurons is still uncertain. Future studies could involve exploring the potential impact of NTN1 knockout on synaptic plasticity, neuronal connectivity, and overall hippocampal network dynamics.

The main cause of insufficient regeneration and recovery after SCI is the imbalance in the microenvironment, as indicated by recent advances in SCI research [28]. It was found that the loss of motor neurons in the anterior horn of spinal cord and impaired motor function recovery occurred in mice lacking NTN1. Furthermore, these mice exhibited larger glial scars in the spinal cord and more severe demyelination reactions, as indicated by the decrease of the myelin-associated protein MBP. These findings highlight the

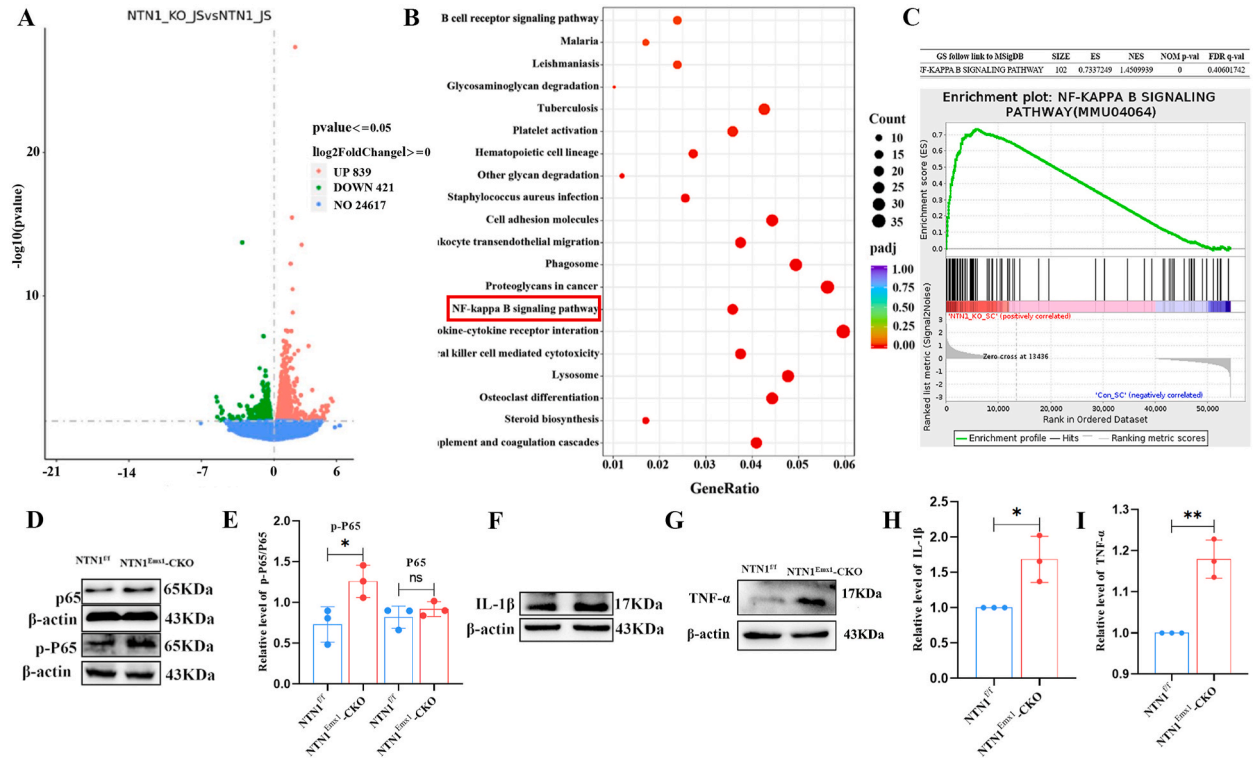


Fig. 8. Effects of NTN1 deficiency on NF-KB signaling pathway after SCI. (A) The differential genes were detected by RNA-seq between two groups. (B) a scatter plot is shown to represent the enrichment of differential genes analyzed by GO analysis. (C) The NF-κB signaling pathway was analyzed using KEGG enrichment analysis. (D) Western blotting was used to detect the expression of p-P65 and P65 in the spinal cord tissue at day3 after SCI. (E) The relative integrated density value (IDV) of p-p65 and p65 was analyzed using Image J. (F–G) Western blotting was used to detect the expression of TNF-α and IL-1β in the spinal cord 3 days after SCI. (H–I) The relative gray value of TNF-α and IL-1 β normalized to β-actin. Data were presented as mean ± S.D. ($n = 3$), ns $p > 0.05$, *p < 0.05, **$p < 0.01$.

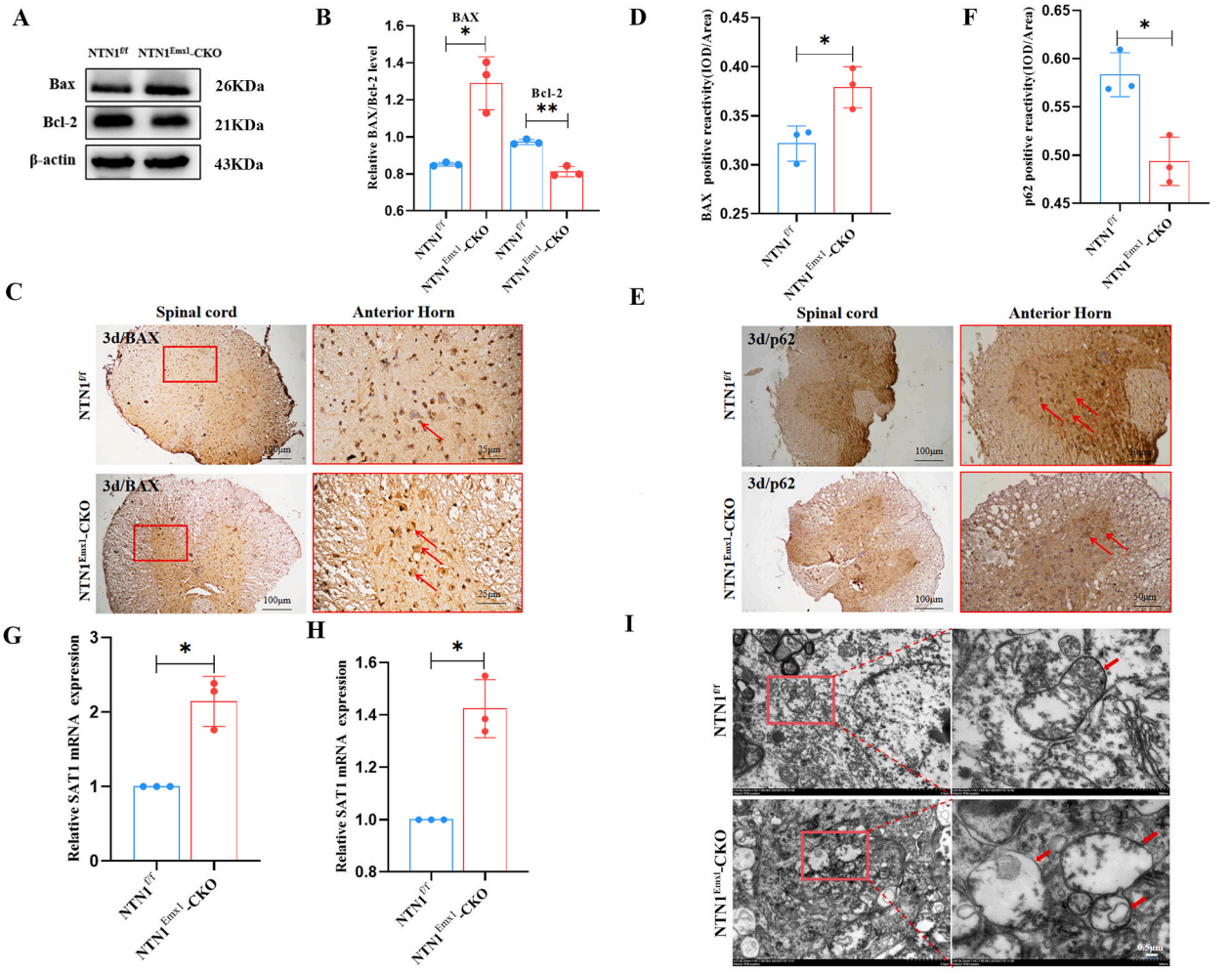


Fig. 9. Effects of SCI on autophagy and apoptosis. (A) The expression of Bcl-2 and BAX in spinal cord were detected by immunoblotting after SCI for 3 days. (B) Quantification of the relative BAX/β-actin and Bcl-2/β-actin. (C, E) IHC for BAX and p62 in the spinal cord at day 3 after SCI. (D, F) Analysis of IHC staining for BAX and p62. (G,H)Relative level of ALOX15 and SAT1 mRNA. (I) Mitochondrial morphology and structure in neurons were observed via transmission electron microscopy. Data are presented as mean ± S.D. ($n = 3$), $^{*}p < 0.05$, $^{**}p < 0.01$.

importance of NTN1 expression in facilitating the recovery process and restoration of functioning after SCI injury.

To further investigate the potential mechanism of neuronal knockout of NTN1 following SCI, we performed RNA sequencing analysis on spinal cord tissue. The results revealed that the NTN1 knockout group samples exhibited differential expression of various genes, predominantly inflammatory factors, through GO enrichment analysis. Moreover, KEGG enrichment analysis demonstrated a strong association between the knockout group data and the NF-κB signaling pathway, which has previously been reported to promote microglia-mediated inflammatory responses, inhibit nerve regeneration, and trigger apoptosis after SCI [29,30]. Furthermore, our results indicated that the expression level of p-p65 were significantly increased. The expression of IL-1β and TNF-α, which are downstream target genes of the NF-κB signaling pathway, was also significantly upregulated. We hypothesize that neuronal knockout of NTN1 may influence the inflammatory response by affecting the NF-κB signaling pathway, subsequently impacting the recovery of neural function. This activation of the inflammatory reaction may promote an increase in inflammatory cells. The results revealed a significant increase in the number of microglia and astrocyte. In the next experiment, we will focus on the corresponding phenotype of microglia to further explain its impact on neuroinflammation.

Research has demonstrated that autophagy, apoptosis, and ferroptosis are the primary factors influencing cell survival in SCI [31]. Autophagy, a cellular process that involves the degradation and recycling of damaged organelles and proteins, has been found to be closely linked to apoptosis in neuronal cells. Numerous studies suggest that dysregulated autophagy can trigger apoptotic pathways, ultimately leading to neuronal cell death [32,33]. Interestingly, conflicting findings have also indicated that autophagy may exert a protective effect against neuronal injury [34–38]. This dual role of autophagy in neuronal cells suggests that its impact on neurological diseases is complex and context-dependent. We found that the level of the autophagy-associated protein p62 was significantly lower in the neurons of mice with NTN1 deficiency following SCI, indicating that NTN1 deficiency may enhance the autophagic pathway.

Nevertheless, further research is needed to explore other markers related to autophagy.

BAX and Bcl-2 as classic pro-apoptotic and anti-apoptotic proteins, directly influence cell apoptosis and indirectly affect functional recovery after SCI [39,40]. Our study showed that the lack of NTN1 could enhance neuronal apoptosis. Ferroptosis is a unique form of cell death that is intricately connected to lipid peroxidation and requires the presence of reactive oxygen species (ROS) accompanied by morphological modifications such as mitochondrial atrophy and increase in membrane density. The previous study confirmed that ferroptosis genes were differentially expressed and then the existence of ferroptosis in neurons following SCI [41]. Gene markers for ferroptosis, including spermidine/spermine N1-acetyltransferase 1 (Sat1) and arachidonate 15-lipoxygenase (Alox15) [42], were found to be significantly upregulated in $NTN1^{Emx1}$-CKO group. Furthermore, mice in the $NTN1^{Emx1}$-CKO group exhibited more significant alterations in mitochondrial morphology and neuronal structure following SCI. The knockout group exhibited more significant alterations in proteins associated with autophagy, apoptosis, and ferroptosis, indicating that neuronal NTN1 deficiency impacts these cellular processes. These findings contribute to a better understanding of the underlying mechanisms involved in the inadequate regeneration and recovery of SCI.

Previous studies have mainly focused on the effects of spinal cord injury on microglia activation in the hippocampus, with limited information on the impact of neuronal changes in brain regions related to learning and memory on the spinal cord. NTN1 is highly expressed in brain neurons and is involved in the development of central nervous system disorders like SCI and Alzheimer's disease (AD). Research has shown that an increase in brain NTN1 levels could be beneficial for SCI recovery. Our study suggests that changes in NTN1 in the hippocampus and cortex also affect the functional recovery of SCI. Therefore, it is essential to consider not only localized spinal cord treatment but also concurrent brain intervention.

5. Conclusion

In summary, our research has shown that NTN1 may also have the ability to regulate the NF-κB signal, which is responsible for mediating the apoptosis and ferroptosis processes and maintaining the stable state of the microenvironment in the central nervous system. These results imply a functional connection between the brain and the spinal cord, as reducing the expression of NTN1 in hippocampal and cortex neurons can hinder the recovery process of spinal cord injury. However, further investigation is necessary to determine the potential efficacy of administering recombinant NTN1 protein directly into the brain in order to improve spinal cord dysfunction, preserve the structural integrity of the hippocampus, and alleviate the learning and memory impairments associated with SCI.

Funding

This work was supported by the National Natural Science Foundation of China, China. (No. 81971827); the Natural Science Foundation for Liaoning Province (No. 2020-MS-146); the Education department Foundation for Liaoning Province (No. LJKMZ20221350).

Ethics statement

All animal experiments conducted in this study strictly adhered to the guidelines set forth by the National Institutes of Health. Additionally, prior approval (No. SYPU-1ACUC-GZR2020R-04.16-201) was obtained from the Animal Care and Use Committee at Shenyang Pharmaceutical University to ensure ethical practices in animal research.

Data availability statement

The raw data supporting the conclusions of this article will be made available on request.

CRediT authorship contribution statement

Xiaojian Qin: Writing – original draft, Methodology, Investigation. **Xiaolan Zhang:** Methodology, Formal analysis, Data curation. **Xiaodong He:** Methodology, Investigation, Formal analysis, Data curation. **Hui Xu:** Methodology, Investigation. **Qiannan Yao:** Methodology, Investigation. **Zifeng Li:** Visualization, Methodology, Investigation. **Yayun Feng:** Methodology, Investigation. **Yichen Zhong:** Methodology, Investigation. **Ziyang Li:** Writing – original draft, Investigation. **Gang Lv:** Supervision, Project administration, Funding acquisition, Conceptualization. **Yanfeng Wang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Gang Lv reports financial support was provided by National Natural Science Foundation of China. Yanfeng Wang reports financial support was provided by Liaoning Provincial Department of Science and Technology. Xiaolan Zhang reports equipment, drugs, or supplies was provided by Shenyang Pharmaceutical University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37388.

References

[1] E. Ilyas, L. David P, G. Zoher, Acute traumatic spinal cord injury, Neurol. Clin. 39 (2021) 471–488, https://doi.org/10.1016/j.ncl.2021.02.004.
[2] N. Tharkika, L. Rylan S, B. Rebecca L, F. Shawn B, P. Benjamin D, N. Randolph J, T. Anne Marion, A.M. Taylor, Distal axotomy enhances retrograde presynaptic excitability onto injured pyramidal neurons via trans-synaptic signaling, Nat. Commun. 8 (2017) 625, https://doi.org/10.1038/s41467-017-00652-y.
[3] Y. Li, H. Xuelian, R. Kawaguchi, Z. Yu, W. Qing, A. Monavarfeshani, Y. Zhiyun, C. Bo, Zhongju Shi, M. Huyan, Z. Songlin, Z. Junjie, J. Anne, S. Vivek, P. Philip G, G. DanielH, H. Zhigang, Microglia-organized scar-free spinal cord repair in neonatal mice, Nature 587 (2020) 613–618, https://doi.org/10.1038/s41586-020-2795-6.
[4] L. Yun, R. Rodney M, K. Niaz, C. Tuoxin, H. Junyun, L. Zhuofan, M. Jessica J, S. Boris, L. Simon, L. Hui, S. Bogdan A, L. David J, F. Alan I, W. Junfang, Delayed microglial depletion after spinal cord injury reduces chronic inflammation and neurodegeneration in the brain and improves neurological recovery in male mice, Theranostics 10 (2020) 11376–11403, https://doi.org/10.7150/thno.49199.
[5] J. Ignacio, D.N. Alejandro F, E. Juan Manuel, L. Florencia, Spinal cord injury leads to hippocampal glial alterations and neural stem cell inactivation, Cell. Mol. Neurobiol. 42 (2022) 197–215, https://doi.org/10.1007/s10571-020-00900-8.
[6] J. Ignacio, P. Luciana, D.N. Alejandro F, Labombarda Florencia, Spinal cord injury impairs neurogenesis and induces glial reactivity in the Hippocampus, Neurochem. Res. 42 (2017) 2178–2190, https://doi.org/10.1007/s11064-017-2225-9.
[7] T. Andrew M, W. Stephen G, Spinal cord injury, dendritic spine remodeling, and spinal memory mechanisms, Exp. Neurol. 235 (2012) 142–151, https://doi.org/10.1016/j.expneurol.2011.08.026.
[8] C. Valentina, L. Gianluca, F. Salvatore, D.'A. Cesare, M. Vincenzo, K. Martin, P. Fulvio, B. Anna, C. Cannizzaro, Prenatal exposure to Δ9-tetrahydrocannabinol affects hippocampus-related cognitive functions in the adolescent rat offspring: focus on specific markers of neuroplasticity, Pharmaceutics 15 (2023) 692, https://doi.org/10.3390/pharmaceutics15020692.
[9] B. Romain, V. Claire, F. Carole, M. Maud, R. Anna Rita, C. Ievgeniia, P. Marie-Laure, A. Annie, K. Olga, C. Fleur, R. Mathieu, H. Maeva, R. Veronika, C. Federica Grazia, L. Julie, B. Birke, R. Michel, F. Hesso, C. Yohann, M. Valbona, D. Thomas, M. Patrick, G. Benjamin, Z. Fabien, P. Romain, Hepatic inflammation elicits production of proinflammatory netrin-1 through exclusive activation of translation, Hepatology 76 (2022) 1345–1359, https://doi.org/10.1002/hep.32446.
[10] P. Davide, D. Gianluca, P. Alex, Di M. Anna, B. Elisa, C. Daniele, P. Andea, B. Mattia, V. William, C. Matteo, C. Antoella, S. Luigi, F. Federico, G. Costanza, V. Nina, R. Michael, Z. Serena, P. Maria Paola, P. Anna, E. Anna, M. Patrick, G. Claudia, A ligand-insensitive UNC5B splicing isoform regulates angiogenesis by promoting apoptosis, Nat. Commun. 12 (2021) 4872, https://doi.org/10.1038/s41467-021-24998-6.
[11] C. Guiqin, K. Seongsu, W. Zhihao, A. Eun Hee, X. Yiyuan, L. Xia, S. Ivette M, M. Fredric P, Z. Zhaohui, Y. Keqiang, Netrin-1 receptor UNC5C cleavage by active δ-secretase enhances neurodegeneration, promoting Alzheimer's disease pathologies, Sci. Adv. 7 (2021) eabe4499, https://doi.org/10.1126/sciadv.abe4499.
[12] R. Punithavthi, M. Riyaz, J. Calpurnia, R. Ganesan, Guidance cue netrin-1 and the regulation of inflammation in acute and chronic kidney disease, Mediat. Inflamm. (2014) 525891, https://doi.org/10.1155/2014/525891.
[13] M. Yan, S. Shifei, C. Shengnan, S. Bin, Netrin-1: a serum marker predicting cognitive impairment after spinal cord injury, Dis. Markers (2022) 1033197, https://doi.org/10.1155/2022/1033197.
[14] B. Liangjie, M. Xifan, W. Yanfeng, Y. Yajiang, B. Yunlong, L. Gang, W. Hongyu, Y. Peng, L. Gang, The role of netrin-1 in improving functional recovery through autophagy stimulation following spinal cord injury in rats, Front Cell Neurosci11 (2017) 350, https://doi.org/10.3389/fncel.2017.00350.
[15] G. Kai, N. Jianbing, D. Xiaoqian, Neuroprotection of netrin-1 on neurological recovery via Wnt/β-catenin signaling pathway after spinal cord injury, Neuroreport 31 (2020) 537–543, https://doi.org/10.1097/WNR.0000000000001441.
[16] Q. Héctor R, Intraspinal administration of netrin-1 promotes locomotor recovery after complete spinal cord transection, J. Neurotrauma 38 (2021) 2084–2102, https://doi.org/10.1089/neu.2020.7571.
[17] L. Xiao, X. Guangyu, L. Zhidi, Z. Fei, L. Siyang, Z. Yuxuan, F. Wei, J. Jianyuan, M. Xiaosheng, S. Jian, Engineered exosomes enriched in netrin-1 modRNA promote axonal growth in spinal cord injury by attenuating inflammation and pyroptosis, Biomater. Res. 27 (2023) 3, https://doi.org/10.1186/s40824-023-00339-0.
[18] G. Yanting, D. Yinfeng, W. Jieru, R. Honglei, K. Raymond C, W. Jian, Interleukin-10 deficiency aggravates traumatic brain injury in male but not female mice, Exp. Neurol. 355 (2022) 114125, https://doi.org/10.1016/j.expneurol.2022.114125.
[19] T. Laura B, V. Alexander G, F. Amanda H, M. Joseph T, Chronic neurobehavioral sex differences in a murine model of repetitive concussive brain injury, Front. Neurol. 10 (2019) 509, https://doi.org/10.3389/fneur.2019.00509.
[20] B. D Michele, F. Lesley C, A. Aileen J, J. Lyn B, M. Dana M, P. Philip G, Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains, J. Neurotrauma 23 (2006) 635–659, https://doi.org/10.1089/neu.2006.23.635.
[21] B. Leo, N. Hironao, E. Michele, W. Johannes, K. Michael, I. Daniel, B. David M, Netrin-1 augments chemokinesis in CD4+ T cells in vitro and elicits a proinflammatory response in Vivo, J. Immunol. 197 (2016) 1389–1398, https://doi.org/10.4049/jimmunol.1502432.
[22] C. Junhui, X. Yong, C. Yan, W. Ting, C. Lei, G. Haoxiang, Y. Shuo, H. Jianqing, S. Dongliang, W. Yuhai, Netrin-1 alleviates subarachnoid haemorrhage-induced brain injury via the PPARγ/NF-KB signalling pathway, J. Cell Mol. Med. 23 (2019) 2256–2262, https://doi.org/10.1111/jcmm.14105.
[23] X. Weikang, Z. Weijiang, M. Xianghe, S. Huifan, H. Peizhi, Spinal cord injury induced Neuregulin 1 signaling changes in mouse prefrontal cortex and hippocampus, Brain Res. Bull. 144 (2019) 180–186, https://doi.org/10.1016/j.brainresbull.2018.12.002.
[24] R. D Madison, A. Zomorodi, G. A Robinson, Netrin-1 and peripheral nerve regeneration in the adult rat, Exp. Neurol. 161 (2000) 563–570, https://doi.org/10.1006/exnr.1999.7292.
[25] T. Bartsch, P. Wulff, The hippocampus in aging and disease: from plasticity to vulnerability, Neuroscience 309 (2015) 1–16, https://doi.org/10.1016/j.neuroscience.2015.07.084.
[26] C. OwenY, N. Susanne, L.B. Marcus, H. Joseph P, S. Maria A, Interaction between the medial prefrontal cortex and hippocampal CA1 area is essential for episodic-like memory in rats, Neurobiol. Learn. Mem. 141 (2017) 72–77, https://doi.org/10.1016/j.nlm.2017.03.019.
[27] R. Maninmran, M. Kiara, A. Namasivayam, M. Lori L, Increased excitability and heightened magnitude of long-term potentiation at hippocampal CA3-CA1 synapses in a mouse model of neonatal hyperoxia exposure, Front. Synaptic Neurosci. 12 (2021) 609903, https://doi.org/10.3389/fnsyn.2020.609903.
[28] F. Baoyou, W. Zhijian, Y. Xue, S. Guidong, C. Xin, Z. Xianhu, Z. Hengxing, N. Guangzhi, K. Xiaohong, F. Shiqing, Microenvironment imbalance of spinal cord injury, Cell Transplant. 27 (2018) 853–866, https://doi.org/10.1177/0963689718755778.
[29] L. Huitao, Z. Jingjing, X. Xingxing, L. Sheng, Y. Danlu, X. Changnan, J. Mengxian, Z. Wenbin, J. Lingting, W. Xiwu, S. Xiya, L. Fayi, W. Wangfei, B. Xiaomei, L. Sijia, Z. Minyu, W. Wei, W. Ying, H. Zhihui, T. Honglin, SARM1 promotes neuroinflammation and inhibits neural regeneration after spinal cord injury through NF-κB signaling, Theranostics 11 (2021) 4187–4206, https://doi.org/10.7150/thno.49054.
[30] X. Shun, W. Jin, Z. Junjie, S. Minghao, J. Jianyuan, S. Jian, Z. Wei, Z. Fan, X. Haocheng, X. Guanyu, Z. Yuxuan, M. Xiaosheng, L. Feizhou, CD73 alleviates GSDMD-mediated microglia pyroptosis in spinal cord injury through PI3K/AKT/Foxo1 signaling, Clin. Transl. Med. 11 (2021) e269, https://doi.org/10.1002/ctm2.269.
[31] X. Yongzhi, X. Haijian, C. Dan, L. Zhengbu, Y. Yi, Mechanisms of autophagy and apoptosis mediated by JAK2 signaling pathway after spinal cord injury of rats, Exp. Ther. Med. 14 (2017) 1589–1593, https://doi.org/10.3892/etm.2017.4674.

[32] D. Sun, X.D. Sun, L. Zhao, D.H. Lee, J.X. Hu, F.L. Tang, J.X. Pan, L. Mei, X.J. Zhu, W.C. Xiong, Neogenin, a regulator of adult hippocampal neurogenesis, prevents depressive-like behavior, Cell Death Dis. 9 (2018) 8. https://10.1038/s41419-017-0019-2.
[33] Y. Yu, X. Wu, J. Pu, P. Luo, W. Ma, J. Wang, J. Wei, Y. Wang, Fei , Lycium barbarum polysaccharide protects against oxygen glucose deprivation/reoxygenation-induced apoptosis and autophagic cell death via the PI3K/Akt/mTOR signaling pathway in primary cultured hippocampal neurons, Biochem. Biophys. Res. Commun. 1495 (2018) 1187–1194. https://10.1016/j.bbrc.2017.11.165.
[34] J. Liu, W. Liu, Y. Lu, H. Tian, C. Duan, L. Lu, G. Gao, X. Wu, X. Wang, H. Yang, Piperlongumine restores the balance of autophagy and apoptosis by increasing BCL2 phosphorylation in rotenone-induced Parkinson disease models, Autophagy 14 (2018) 845–861. https://10.1080/15548627.
[35] L. Qiao, J. Fu, X. Xue, Y. Shi, L. Yao, W. Huang, J. Li, D. Zhang, N. Liu, X. Tong, Y. Du, Y. Pan, Neuronalinjury and roles of apoptosis and autophagy in a neonatal rat model of hypoxia-ischemia-induced periventricular leukomalacia, Mol. Med. Rep. 17 (2018) 5940–5949. https://10.3892/mmr.2018.8570.
[36] L. Yun, L. Zhuofan, R. Rodney M, H. Junyun, L. Hui, C. Harry Mc, L. Marta M, W. Junfang, Impairment of autophagy after spinal cord injury potentiates neuroinflammation and motor function deficit in mice, Theranostics 12 (2022) 5364–5388, https://doi.org/10.7150/thno.72713.
[37] L. Dachuan, L. Xiao, X. Guangyu, L. Siyang, G. Zhaoyang, L. Feizhou, X. Xinlei, J. Jianyuan, W. Hongli, Z. Fei, M. Xiaosheng, Dihydroorotate dehydrogenase regulates ferroptosis in neurons after spinal cord injury via the P53-ALOX15 signaling pathway, CNS Neurosci. Ther. 29 (2023) 1923–1939, https://doi.org/10.1111/cns.14150.
[38] X. Yu, G. Yibo, W. Hui, Z. Haojie, Q. Jianjun, L. Feida, H. Xinli, C. Yituo, S. Haipeng, L. Yao, W. Xiangyang, X. Huazi, K. Jianzhong, C. Yuepiao, W. Aimin, N. Wenfei, X. Jian, Z. Kailiang, Cyclic helix B peptide alleviates proinflammatory cell death and improves functional recovery after traumatic spinal cord injury, Redox Biol. 64 (2023) 102767, https://doi.org/10.1016/j.redox.2023.102767.
[39] R. Yuluo, L. Wei, W. Jiaxing, F. Jin, L. Yongjun, L. Linwei, K. Fanqi, C. Jian, T. Pengyu, C. Weihua, Neural stem cell-derived small extracellular vesicles attenuate apoptosis and neuroinflammation after traumatic spinal cord injury by activating autophagy, Cell Death Dis. 10 (2019) 340, https://doi.org/10.1038/s41419-019-1571-8.
[40] W. Chao, Z. Lu, N. Jean De La Croix, H. Aubryanna, S. Guodong, C. Changhong, Z. Chen, L. Ronghan, L. Chuanju, Progranulin deficiency exacerbates spinal cord injury by promoting neuroinflammation and cell apoptosis in mice, J. Neuroinflammation 16 (2019) 238, https://doi.org/10.1186/s12974-019-1630-1.
[41] L. Kewei, L. Zhong, W. Peipei, C. Shenyuan, W. Min, L. Wenhui, Z. Leilei, G. Song, L. Yanbin, L. Pengcheng, Z. Beiting, T. Lin, D. Hui, Q. Hui, F. Qiang, Micro electrical fields induced MSC-sEVs attenuate neuronal cell apoptosis by activating autophagy via lncRNA MALAT1/miR-22-3p/SIRT1/AMPK axis in spinal cord injury, J. Nanobiotechnol. 21 (2023) 451, https://doi.org/10.1186/s12951-023-02217-2.
[42] W. Kexing, J. Min, Z. Hong, L. Yuye, W. Suixi, Z. Kailing, W. Zixiao, Z. He, Z. Xunan, L. Yi, P. Lei, W. Caihua, L. Yongmin, L. Man, Electroacupuncture alleviates neuropathic pain by suppressing ferroptosis in dorsal root ganglion via SAT1/ALOX15 signaling, Mol. Neurobiol. 60 (2023) 6121–6132, https://doi.org/10.1007/s12035-023-03463-z.