



Electroacupuncture Ameliorates NLRP3-mediated Pyroptosis in Spinal Cord Injury Rats by Reshaping The Gut Microbiota*

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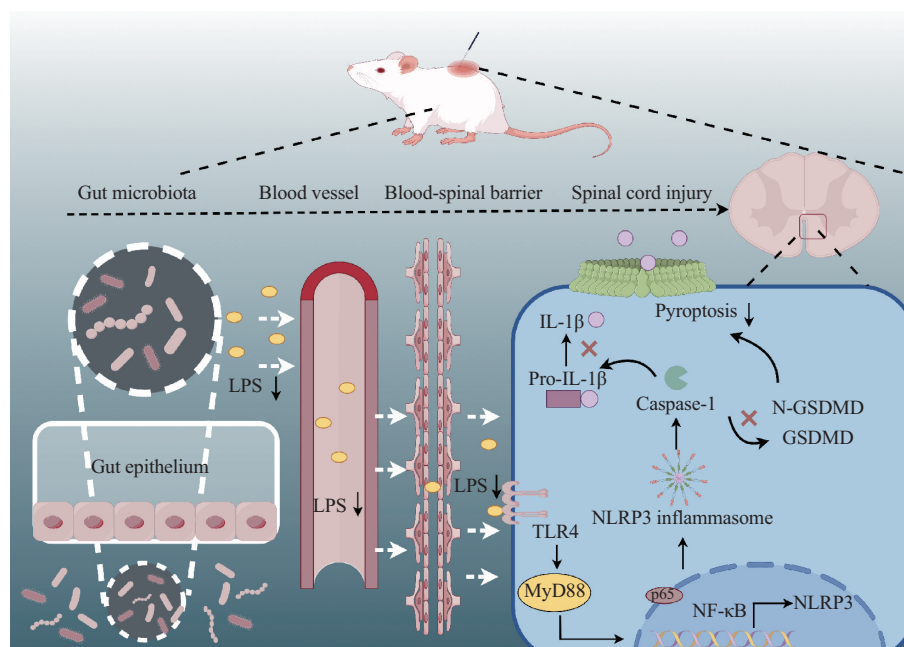
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Graphical abstract



Abstract Objective Spinal cord injury (SCI) directly impairs the regulatory function of the autonomic nervous system, induces intestinal dysfunction, and significantly reduces patients' quality of life. Preclinical studies have shown that electroacupuncture (EA) therapy can regulate the brain-gut axis and is used to treat central nervous system diseases such as major depressive disorder, Alzheimer's disease and Parkinson's disease. Recent research has established that fecal microbiota transplantation (FMT) from EA-treated SCI rats restored intestinal motility and colonic morphology. However, it remains unclear whether the regulation of gut microbiota by EA therapy directly contributes to neural repair after SCI. This study aims to explore whether gut microbiota mediates

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the neuroprotective effect of EA in the treatment of SCI and its possible mechanism. **Methods** The study employed RNA transcriptome analysis of spinal cord tissue to characterize gene expression profiles and to identify key signaling pathways following EA treatment for SCI. Hematoxylin-Eosin (HE) staining and Nissl staining were used to observe the morphological changes in spinal cord tissue. Western blot (WB) and enzyme-linked immunosorbent assay (ELISA) were applied to detect the effects of EA on the expression of proteins related to nucleotide-binding domain leucine-rich repeat and pyrin domain-containing receptor 3 (NLRP3)-dependent pyroptosis. Using 16S rDNA sequencing, the study observed alterations in gut microbiota diversity and community composition in SCI rats. Prior to establishing SCI models, rats were pretreated with an antibiotic cocktail to induce gut dysbiosis, and the effects on intestinal function and spinal cord neural repair were evaluated. FMT was performed to investigate the regulatory effects of post-EA FMT on motor function, general status, liver and spleen indices, and NLRP3-mediated pyroptosis in SCI rats. **Results** EA improved motor function and reduced regulated neuronal cell death in SCI rats. Transcriptomic analysis demonstrated the activation of immune- and inflammation-related pathways post-SCI, including NOD-like receptors, nuclear factor-kappa B (NF- κ B), and Toll-like receptor (TLR) pathways. EA primarily influenced intestinal inflammation and autoimmune functions. 16S rDNA sequencing illustrated that EA did not alter the diversity of gut microbiota. However, EA altered the gut microbiota composition in SCI rats, increasing *Lactobacillus* and *Akkermansia* genera while rebalancing the Firmicutes/Bacteroidetes ratio. Furthermore, depletion of gut microbiota by antibiotics disrupted the intestinal barrier, reduced the expression of intestinal barrier proteins Zonula Occludens-1 (ZO-1) and Occludin, elevated serum lipopolysaccharide-binding protein (LBP) levels, exacerbated spinal cord tissue damage, and hindered motor function recovery in SCI rats. FMT from donors treated with EA reduced LBP levels in the intestine, blood, and spinal cord of rats, inhibited the TLR4 myeloid differentiation primary response protein 88 (MyD88)-NF- κ B pathway and NLRP3-dependent pyroptosis, and improved motor function. On the other hand, FMT treatment resulted in decreased body weight and food intake, whereas FMT using EA-treated donors effectively alleviated these alterations. **Conclusion** EA effectively alleviated neuroinflammatory responses in rats with SCI, primarily through regulating the gut microbiota and suppressing the NLRP3-dependent pyroptosis signaling pathway.

Key words electroacupuncture, spinal cord injury, fecal microbiota transplantation, gut microbiota, pyroptosis, NLRP3 inflammasome

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Spinal cord injury (SCI) is a disabling neurological disorder characterized by motor, sensory, and autonomic deficits that severely compromise patients' self-care and social participation. With lifetime treatment costs exceeding \$1 million per patient^[1], SCI imposes a substantial socioeconomic burden. Current therapies remain unsatisfactory because the neural repair mechanisms are complex. Excessive neuroinflammation plays a central role in secondary SCI^[2], spreading to adjacent tissue, causing cell death, and impairing axonal regeneration^[3]. The nucleotide-binding domain leucine-rich repeat and pyrin domain-containing receptor 3 (NLRP3) inflammasome is central to this process: NLRP3 senses danger signals, recruits apoptosis-speck compound (ASC) and pro-caspase-1, and promotes caspase-1 activation^[4], which matures proinflammatory cytokines (interleukin (IL)-1 β , IL-18) and cleaves gasdermin D (GSDMD) to trigger pyroptosis^[5-6]. NLRP3-mediated pyroptosis exacerbates neuronal loss and worsens motor deficits in SCI models^[6]. Pharmacological inhibition of this pathway has attenuated neuroinflammation and improved functional outcomes^[7-9], identifying NLRP3 as a promising therapeutic target for SCI management^[10].

The brain-gut axis mediates bidirectional communication between the central nervous system and the gastrointestinal tract through neural,

endocrine, and immune pathways. This regulatory network extends to spinal cord-gut interactions via autonomic connections^[11]. Clinically, 62.5% of SCI patients develop gastrointestinal dysfunction, and 30% rate it as more distressing than bladder or sexual impairments^[12]. SCI induces gut dysbiosis, which is characterized by increases in proinflammatory and pathogenic bacteria and decreases in butyrate-producing species^[13-16]; dysbiosis severity correlates with injury magnitude^[14, 17]. This microbial imbalance promotes bacterial translocation that damages intestinal and hepatic tissues^[18-19] and activates Toll-like receptor 4 (TLR4)-myeloid differentiation primary response protein 88 (MyD88)-nuclear factor-kappa B (NF- κ B) inflammatory pathways, exacerbating neuroinflammation^[20-21]. Recently, some researchers have proposed a "microbiota-gut-inflammasome-brain axis" in central nervous system diseases, assigning the NLRP3 inflammasome a central role in coordinating host physiology and shaping both peripheral and central immune and inflammatory responses^[22]. Clinical studies have reported that Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), and autism spectrum disorder (ASD) are associated with altered gut microbiota composition and activation of NLRP3 inflammasomes in the gut, periphery, and brain^[22]. Whether gut dysbiosis after SCI exacerbates neuroinflammation by promoting

NLRP3-mediated pyroptosis remains to be determined.

Electroacupuncture (EA), a well-established complementary intervention, has neuroprotective potential in SCI through multiple mechanisms, including attenuating the secondary injury microenvironment, suppressing neuroinflammatory cascades, and promoting axonal regeneration^[23-24]. Our previous studies demonstrated that EA exerted therapeutic effects in SCI rats by inhibiting NLRP3 inflammasome activation^[25]. On the other hand, studies have shown that EA increases microbial diversity and enriches beneficial bacterial populations, effectively treating gastrointestinal disorders such as enteritis, irritable bowel syndrome, and constipation^[26-28]. Preclinical studies have increasingly indicated that EA can regulate the brain-gut axis to treat neurological diseases such as major depressive disorder^[29], AD^[30], and PD^[31]. Recent research has established that fecal microbiota transplantation (FMT) from EA-treated SCI rats restored intestinal motility and colonic morphology^[32]. However, it remains unclear whether EA-mediated modulation of the gut microbiota directly contributes to neural repair in SCI. In the present study, we sought to investigate the hypothesis that EA exerts an anti-pyroptotic effect by regulating the gut microbiota in rats with SCI.

1 Materials and methods

1.1 Animals and model preparation

Female rats are preferred for SCI models because of their relatively smaller and straighter urethra, which decreases the likelihood of urinary tract infections^[33]. Adult female Sprague-Dawley rats weighing 180–220 g are kept in controlled conditions with a 12-h light/dark cycle and a constant ambient temperature of (22±1)°C. All animal procedures were approved by the Experimental Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (Approval No: TCM-LAEC2021220).

Rats were anesthetized with isoflurane (3% induction) and maintained on 1.2% oxygen-rich air *via* a face mask before surgery. The dorsal fur of each rat was shaved in a sterile field, and a midline incision through the skin and muscle was made to perform a laminectomy at thoracic 9–11 (T9–T11). An aneurysm clip (70 g, FT228T, Peter Lazic GmbH, Tuttlingen, Germany) was applied to the spinal cord at T10 for 30 s to create the SCI model. After surgery, the underlying muscles and skin were sutured at the incision site. Animals were placed in a temperature-

controlled chamber until normothermia was restored. Rats received 0.5 ml of Ringer's solution subcutaneously once daily for 5 consecutive days, and manual bladder expression was performed twice daily for 7 d beginning 24 h after operation.

1.2 Animal grouping and experimental design

Experiment 1. Rats were randomly assigned to three groups: Sham, SCI, and SCI+EA. The SCI model was produced using aneurysm clips. In the Sham group, only a T10 laminectomy was performed without inducing SCI. Rats in the SCI+EA group received EA after SCI. The SCI+EA group received EA for 30 min at T9–T11 Jia-Ji, Du Meridian 14 (DU14), and Du Meridian 4 (DU4) following SCI.

Experiment 2. To investigate the effect of gut microbiota dysbiosis on neurological recovery after SCI, we disrupted the gut microbiota with an antibiotic cocktail. Rats received drinking water supplemented with antibiotics (AB) (1 g neomycin sulfate, 0.5 g vancomycin, 1 g ampicillin, and 1 g metronidazole per liter) for 14 d prior to SCI induction. The rats were then randomly divided into two groups: SCI and SCI+AB.

Experiment 3. SCI rats were randomly assigned to three groups: SCI+EA, FMT(SCI)-SCI, and FMT(EA)-SCI. All rats received drinking water containing the same antibiotic cocktail described in Experiment 2 for 14 d before SCI modeling. At 5 d post-SCI, feces were collected for fecal transplantation. In the FMT(SCI)-SCI group, rats received daily gavage of their own feces. Rats in the FMT(EA)-SCI group received daily fecal transplants from donor rats that had undergone EA. The SCI+EA group received daily EA sessions and oral gavage with normal saline. The comprehensive experimental design are illustrated in Figure 1.

1.3 EA treatment

After a five-day postoperative recovery period, the rats received EA treatment. Each animal was placed in a specially designed rat suit on the operating platform. Following standard sterilization, sterile disposable acupuncture needles (0.25 mm×13 mm) were inserted to a depth of 4 mm at the T9–T11 Jia-Ji, DU14, and DU4 acupoints. Electrode connections are shown in Figure 2. Stimulation was delivered with a Hwato Electroacupuncture Therapy Instrument (model SDZ-V; Suzhou Medical Supplies Factory Co. Ltd.). All acupoints were stimulated at 2 mA using a sparse-dense wave pattern (2 Hz/15 Hz).

1.4 FMT intervention

Fecal material was collected and processed as previously described^[21]. Fresh fecal pellets from the

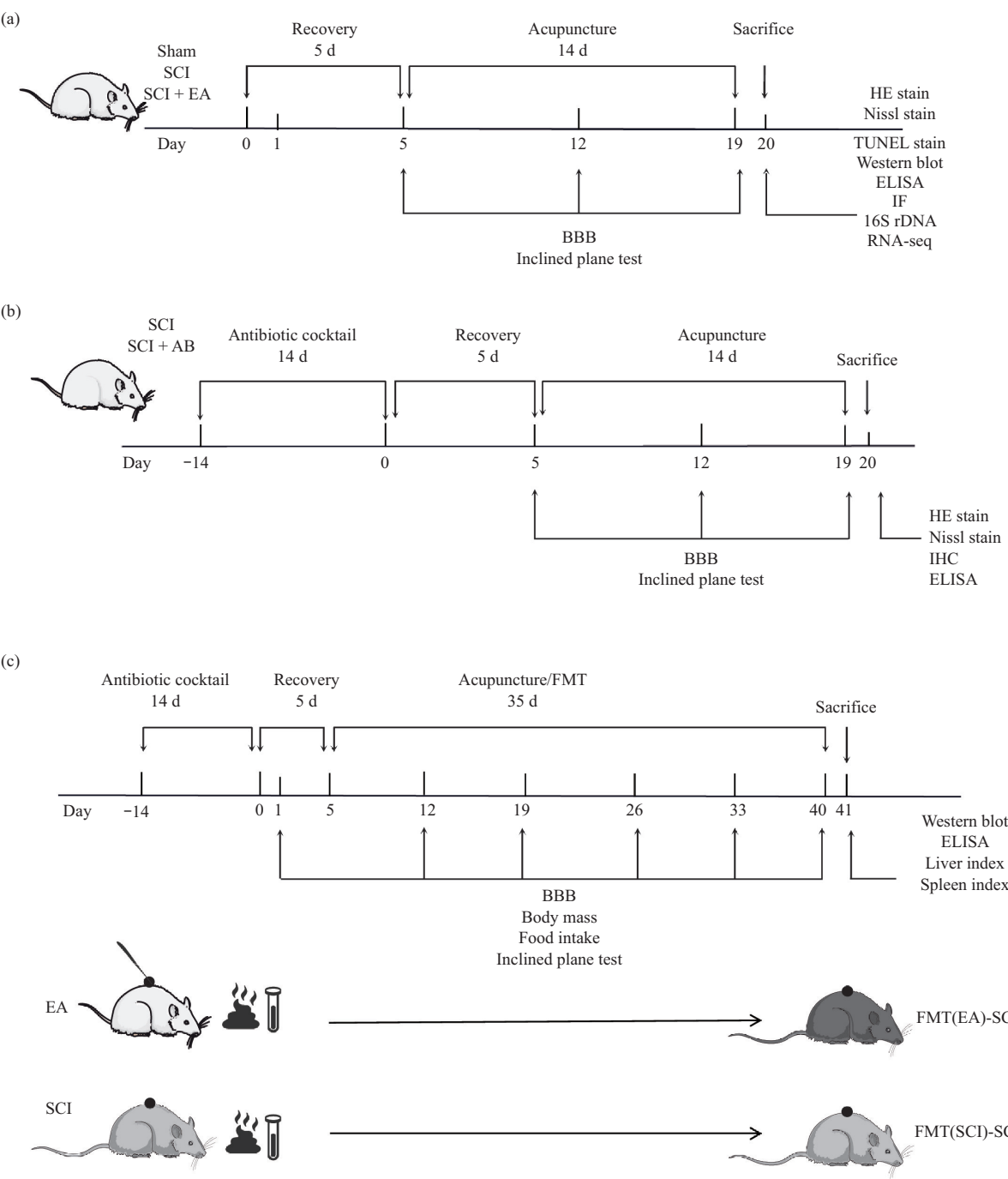


Fig. 1 Schematic diagram illustrating the comprehensive experimental design

(a) Experimental flowchat of electroacupuncture intervention; (b) flowchat of critibiotic intervention; (c) flowchat of fecal microbiota transplahtation. BBB: Basso-Beattie-Bresnahan.

rats were collected daily, weighed, and immediately stored at -80°C . For transplantation, feces were diluted 1 : 10 in sterile normal saline and homogenized on a TS100 shaker. The suspension was centrifuged at 1 200 r/min for 3 min, filtered through four layers of sterile gauze, and administered at a dose of 1 ml per 100 g of body mass. The fecal suspension was administered once daily for 35 consecutive days

by oral gavage using a sterile gavage needle. After each administration, rats were observed for adverse reactions, such as vomiting or convulsions, and were returned to their housing only once conditions were stable.

1.5 Locomotion recovery assessment

Basso-Beattie-Bresnahan (BBB) scores were used to assess motor function in the SCI rats. Scores

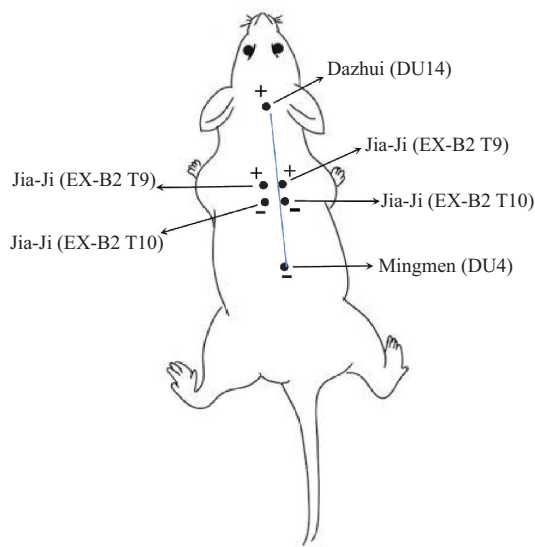


Fig. 2 Acupuncture protocol

SCI+EA group acupuncture point: T9-T11 Jia-Ji (EX-B2 T9; EX-B2 T10), Dazhui (DU14), Mingmen (DU4).

were calculated on days 1, 5, 12, 19, 26, 33, and 40 after SCI in the open field. Two trained evaluators, blinded to the experimental details, independently conducted all behavioral assessments. Each assessment lasted 4 min.

The Rivlin inclined plane test was used to evaluate limb muscle strength^[34]. Rats were placed on a rubber inclined plane, and the inclination angle was increased by 5° increments. The highest angle each rat maintained for 5 s was recorded. This procedure was repeated 3 times, and the average value was calculated. The interval between measurements was at least 5 min.

1.6 Microbiota characterization and bioinformatics analysis

Under sterile conditions, fecal samples were collected from the rats. The intestinal microbiota was analyzed *via* advanced high-throughput sequencing technology targeting the 16S rDNA. DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB). The purity and concentration of the extracted DNA were assessed *via* agarose gel electrophoresis. The V3-V4 region was the target region for amplification. Sequencing was carried out on the Illumina NovaSeq platform (Illumina, San Diego, CA, USA). Alpha diversity was evaluated using the Abundance-based Coverage Estimator (ACE), and Chao1 indices. Beta diversity was analyzed using principal component analysis (PCA).

T-tests and Tukey tests were employed to analyze whether there were significant differences in species α and β diversity among the groups. Using the linear discriminant analysis effect size (LEfSe), we identified key microbial community differences between groups. On the basis of taxonomic annotation, the top 10 most abundant species at each level (phylum, order, family) were selected to generate stacked bar plots of relative abundance, and performed to identify taxa exhibiting significant differences between groups. Tax4Fun was used to predict the functional profiles of the gut microbiota.

1.7 RNA sequencing

After RNA samples were qualified, mRNA was enriched using magnetic beads with oligo(dT). mRNA was fragmented with fragment buffer and reverse-transcribed to synthesize cDNA. After library construction, preliminary quantification was performed with a Qubit 2.0 fluorometer. Qualified libraries were sequenced on the HiSeq X-Ten platform (Illumina). Raw sequencing data were filtered using the next-generation sequencing (NGS) QC Toolkit (v2.3.3), and alignment was performed with TopHat (v2.0.13). Clean reads for further analysis were obtained after filtering the raw data, verifying the sequencing error rate, and examining the GC content distribution. To ensure comparability of gene expression across treatments, expression levels were normalized using fragments per kilobase of transcript per million mapped reads (FPKM). Differential expression analysis was performed using the NOISeq package. Genes were considered differentially expressed if the fold change exceeded 2 and the probability exceeded 0.8. Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene set enrichment analysis (GSEA) analyses were performed.

1.8 Spleen and liver index

The spleen index was calculated as spleen weight divided by body weight, and the liver index was calculated as liver weight divided by body weight^[35].

1.9 Hematoxylin-Eosin (HE) staining and Nissl staining

Five-micrometer paraffin-embedded sections of the spinal cord were mounted onto slides. HE and Nissl staining were performed according to the manufacturer's instructions. After staining, dehydration, and clearing, sections were examined with an optical microscope (Olympus, Tokyo, Japan). Quantification of ventral horn neurons and Nissl capsule density was performed using the Image-Pro Plus software (Rawak Software, Stuttgart, Germany).

1.10 Immunofluorescence staining/immunohistochemistry

For immunofluorescence staining, spinal cord sections and colon tissue were fixed in 4% paraformaldehyde for 10 min. Sections were then permeabilized with 0.1% Triton X-100 for 10 min and blocked with 2% bovine serum albumin (BSA) for 1 h. Next, sections were incubated with primary antibodies overnight at 4°C. After phosphate-buffered saline (PBS) washes, secondary antibodies were applied and samples were incubated for 1 h. Immunofluorescence images were acquired with an Olympus fluorescence microscope (Tokyo, Japan). Semiquantitative analysis and mean fluorescence intensity were determined using the Image-Pro Plus software. Primary antibodies were mouse anti-ionized calcium-binding adapter molecule 1 (Iba-1) (1 : 200, ab283319, Abcam, UK), rabbit anti-GSDMD (1 : 200, PA5-116815, Thermo Fisher, USA), and rabbit anti-NLRP3 (1 : 200, ab263899, Abcam, UK). Secondary antibodies were goat anti-mouse IgG (1 : 200, ab150113, Abcam, USA) and goat anti-rabbit IgG (1 : 200, ab150077, Abcam, UK). The apoptosis index was determined by counting Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells in the ventral horn region using fluorescence microscopy (Olympus, Japan). Data analysis was performed using Image-Pro Plus software.

Colon tissue immunohistochemistry was performed on paraffin sections mounted on positively charged glass slides. Sections were dewaxed in xylene and rehydrated through a graded alcohol series. Antigen retrieval was carried out in citrate buffer (pH 6.0) by boiling in a water bath for 15 min, then allowed to cool to room temperature without drying. Sections were rinsed three times with PBS for 3 min each to remove residual buffer. Endogenous peroxidase activity was blocked by incubating sections with 3% hydrogen peroxide at room temperature in the dark for 10 min. After blocking with serum for 1 h, sections were incubated overnight at 4°C with primary antibodies Zonula Occludens-1 (ZO-1) (1 : 1 000, Abcam, UK) and Occludin (1 : 1 000, Abcam, UK).

1.11 Western blot (WB)

Tissues from the spinal cord were lysed in a radioimmunoprecipitation assay (RIPA) buffer, and the crude lysates were centrifuged to collect the supernatants. Protein concentrations were determined by the bicinchoninic acid (BCA) method. Target proteins were separated by sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were incubated with the following primary antibodies: rabbit anti-NLRP3 (1 : 1 000, ab263899, Abcam, UK), rabbit anti-ASC (1 : 1 000, ab283684, Abcam, USA), rabbit anti-cleaved caspase-1 (1 : 1 000, ab207802, Abcam, USA), mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1 : 1 000, ab8245, Abcam, UK), and rabbit anti-GSDMD (1 : 1 000, PA5-116815, Thermo Fisher, USA). A goat anti-rabbit IgG secondary antibody (1 : 2 000, ab150077, Abcam, UK) was used. Bands were visualized with Western LightningTM Chemiluminescence Reagent (PerkinElmer, America) and an enhanced chemiluminescence (ECL) imaging system (Shanghai Qinxiang Scientific Instrument Co., Ltd., Shanghai, China). Western blot quantification was performed using Image-Pro Plus software.

1.12 Enzyme-linked immunosorbent assay (ELISA)

Spinal cord, colon tissue, and serum samples were placed in PBS and homogenized. Concentrations of IL-18) IL-1 β , IL-6, tumor necrosis factor- α (TNF- α), and lipopolysaccharide-binding protein (LBP) were quantified using ELISA kits (Meimian, Jiangsu, China).

1.13 Statistical analysis

All values are reported as mean $\pm$ standard error of the mean (SEM). For normally distributed data, differences between two groups were assessed by a two-tailed unpaired Student's *t* test. Differences among multiple groups were assessed by one-way or two-way analysis of variance. Non-normally distributed data were analyzed using the Mann-Whitney *U* test or the Kruskal-Wallis test with Dunn's post hoc analysis. *P*<0.05 was considered statistically significant.

2 Results

2.1 Electroacupuncture improves motor function and protects neurons in SCI rats

To evaluate the therapeutic efficacy of EA in SCI, we performed behavioral tests and histological examinations on three groups of rats. Compared with the Sham group, the SCI group showed lower BBB scores and reduced angles in the Rivlin inclined plate test, indicating significant motor impairment after SCI. Over time, BBB scores and the Rivlin inclined plate test improved in both SCI+EA and SCI groups. Notably, on Day 12 and 19 post-SCI, both measures were higher in the SCI+EA group than in the SCI

group ($P<0.05$) (Figure 3a, b). Spinal cord tissue from the Sham-operated group displayed normal morphology, whereas the SCI group exhibited severe structural damage, including progressive inflammatory cell infiltration and tissue cavitation (black arrows) (Figure 3d). EA treatment preserved spinal cord structural integrity. Nissl staining showed a marked decrease in surviving neurons in the ventral horn of the SCI group, which was alleviated by EA (Figure 3c). As indicated by the red arrow, neuronal

cell bodies became shrunken and deformed, with irregular contours and markedly reduced volume, and neuronal staining was markedly lighter after SCI (Figure 3e). TUNEL staining showed that the number of TUNEL-positive cells increased markedly after SCI. EA treatment reduced the number of regulated cell death compared with the SCI group ($P<0.05$ vs the SCI group) (Figure 3f). These findings indicate that EA exerted neuroprotective effects after SCI and promoted recovery of hindlimb motor function.

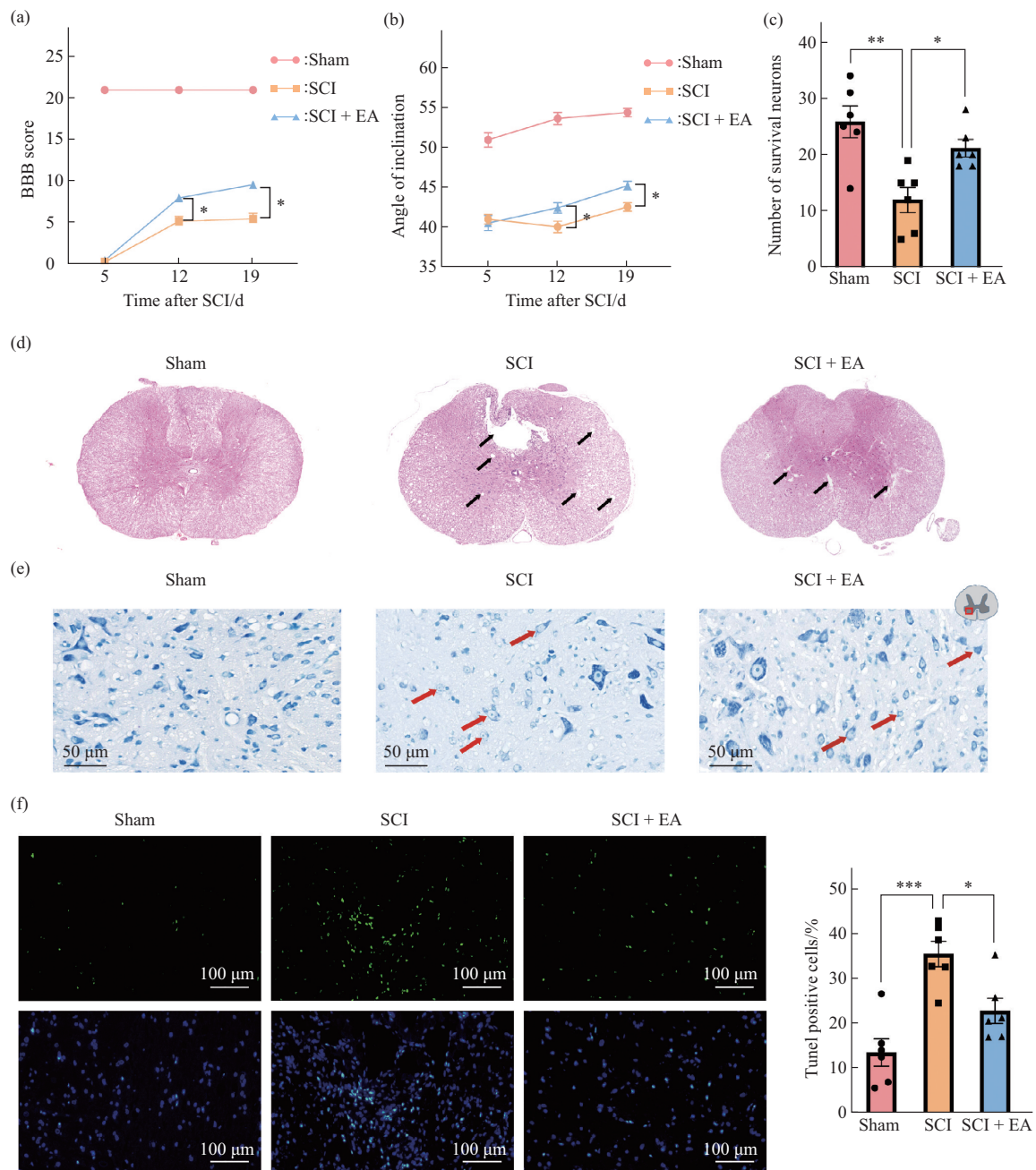


Fig. 3 Electroacupuncture improves motor function and protects neurons in SCI rats

(a, b) BBB scores and results of the Rivlin inclined-plate test at 5 d, 12 d, and 19 d post-SCI ($n=15$). (c) Quantitative assessment of Nissl staining in ventral motor neurons. (d) HE staining; black arrows indicate the tissue cavity. (e) Nissl staining. Representative images of the ventral horn at the lesion site. Red arrows indicate dead neurons. (f) TUNEL-positive cells in the ventral horn (green fluorescence), $n=6$. Data are expressed as mean $\pm$ SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

2.2 EA regulates differentially expressed genes (DEGs)

To delve deeper into the mechanism of EA-induced neuroprotection, we conducted transcriptome analysis using RNA sequencing in three groups (Sham, SCI, and SCI+EA). Our findings indicate that EA modulates numerous genes and signaling pathways. SCI led to the upregulation of 3 409 genes and the downregulation of 3 099 genes compared to the sham group. After EA treatment, 336 genes were

upregulated and 319 were downregulated (Figure 4a, b). Subsequent KEGG pathway enrichment analysis of these rescued genes highlighted that the most significantly enriched pathways in the SCI group were linked to immune inflammation, such as the NOD-like receptor signaling pathway and the Toll-like receptor signaling pathway (Figure 4c). Conversely, the SCI+EA group showed predominant enrichment in pathways associated with intestinal inflammation and autoimmunity, offering valuable insights for further

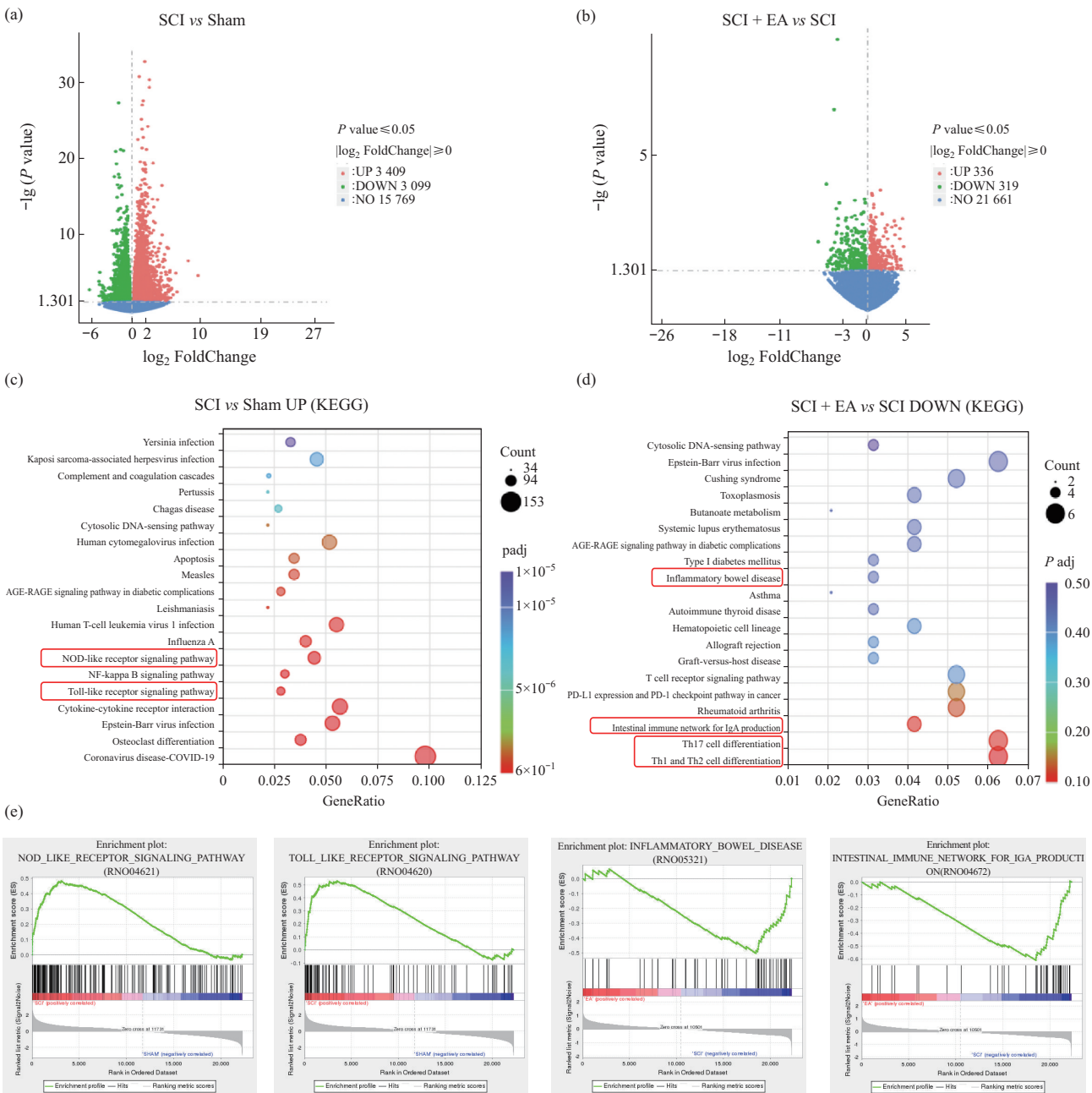


Fig. 4 EA regulates differentially expressed genes (DEGs) and intestinal immune-related signaling pathways (a, b) Statistics on the number of DEGs across various comparison combinations are presented. (c) The top significantly enriched KEGG signaling pathways that are upregulated following SCI are shown. (d) The most significant KEGG pathway rescued by EA treatment is highlighted. (e) GSEA analysis reveals the gene categories enriched with upregulated or downregulated genes in the SCI and SCI+EA groups. $n=5$ in each group. The red boxes represent shared enriched immune-related biological processes and pathways.

exploration (Figure 4d). These findings were corroborated by GSEA analysis (Figure 4e). Based on these findings, we hypothesize that EA could enhance neurological function post-SCI through pathways involving intestinal immune inflammation. Furthermore, we observed that EA improved intestinal function in SCI rats. Specifically, compared to the Sham group, the SCI+EA group exhibited an increased fecal water content and restored normal small intestinal transit rates (Figure S1a, b). Additionally, EA enhanced the expression of intestinal barrier proteins ZO-1 and Occludin (Figure S1c, d).

2.3 EA regulates the diversity and structure of the gut microbiota

The interaction between the gut microbiota and gut-mediated immunity influences the central nervous system. We conducted 16S rDNA sequencing on fecal samples from SCI rats to evaluate EA's potential to modulate the gut microbiota. The ACE and Chao1 indices, representing α diversity, were significantly higher in the SCI group ($P < 0.05$). However, EA treatment resulted in a non-significant decrease in both indices (Figure 5a, b). Principal coordinate analysis (PCoA) was utilized to assess β diversity,

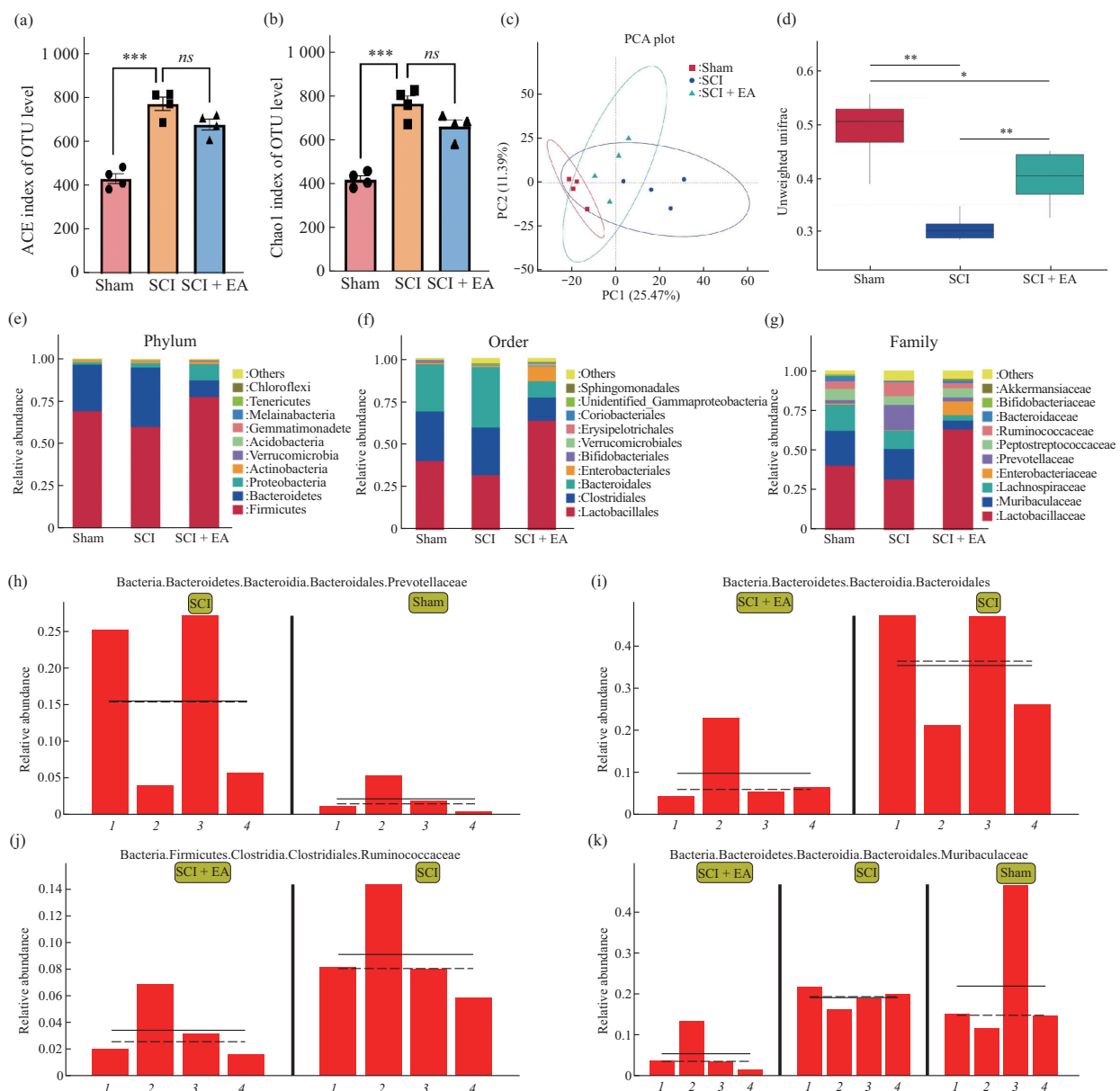


Fig. 5 EA regulates the diversity and structure of the gut microbiota

(a) ACE. (b) Chao1. (c) Principal coordinate analysis (PCoA). (d) Unweighted Tukey HSD of beta diversity. (e) Phylum-level representation of intestinal microbiota depicted in bar charts. (f) Order level. (g) Family level. (h–k) LefSe analysis was conducted to examine the bacterial community and identify the differences among groups. The complete taxonomic hierarchy of bacteria is ordered as domain, phylum, class, order and family. The data are expressed as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant, $n = 4$ in each group.

revealing distinct clustering patterns between groups (Figure 5c, d). Stacked column charts illustrating species distribution depicted the composition of gut microbiota at the phylum, order, and family levels (Figure 5e–g). Key microbial community differences between the groups were identified using LEfSe. The LEfSe analysis revealed significant differences in Prevotellaceae bacteria between the Sham and SCI groups, distinct *Bacteroidales* and Ruminococcaceae bacteria communities between the SCI and SCI+EA groups, and notable variations in Muribaculaceae among all three groups (Figure 5h–k). It was observed that at the phylum level, the Firmicutes/Bacteroidetes ratio decreased in the SCI group compared to the Sham group, but increased after EA intervention (Figure 6a). At the order level, EA resulted in the downregulation of Bacteroidales abundance and the upregulation of Lactobacillales abundance compared to the SCI group (Figure 6b, c). Furthermore, at the genus level, EA upregulated *Lactobacillus* and *Akkermansia* while downregulating the abundance of *Alloprevotella*, *Dorea*, *Clostridiales* and *Blautia* compared to the SCI group (Figure 6d–i). *Alloprevotella* and *Dorea* bacteria are closely associated with intestinal inflammatory responses. EA has been shown to significantly enhance the presence of beneficial bacteria such as *Lactobacillus* and *Akkermansia*, which are known to produce health-promoting short-chain fatty acids like butyric acid and propionic acid^[36–37]. These findings indicate that the gut microbiota could play a crucial role in mediating the positive effects of EA as a primary therapeutic target. Furthermore, utilizing Tax4Fun to predict microbiota functions revealed that in the SCI group, pathways including pyruvate metabolism, glycolysis/gluconeogenesis, and quorum sensing were downregulated, whereas EA led to an increase in the enrichment of these pathways (Figure 6j). An integrated analysis was conducted to investigate the relationships between gut microbiota and host gene expression by examining differentially expressed genes from transcriptomic data alongside microbial abundance at the genus level. The findings were presented using a correlation network diagram and a heatmap. The network diagram revealed a positive correlation between the Pycard gene (ASC protein) and *Bacteroides* and *Blautia*, while showing a negative correlation with *Lactobacillus*, *Romboutsia*, and *Enterobacteriaceae* (Figure S2a). The gene heatmap illustrated a positive correlation of Pycard (ASC protein) with *Alloprevotella*, *Lysobacter*, *Microvirga*, and *Nonomurda* (Figure S2b).

2.4 EA attenuates NLRP3-mediated pyroptosis and neuroinflammation

The ASC protein, derived from the *Pycard* gene, serves as a fundamental element of the NLRP3 inflammasome. We assessed whether EA could impede NLRP3-dependent pyroptosis and neuroinflammation by evaluating the expression of crucial pyroptosis-related proteins and inflammatory mediators. Protein levels of NLRP3, ASC, cleaved-caspase-1, and GSDMD were elevated in the SCI group compared with the Sham group (Figure 7a–d), but EA treatment reversed these elevations. Immunofluorescence analysis revealed reduced levels of NLRP3/GSDMD and GSDMD/Iba-1 in the SCI+EA group in comparison to the SCI group (Figure 7i–l). Additionally, ELISA results (Figure 7e–h) showed that EA treatment led to decreased concentrations of proinflammatory cytokines, such as IL-1 β , IL-18, TNF- α , and IL-6 post-SCI ($P<0.05$).

2.5 Antibiotic-induced gut dysbiosis reduces intestinal barrier protein expression and impairs neurological recovery in rats with SCI

To investigate the specific impact of microbiota changes on intestinal and spinal cord neural functions post-SCI, we induced gut dysbiosis with antibiotics. By Day 19 post-injury, the SCI+AB group showed reduced expression levels of intestinal barrier proteins ZO-1 and occludin compared to the SCI group (Figure 8a, b). Particularly, there was a significant decrease in occludin protein expression ($P<0.05$; Figure 8b). Remarkably, serum LBP levels were notably elevated in the SCI+AB group rats ($P<0.05$; Figure 8e), indicating that gut dysbiosis accelerates intestinal barrier breakdown in SCI rats, leading to increased leakage of microbial metabolite lipopolysaccharide (LPS). Regarding neurological function, the SCI group exhibited significantly higher BBB scores and inclination angles than the SCI+AB group ($P<0.05$; Figure 8c, d). Histopathological examination using HE and Nissl staining (Figure 8f) revealed larger cavitory areas (black arrows) and a significant decrease in the number of surviving neurons (red arrows) in the SCI+AB group compared to the SCI group (Figure 8g, $P<0.05$). These findings collectively indicate that antibiotic-induced disruption of gut microbiota worsens gut permeability and hinders motor function recovery post-SCI.

2.6 Fecal microbiota transplantation following EA enhances neurological recovery, liver and spleen index and general condition after SCI

To further validate the role of gut microbiota in mediating the neuroprotective effects of EA, we

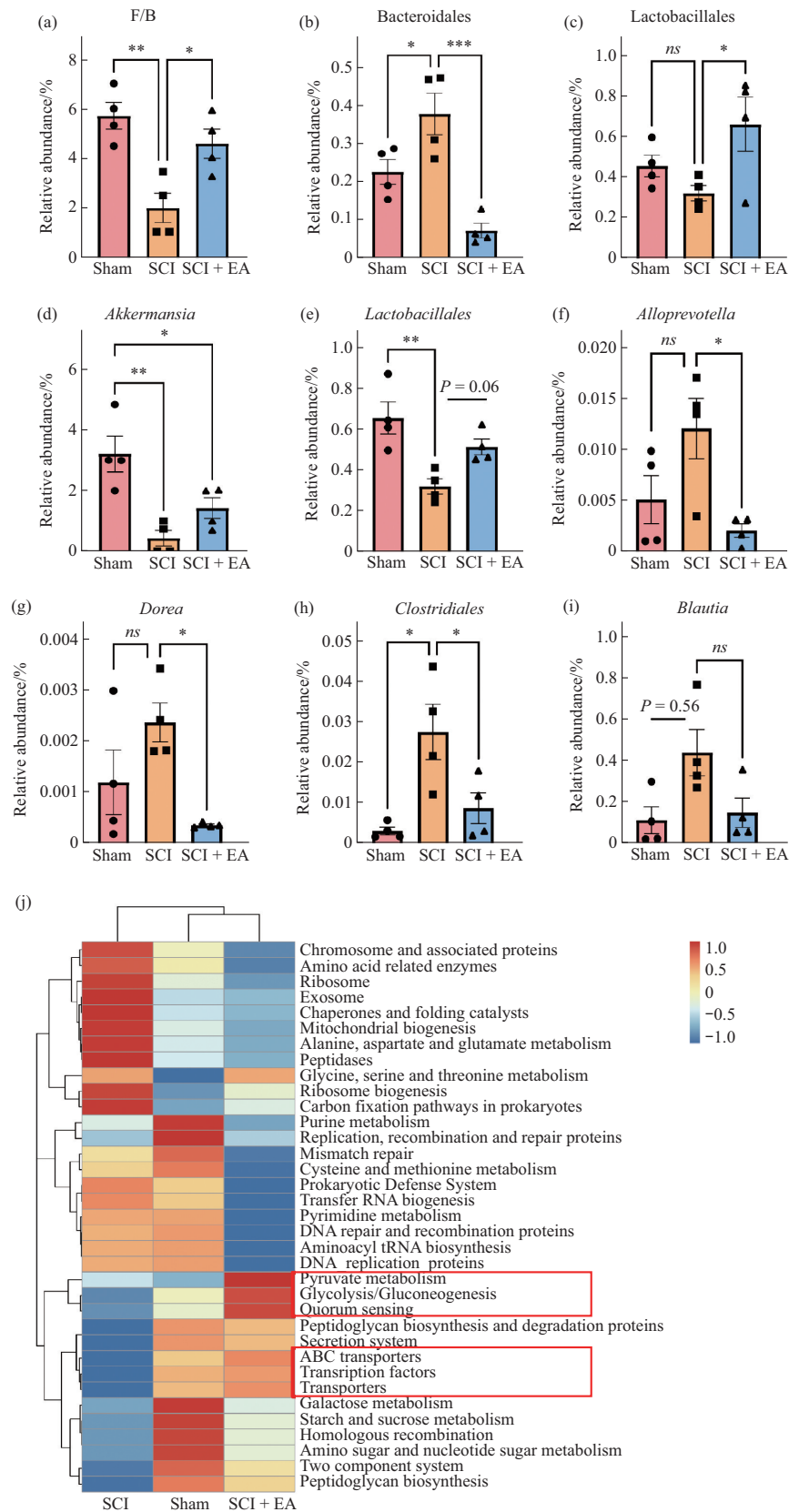


Fig. 6 EA influences the representation of specific differential bacterial taxa and modulates Tax4Fun-predicted functional KEGG pathways

(a) The ratio of Firmicutes to Bacteroidetes (F/B) at the phylum level. (b, c) The relative abundance of Bacteroidales and Lactobacillales at the order level. (d-i) The relative abundance of gut microbiota at the genus level, including *Akkermansia*, *Lactobacillus*, *Alloprevotella*, *Dorea*, Clostridiales and *Blautia*. (j) Changes in the KEGG pathways among the three groups. The data are expressed as mean±SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant, $n = 4$ in each group. The red box represents the pathways and molecules that are enhanced by EA stimulation.

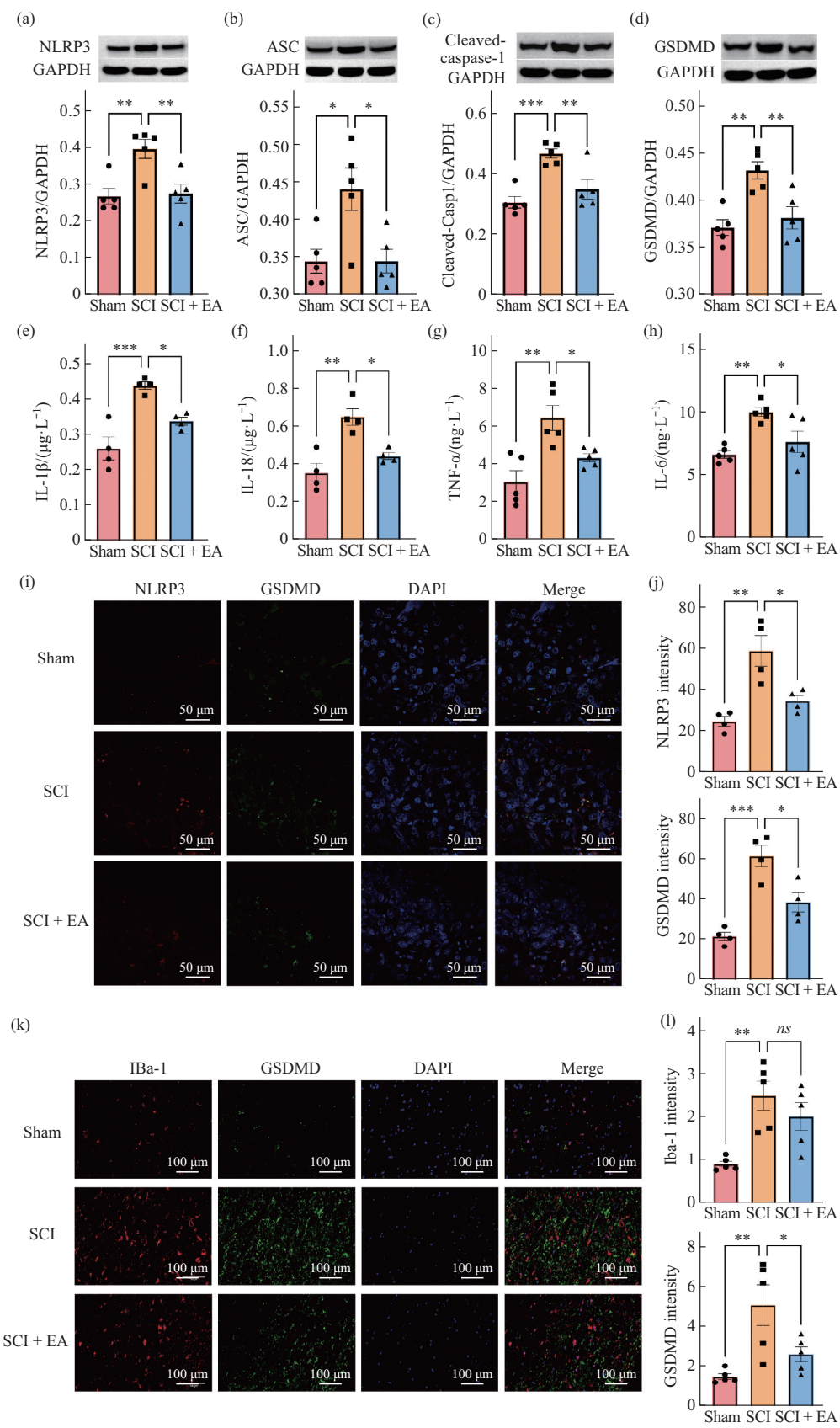


Fig. 7 EA attenuates NLRP3-mediated pyroptosis and neuroinflammation

(a–d) Western blot analysis quantifies the expression of NLRP3, ASC, cleaved-caspase-1, and GSDMD. (e–h) ELISA was utilized to determine the protein levels of IL-1β, IL-18, TNF-α, and IL-6. (i, j) Immunofluorescence staining depicted NLRP3 (red) and GSDMD (green) along with the average fluorescence intensity of NLRP3/GSDMD. (k, l) Immunofluorescence staining of GSDMD (green)/Iba-1 (red) and the average fluorescence intensity. The data are presented as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *ns*, not significant.

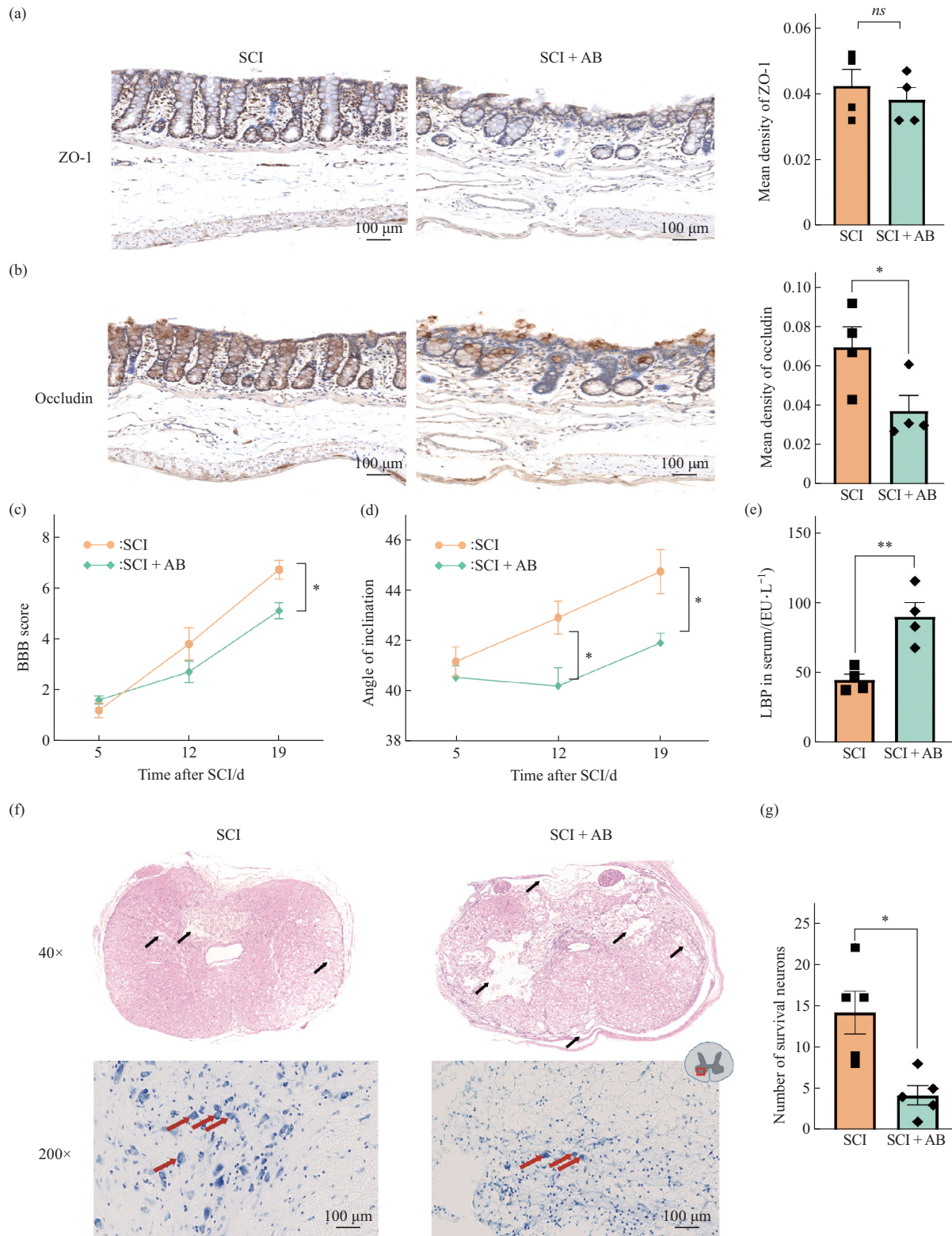


Fig. 8 Antibiotic-induced gut dysbiosis reduces intestinal barrier protein expression and impairs neurological recovery in rats with SCI

(a, b) Representative images of ZO-1, occludin and statistical analysis for the protein expression in the colon using immunohistochemical methods. ($n=4/\text{group}$). (c) BBB scores at 5 d, 12 d, and 19 d post-SCI; $n=10$ per group. (d) Rivlin inclined-plate test results of the two groups at 5, 12, and 19 d after SCI. (e) Serum levels of LBP were detected using ELISA assay kits ($n=4/\text{group}$). (f) Representative images of Nissl-stained sections, and HE-stained transverse sections at magnifications of $\times 40$ and $\times 200$, $n=5$ in each group. (g) Quantitative analysis of Nissl staining in the ventral horn of the spinal cord. The data are presented as the mean $\pm$ SEM. * $P<0.05$, ** $P<0.01$, ns, not significant.

conducted FMT experiments. Rats in the FMT(SCI)-EA group showed improved performance in the Rivlin inclined plate test compared to those in the FMT(SCI)-SCI group, with significant differences at 33 and 40 d post-FMT (Figure 9b, $P<0.05$). The BBB scores of the FMT(EA)-SCI group demonstrated an increasing trend compared to the FMT(SCI)-SCI group, although the difference was not significant (Figure 9a, $P>0.05$). This discrepancy could be attributed to transient adverse effects induced by the FMT procedure itself,

potentially affecting the specific behavioral assessments results. At 40 d post-SCI, rat spleens and livers were collected. The FMT(SCI)-SCI group exhibited elevated liver indices compared with the sham group (Figure 9d). Treatment with FMT(EA)-SCI led to a decrease in spleen indices (Figure 9c). Both FMT groups experienced a reduction in body weight and food intake during the FMT procedure (Figure 9e, f). These findings suggest that FMT after SCI may lead to specific adverse effects that require

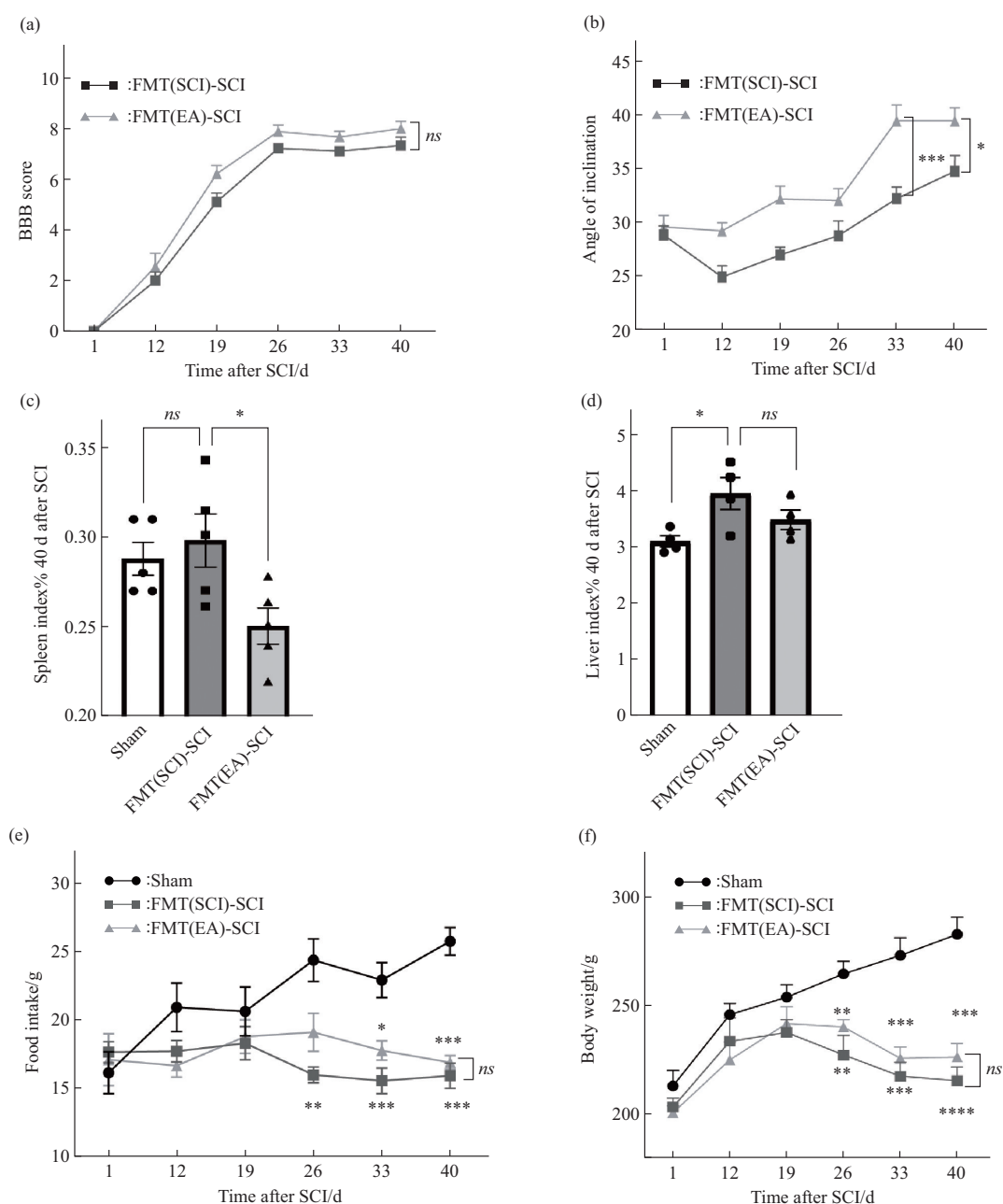


Fig. 9 Fecal microbiota transplantation following EA enhances neurological recovery, liver and spleen index and general condition after SCI

(a, b) The BBB score and Rivlin inclined-plate test results of the two groups were assessed at 1, 12, 19, 26, 33, and 40 d post-SCI, with $n=9$ per group. (c, d) Spleen and liver indices were measured at 40 d post-SCI, with $n=5$ per group. (e, f) Food intake and body weight of the rats were recorded on 1, 12, 19, 26, 33, and 40 d post-SCI. The data are presented as the mean $\pm$ SEM, with $n=9$ per group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. (*vs Sham in figure e, f).

careful consideration. Nonetheless, FMT from EA-treated donors seems to mitigate these side effects and enhance the restoration of motor function post-SCI. These outcomes additionally reinforce the role of the gut microbiota in modulating the neuroprotective benefits of EA.

2.7 Fecal microbiota transplantation following EA treatment alleviates TLR4/MyD88/NF- κ B signaling pathway and NLRP3-dependent pyroptosis in SCI rats

To further investigate whether EA-mediated modulation of the gut microbiota inhibits the TLR4/MyD88/NF- κ B signaling pathway and NLRP3-dependent pyroptosis, spinal cord tissues were harvested from two groups of SCI rats that had undergone FMT for analysis using western blot and ELISA. The protein levels of TLR4, MyD88, NF- κ B, p-NF- κ B, NLRP3, ASC, cleaved-caspase-1, GSDMD, IL-1 β , and IL-18 were significantly lower in the FMT (EA) -SCI group compared to the FMT(SCI) -SCI group ($P < 0.05$), as shown in Figure 10. These results indicate that FMT from EA-treated donors suppresses the TLR4/MyD88/NF- κ B signaling pathway and NLRP3-mediated pyroptosis following SCI.

2.8 Fecal microbiota transplantation post EA treatment decreased IL-6, TNF- α , and LBP levels in the blood, intestinal tissue, and spinal cord of SCI rats

Pro-inflammatory microbial byproducts, such as LPS and related cytokines, can breach the compromised intestinal barrier, triggering systemic inflammatory responses. LBP, known for its stability and detectability, serves as a reliable indicator of LPS levels. Through ELISA analysis, we quantified inflammatory cytokines and LBP concentrations in colon, serum, and spinal cord samples (Figure 11). Relative to the FMT(SCI)-SCI group, the FMT(EA)-SCI group exhibited significantly reduced LBP, TNF- α , and IL-6 levels in the colon, serum, and spinal cord. These findings suggest that FMT from EA-treated donors mitigates the excessive release of inflammatory cytokines and restricts LPS translocation into systemic circulation and spinal cord tissue.

3 Discussion

Accumulating evidence confirms that acupuncture affects the gut-brain axis to improve neurological disorders such as AD and PD^[38-39]. However, it is unclear whether EA enhances neurological recovery after SCI by regulating the gut

microbiota. Our study found that inducing gut dysbiosis with antibiotics before SCI disrupts the intestinal barrier, and worsens motor deficits and neuroinflammation. Importantly, EA treatment alters the composition of gut microbiota, reduces systemic concentrations of LBP, and decreases inflammatory responses. This leads to the inhibition of the TLR4/MyD88-NF- κ B signaling pathway and NLRP3-mediated pyroptosis in spinal cord tissues, promoting neural repair. These results highlight the modulation of gut microbiota as one of the key mechanism contributing to the therapeutic effects of EA in SCI.

To elucidate the regulatory mechanisms underlying EA efficacy, we first performed transcriptome sequencing. The analysis showed that SCI primarily activated immune-and inflammation-related pathways, including NOD-like receptor signaling, NF- κ B signaling, and Toll-like receptor transduction. By contrast, EA modulated the intestinal immune network, suppressed the differentiation of T helper 17 (Th17), T helper 1 (Th1), and T helper 2 (Th2) cells, and reduced proinflammatory cytokine production. Given EA's established ability to regulate the gut-brain axis, we propose that the microbiota mediate EA's attenuation of neuroinflammatory responses after SCI. Furthermore, we validated the sequencing results. The NLRP3 inflammasome, which is activated after SCI and is recognized as a critical contributor to secondary injury^[2, 40]. Consistent with previous work^[41], we also observed that EA inhibited the activation of the NLRP3 inflammasome and pyroptosis after SCI. These findings indicate an important role for EA in regulating neuroimmune inflammatory responses, although the specific mechanism by which it modulates pyroptosis remains unclear.

Recent clinical studies have confirmed that SCI induces intestinal dysfunction and gut dysbiosis^[14, 42]. Echoing the observations of Kigerl *et al.*^[43] and Jing *et al.*^[44], our data showed marked increases in gut bacterial abundance and a pronounced shift in microbial composition in SCI rats relative to sham controls. Although EA did not produce a significant change in α diversity, it substantially reshaped the overall structure of the intestinal microbiota. Specifically, 16S rRNA analysis indicated that EA restored the Firmicutes-to-Bacteroidetes ratio in SCI rats and increased the relative abundances of *Lactobacillus* and *Akkermansia*. Within the gut-brain axis framework, short-chain fatty acids (SCFAs) - metabolic products of the gut microbiota-mediate bidirectional communication between the gut and the

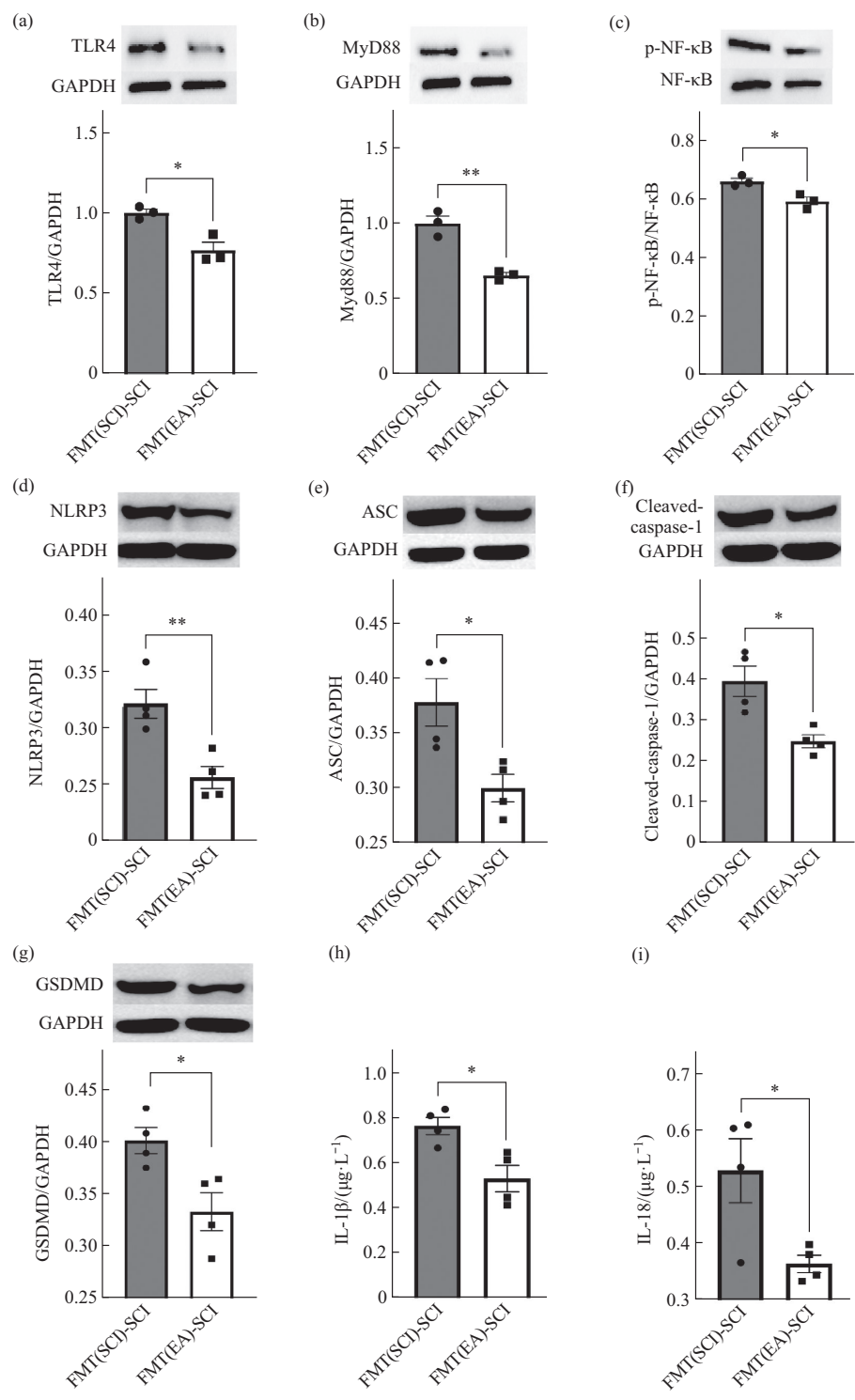


Fig. 10 Electroacupuncture fecal transplantation alleviated TLR4/MyD88/NF-κB signaling pathway and NLRP3-dependent pyroptosis in SCI rats

(a–g) Western blot analysis quantified TLR4, Myd88, p-NF-κB/NF-κB, NLRP3, ASC, cleaved-caspase-1, and GSDMD. (h, i) ELISA measured the protein concentrations of IL-1β and IL-18. The data are expressed as mean±SEM. **P*<0.05, ***P*<0.01.

brain through multiple mechanisms^[45]. Several studies have reported that probiotic populations elevate SCFAs concentrations, attenuate neuroinflammation, and ameliorate depressive-like behaviors^[46–47]. For example, Xu *et al.*^[48] demonstrated that supplementation with *Akkermansia muciniphila* increased butyrate levels in the intestine and brain, and that exogenous butyrate administration reduced

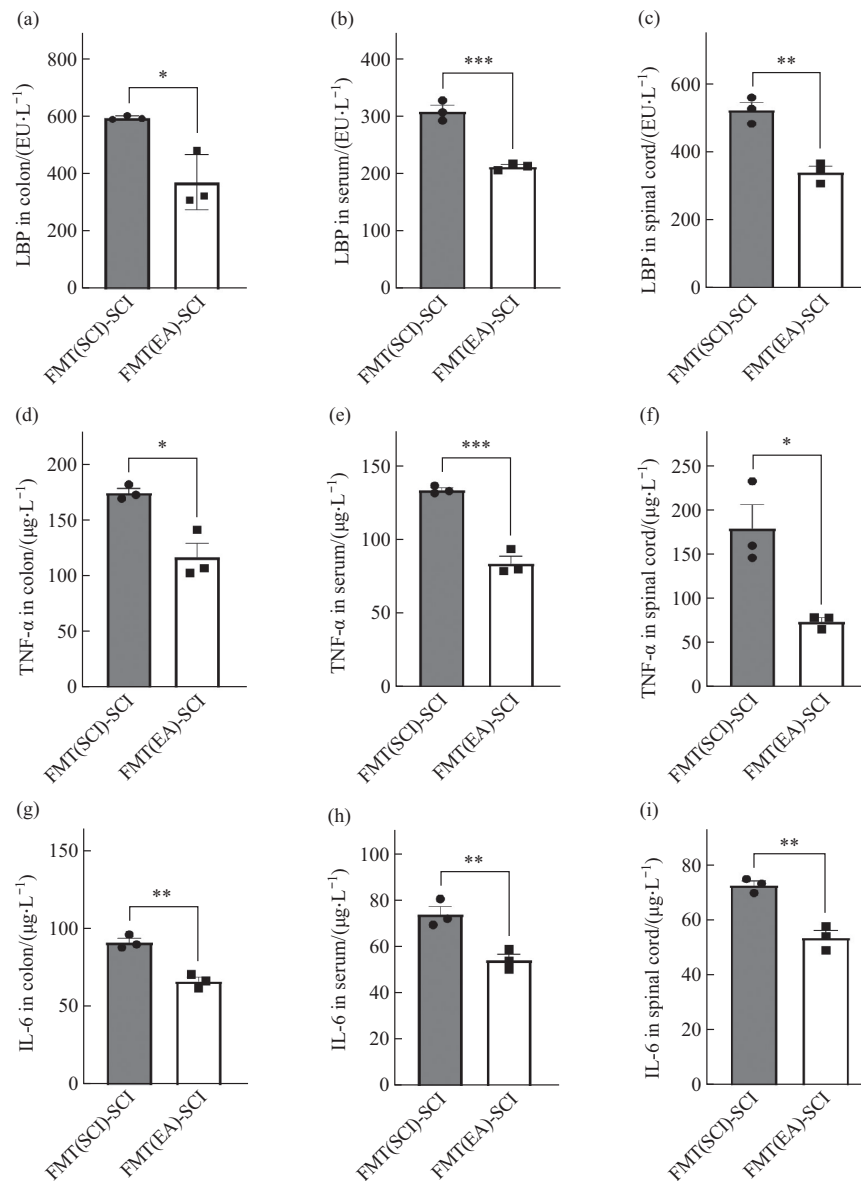


Fig. 11 Fecal microbiota transplantation post EA treatment decreased IL-6, TNF- α , and LBP levels in the blood, intestinal tissue, and spinal cord of SCI rats

(a–c) LBP levels were measured in the colon, serum, and spinal cord tissues. (d–f) TNF- α levels were assessed in the colon, serum, and spinal cord tissues. (g–i) IL-6 levels were evaluated in the colon, serum, and spinal cord tissues. The data are expressed as the mean $\pm$ SEM. * P <0.05, ** P <0.01.

dopaminergic neuron injury by inhibiting microglial activation and the production of neuroinflammatory mediators. Similar studies have shown that dietary supplementation with the microbial metabolite butyrate improves cognitive function in chronic alcohol-induced central nervous system injury by modulating the gut-brain axis; this effect may involve butyrate regulation of the G protein-coupled receptor 109A (GPR109A)/peroxisome proliferator-activated receptor gamma (PPAR- γ)/TLR4-NF- κ B signaling pathway to suppress microglia-mediated neuroinflammation^[49]. Currently, research has found

that EA can modulate intestinal microbial metabolites, including butyrate, to relieve constipation^[27]. In treatments for central nervous system disorders, EA reduced post-stroke spasticity by attenuating inflammatory responses, inhibiting the NF- κ B/NLRP3 signaling pathway, and altering the gut-brain axis through increased intestinal levels of n-propyl acetate and n-propyl butyrate^[50].

Guo *et al.*^[51] clarified that such microbial imbalance accelerates senescence of intestinal mucosal immune cells (*e.g.*, macrophages and T lymphocytes), downregulates tight junction proteins

(*e.g.*, occludin and claudin-1), and compromises mucosal immune defenses, thereby allowing intestinal bacteria, metabolites, and inflammatory mediators to translocate to peripheral sites. In our study, we disrupted the gut microbiota with antibiotics and found that intestinal barrier injury in rats with SCI was more severe. Levels of LBP in the serum increased, structural and morphological damage in the spinal cord were more pronounced, and the animals' motor function declined significantly. These findings indicate that dysbiosis exacerbates the leakage of harmful microbial metabolites into the central nervous system. As a major component of Gram-negative bacterial cell walls, LPS activates the NLRP3 inflammasome either via noncanonical pathways^[52] or through TLR-NF- κ B signaling cascades, thereby amplifying neuroinflammatory responses. Notably, Rong *et al.*^[20] reported that gut dysbiosis exacerbates post-SCI neuroinflammation by initiating TLR4-MyD88-dependent neuronal apoptosis. Among the dysbiotic taxa, *Alloprevotella* (a genus in Bacteroidetes) and *Bacteroides* (for example, *B. fragilis*) constitute the principal LPS-producing populations, and their increased abundance strongly correlates with elevated systemic LPS levels^[53]. Functional prediction of our gut microbiota indicated that EA enriched pathways for ABC transporters and quorum sensing. Gut microbes regulate LPS production and release *via* quorum-sensing molecules (*e.g.*, Autoinducer-2), thereby modulating the intensity of TLR4 signaling^[54]. ATP-binding cassette(ABC) transporters (*e.g.*, ATP-binding cassette subfamily A member 7 (ABCA7)) move lipids across membranes, influence amyloid-beta (A β) peptide processing and clearance, inhibit NLRP3 inflammasome activation in microglia, and thereby regulate microglial and T cell functions to alleviate neuroinflammation in AD. EA treatment significantly reduced the abundance of these pathogenic bacteria, matching the observed decreases in LBP levels in gut, blood, and spinal cord tissues^[55]. This remodeling of the microbiota likely contributed to the suppression of systemic and neuroinflammatory responses and ultimately attenuated NLRP3-dependent pyroptosis in spinal microglia.

Previous studies have found that interventions to restore the microbiota-including probiotic supplementation^[43], and FMT from healthy donors into SCI models show therapeutic potential for nerve repair^[21, 56]. Our experiment showed the therapeutic advantages of EA FMT in improving some motor functions and reducing systemic immune-inflammatory responses. Specifically, FMT with EA treatment downregulated the liver and spleen indices,

inhibited the expression of TLR4/MyD88-NF- κ B NLRP3 pyroptosis-related proteins, and improved the performance in the Rivlin inclined-plate test, but failed to improve the BBB motor score. This behavioral difference may stem from the following reasons. (1) The BBB score relies on the subjective judgment of observers and is influenced by factors such as the individual activity status of rats. For rats with chronic SCI, habitual upper limb support, reduced hindlimb activity, or even immobility may occur, which could interfere with observers' accurate assessment of fine motor function in the hindlimbs and mask potential subtle intergroup differences. In contrast, Rivlin inclined-plate test uses the maximum tilt angle at which rats can maintain balance as a quantitative indicator, yielding relatively objective and reproducible results. (2) The FMT procedure itself can induce transient adverse reactions, including weight loss and systemic inflammatory and immune responses, which may confound outcome assessment. Clinical data indicate that 5.8% of FMT recipients experience mild adverse events, with diarrhea and abdominal pain occurring in 10% and 7% of cases, respectively^[57]. Although FMT has therapeutic potential, it has been associated with a high incidence (37%) of severe (grade 3–4) immune-related adverse events, particularly colitis and hepatitis^[58–59]. In our study, rats that underwent FMT exhibited decreased body weight and food intake and increased liver and spleen indices. Such complications may have impacted motor-function testing in SCI rats, producing inconsistencies between the BBB and inclined-plane outcomes. (3) We propose that the BBB score primarily assesses fine motor function, including the range of motion of hindlimb joints, gait coordination, and toe placement and grasping. Its most sensitive targets are corticospinal tract regeneration, reconstruction of motor neuron circuits, and the efficiency of nerve signal conduction^[60–61]. By contrast, Rivlin *et al.*^[62] inclined-plate test predominantly evaluates trunk core muscle strength, limb muscle tone, grasping ability, postural balance, and resistance to fatigue; it is therefore especially sensitive to the functional state of skeletal muscles, trunk stability, and posture-regulating pathways mediated by spinal interneurons. We hypothesize that EA's modulation of the gut microbiota acts on the local inflammatory microenvironment of peripheral skeletal muscle and the spinal cord through metabolites (*e.g.*, SCFAs), anti-inflammatory signaling, and immunomodulatory effects. These actions improve skeletal muscle atrophy, increase muscle strength, and normalize muscle tone, thereby

enhancing trunk stability and limb grasping in rats. Nonetheless, this intervention may exert a comparatively modest effect on corticospinal axon regeneration and on the reconstruction of fine motor neuron circuits. In subsequent studies, we plan to perform gene sequencing of spinal cord tissues from the two FMT groups to further test the hypotheses described above. We will also extend the observation period after FMT and adopt multimodal motor-function assessment methods to more thoroughly evaluate the therapeutic effects of EA-FMT.

EA directly affected intestinal transit in SCI rats and promoted colonic expression of the tight-junction proteins ZO-1 and occludin (Figure S2). This effect may arise from EA acting on the vagus and/or sympathetic nerves *via* acupoint stimulation. By modulating neuromodulatory pathways, EA likely stabilized microbial community structure and rebalanced pro-and anti-inflammatory bacterial populations through effects on interactions between the intestinal immune network and the microbiota. Studies indicate that signals from acupuncture at somatic acupoints travel to brainstem nuclei *via* vagal afferent fibers and, after central integration, regulate gastrointestinal motility, the intestinal mucosal barrier, and microbiota composition *via* vagal efferent fibers; concurrently, they activate the cholinergic anti-inflammatory pathway, suppress peripheral and central inflammation, and thereby reduce inflammatory damage to nerves and the intestine^[63]. In central nervous system disorders, electroacupuncture altered the gut microbiota to improve social behavior in autistic mice, and this efficacy depended on an intact vagus nerve and its regulation of the gut-brain axis^[64]. Beyond the vagus nerve, the sympathetic nervous system is equally important: EA-induced hypothalamic activation of sympathetic outflow modified the local intestinal microenvironment through neurotransmitter release, which in turn reshaped the gut microbiota structure^[65-66]. From the standpoint of modern anatomy, sympathetic preganglionic neurons that innervate the colon reside in the intermediolateral column of the thoracolumbar spinal cord. After synapsing in the superior and inferior mesenteric ganglia, these neurons extensively innervate tissues and cells across all colonic layers. The Jia-Ji acupoints lie within the distribution of the sympathetic trunk, whereas the Dazhui acupoint lies anatomically adjacent to the main trunk and branches of the cervical vagus nerve and is among the most commonly used points in acupuncture to stimulate vagal fibers. EA at these acupoints can effectively

stimulate the sympathetic and/or vagus nerves, improve the local intestinal immune microenvironment, and alter the abundance and structure of the gut microbiota.

4 Conclusion

Our results demonstrated that EA improved motor function and preserved spinal cord tissue morphology after SCI. EA treatment increased the abundance of *Lactobacillus* and *Akkermansia* while decreasing the levels of *Dorea* and *Alloprevotella*. Administration of antibiotics prior to SCI impeded recovery of spinal nerve function. FMT from EA-treated mice into SCI rats attenuated these adverse effects and suppressed TLR4/MyD88/NF- κ B signaling as well as NLRP3-dependent pyroptosis. Together, these findings indicate that EA exerts neuroprotective effects by modulating the gut microbiota. The study clarified molecular mechanisms underlying electroacupuncture intervention in SCI and established a theoretical basis for its clinical application and future research.

Supplementary Available online (<http://www.pibb.ac.cn>, <http://www.cnki.net>):
PIBB_20260020_Figure_S1.pdf
PIBB_20260020_Figure_S2.pdf

Data availability The original contributions presented in the study are included in the article/supplementary material, and further inquiries can be directed to the corresponding authors.

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电针通过调节肠道菌群缓解NLRP3介导的 脊髓损伤大鼠的细胞焦亡*

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摘要 目的 明确肠道菌群是否介导电针对脊髓组织小胶质细胞核苷酸结合结构域富含亮氨酸重复序列和含热蛋白结构域受体3 (NLRP3) 依赖性细胞焦亡的抑制调控进而实现神经保护作用。**方法** 首先采用脊髓组织RNA转录组检测明确电针对脊髓损伤组织基因表达及关键信号通路影响; 其次运用组织学与分子生物学技术验证电针神经保护作用与关键通路; 随后采用16S rDNA测序观察脊髓损伤 (SCI) 大鼠肠道菌群多样性及组成变化; 最后采用抗生素与粪菌移植技术阐明电针调节肠道菌群抗焦亡的具体机制。**结果** 电针可提升SCI大鼠运动功能, 改善脊髓组织形态, 减轻神经元调节性死亡。转录组数据显示, SCI后免疫炎症相关信号通路被激活, 而电针主要调控肠道炎症及自身免疫相关分子与通路。16S rDNA测序表明, 电针可重塑SCI大鼠肠道菌群结构, 如提升乳杆菌属、阿克曼氏菌属丰度, 逆转厚壁菌门拟杆菌门比例失衡。抗生素耗竭肠道菌群破坏肠道屏障蛋白 (ZO-1 和闭合蛋白) 的表达, 升高血清脂多糖结合蛋白 (LBP) 水平, 并加重脊髓损伤运动功能障碍。电针供体粪菌移植可降低肠道、血液、脊髓组织LBP含量, 并且抑制TLR4-Myd88-NF- κ B通路从而缓解NLRP3依赖细胞焦亡, 促进神经功能恢复。**结论** 电针通过调节肠道菌群抑制NLRP3依赖的细胞焦亡通路缓解SCI大鼠神经炎症反应。

关键词 电针, 脊髓损伤, 粪菌移植, 肠道菌群, 细胞焦亡, NLRP3炎症小体

中图分类号 R245.97, R651.2, Q939.9

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