• Basic Research •

Impaired corneal nerve repair and regeneration after injury in mouse sleep deprivation model

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Abstract

● **AIM:** To investigate the impact of sleep deprivation (SD) on corneal nerve repair and regeneration following injury and to elucidate the underlying molecular mechanisms.

● **METHODS:** Female C57BL/6 mice, aged 10-12wk, were allocated to either the SD group or the normal control (NC) group based on sleep duration. A corneal injury model was established to evaluate the rate of corneal epithelial healing and the dynamics of wound repair. Immunofluorescence staining assessed changes in corneal basal nerve fiber density. Corneal sensitivity was measured using the Cochet-Bonnet esthesiometer. A quantitative polymerase chain reaction was conducted to determine changes in the expression of nerve growth factor (NGF) in mouse corneal tissues. Furthermore, RNA-sequencing was performed for in-depth analysis of the corneal transcriptome following epithelial injury.

● **RESULTS:** On day 6 post-injury, the SD group exhibited significantly lower central and peripheral corneal nerve fiber density compared with the NC group. Corneal sensitivity in the SD group was substantially lower than in the NC group. NGF expression in the cornea was significantly lower in the SD group compared with the NC group. Additionally, the corneal transcriptome of the SD group exhibited significant alterations compared to the NC group, notably a downregulation of nerve-related gene expression following SD treatment. Pathway analysis further revealed that SD inhibited multiple pathways associated with nerve repair.

● **CONCLUSION:** These findings demonstrate that SD significantly delays repair and regeneration following corneal nerve injury, providing novel theoretical perspectives into its detrimental effect on ocular health.

● **KEYWORDS:** cornea; nerves; regeneration; sleep deprivation

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INTRODUCTION

The cornea, the outermost transparent layer of the eye, is one of the most densely innervated tissues in the body[1-2]. Corneal nerves primarily originate from the trigeminal nerve and are responsible for sensory conduction. Additionally, a limited number of sympathetic nerve fibers originate from the superior cervical ganglion[3]. These nerve fibers are essential for maintaining corneal homeostasis, as they release various neurotrophic factors that provide nutritional support, regulate the function of the corneal epithelium and stroma, sustain physiological renewal, and promote wound healing[4]. Mechanical injury to the cornea frequently results in concurrent damage to corneal nerves, and such nerve injury often induces pathological alterations in the corneal epithelium and stroma[5-6].

Corneal nerve repair is a complex, multifactorial process involving cytokine secretion by corneal cells, epithelial differentiation, immune cell recruitment, and the release of growth factors within the subbasal nerve plexus. In addition to local cellular and inflammatory responses, corneal homeostasis is influenced by systemic physiological rhythms[7]. The ocular surface exhibits circadian variation in epithelial renewal, tear film dynamics, corneal thickness, and wound repair capacity, suggesting that coordinated sleep–circadian

regulation may contribute to the maintenance and restoration of corneal integrity[8-9]. Therefore, disruption of sleep and circadian homeostasis may interfere with the reparative microenvironment required for corneal epithelial healing and nerve regeneration.

Recently, human sleep patterns have substantially changed, marked by a widespread decline in total sleep duration. Chronic sleep deprivation (SD) leads to diverse physiological and psychological impairments, elevating the incidence of multiple disorders, including cardiovascular disease[10]. SD is a condition in which the body fails to obtain adequate sleep due to various factors, thereby disrupting psychological and physiological functions. Increasing evidence highlights a complex relationship between SD and sympathetic nervous system (SNS) activity. Insufficient sleep may promote sympathetic hyperactivity and dysregulation, as indicated by elevated plasma norepinephrine levels, reflecting increased systemic SNS tone[11]. Furthermore, SNS activation may trigger pro-inflammatory responses[12].

Previous research demonstrated that SD significantly impairs corneal epithelial wound healing by activating the SNS[13]. Although the link between corneal epithelial repair and nerve repair is well-established, the impact of SD on peripheral nerves, particularly corneal innervation, remains poorly understood despite extensive evidence of its detrimental effects on brain function. Therefore, this study investigated the molecular mechanisms by which SD impairs corneal nerve repair and regeneration, aiming to elucidate its adverse effects on corneal nerve recovery and provide foundational insights for future therapeutic strategies targeting corneal trauma-associated disorders.

MATERIALS AND METHODS

Ethical Approval Female C57BL/6 mice aged 10–12wk were housed in a specific pathogen-free facility at 24±2°C with free access to food and water. All animal experimental procedures were approved by the Experimental Animal Ethics Committee of Jinan University (approval number: JN-A-2002-01) and conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Reagents Phosphate-buffered saline (PBS) was obtained from Guangzhou RuiTai Biotechnology Co., Ltd. Triton X-100 (10%, v/v), fluorescein sodium, and bovine serum albumin (BSA) were procured from Guangzhou Sijia Biotechnology Co., Ltd. A Cochet–Bonnet esthesiometer (Model 8630-1490-29) was sourced from Luneau SAS. A DeltaVision™ Elite high-resolution microscope was provided by GE, and a fast laser-scanning confocal microscope was acquired from Leica. Anti-β-tubulin III antibody (Cat. No.AB15708A4) was supplied by Sigma-Aldrich, and anti-nerve growth factor (NGF) was obtained from BOSTER. A trephine and a Golgi knife were acquired from Malvern, PA. The ReverTra Ace reverse transcription quantitative polymerase chain reaction (RT-qPCR) kit (Code No.FSQ-101) and THUNDERBIRD SYBR qPCR Mix (Code No.QPS-201) were sourced from TOYOBO.

Sleep Deprivation Mouse Model Mice were randomly assigned to the normal control (NC) group and the SD group. SD was induced using a modified multiple-platform method. Before formal SD, mice underwent a 2-day habituation period, during which they were exposed to the apparatus for 2h per day and retained 22h of sleep opportunity. During the progressive sleep restriction phase, daily sleep opportunity was gradually reduced from 12h to 4h between days 1 and 5. Specifically, mice were allowed 12, 10, 8, 6, and 4h of sleep opportunity on days 1, 2, 3, 4, and 5, respectively. From day 5 to day 10, mice were maintained under chronic sleep restriction with 4h of sleep opportunity per day. The detailed experimental timeline is shown in Figure 1A.

Corneal Trauma Animal Model The corneal wound model in mice was established as previously described. Briefly, mice were anesthetized by intraperitoneal injection of sodium phenobarbital (25–50 mg/kg). Under the anatomical microscope, a 2-mm circular area was marked in the central cornea using a trephine, followed by mechanical removal of the corneal epithelium within the marked area using a golf knife. Corneal epithelial defects were assessed every 6h using 2% fluorescein sodium staining, and wound healing was quantitatively analyzed through standardized imaging.

RNA Sequencing Analysis As previously described[14], corneal tissue samples from NC and SD mice were rapidly collected and immediately frozen in liquid nitrogen. Samples were submitted to Beijing Genomics Institute Shenzhen for liquid nitrogen grinding, cell lysis, and RNA extraction and purification[15]. Complementary DNA (cDNA) library construction and sequencing were performed using the BGISEQ-500 sequencing platform. For RNA sequencing, three biological replicates were included in each group. Each biological replicate consisted of independently collected corneal tissue samples from mice in the corresponding experimental group.

Immunofluorescence Staining As previously described[15-17], mouse eyeballs were quickly collected and fixed in 2% formaldehyde for 45min. Eyeballs were dissected to isolate intact corneas. Tissues were blocked with 2% BSA for 15min and permeabilized with 0.2% Triton X-100 for 2.5h. Corneal nerves were labeled with β-tubulin III monoclonal antibody (1:40 dilution). After staining, samples were washed three times with PBS for 5min each. Corneas were radially sectioned into four segments, epithelial side facing up, and arranged in a plum-blossom pattern. Mounting was performed using

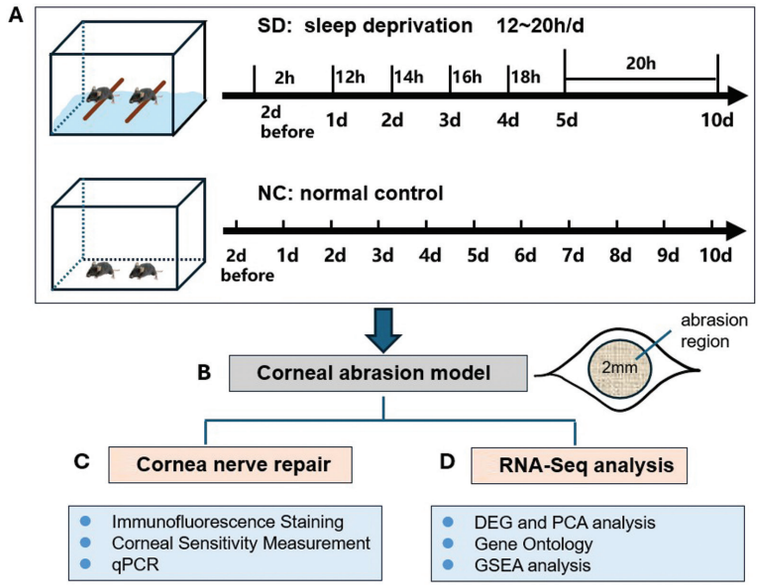


Figure 1 Experimental framework and analysis workflow A: Experimental timeline of the SD protocol, mice in the SD group underwent a 2-day habituation period, followed by progressive sleep restriction from days 1 to 5. The numbers above the timeline indicate the daily sleep opportunity: 12, 10, 8, 6, and 4h on days 1–5, respectively. From day 5 to day 10, mice were maintained under chronic sleep restriction with 4h of sleep opportunity per day. NC mice were maintained under standard housing conditions throughout the experiment; B: Establishment of a mouse model for corneal epithelial injury repair; C: The repair of corneal nerves was observed by immunofluorescence staining, corneal sensitivity measurement, and quantitative real-time polymerase chain reaction (qPCR) techniques; D: RNA-Seq was performed to comprehensively profile the transcriptomic landscape underlying SD-induced modulation of corneal nerve regeneration. SD: Sleep deprivation; DEG: Differentially expressed gene; PCA: Principal component analysis; GSEA: Gene Set Enrichment Analysis; NC: Normal control.

4',6-diamidino-2-phenylindole (DAPI)-containing anti-fade buffer, and slides were stored at 4℃, protected from light. NGF distribution in the cornea was assessed using anti-NGF monoclonal antibodies and Alexa Fluor 488[18]. To evaluate changes in subbasal corneal nerve density, high-resolution imaging was conducted, as previously reported[19]. Whole-cornea nerve plexuses were scanned at 40× magnification using a Delta Vision Elite microscope coupled with a high-speed laser scanning confocal system. Nerve fiber length (mm/mm^2) was quantified in central (2 mm diameter) and peripheral corneal regions using ImageJ software (version 1.53, NIH) following established tracing protocols.

Corneal Sensitivity Measurement Corneal sensitivity was measured following a standardized protocol[17]. A Cochet–Bonnet esthesiometer was used to stimulate the corneal surface. A 6-cm nylon filament was set as the maximum length, and the filament length that elicited a blink reflex was recorded as the corneal sensitivity value. All corneal sensitivity measurements were conducted by the same researcher at the same time of day to minimize experimental variability.

Reverse Transcription Quantitative Polymerase Chain Reaction As previously described[20], eyeballs were collected from NC and SD mice, and corneal tissue with the limbus was isolated. Samples were placed in Buffer RZ and homogenized using a TissueRuptor. Total RNA was extracted using the RNA simple Total RNA Kit and then reverse-transcribed to synthesize cDNA. Relative expression levels of target genes were quantified using THUNDERBIRD SYBR qPCR Mix and normalized to glyceraldehyde-3-phosphate dehydrogenase. Gene-specific primer sequences used for amplification are listed in Table 1.

Statistical Analysis Statistical analysis was conducted using the Statistical Package for the Social Sciences (version 22.0, IBM, USA), and data visualization was performed using GraphPad Prism software (version 8.0, GraphPad, USA). The Shapiro–Wilk test was used to assess data normality, and Levene's test was applied to evaluate the homogeneity of variances. Results are presented as mean±standard deviation. Group comparisons were performed using the analysis of variance, followed by post hoc tests and independent-sample *t*-tests. A $P<0.05$ was considered statistically significant.

RESULTS

Sleep Deprivation Significantly Delays Corneal Nerve Repair in Mice The experimental workflow for evaluating the effects of SD on corneal nerve repair after injury is illustrated in Figure 1. To examine the impact of SD, the kinetics of corneal epithelial wound repair were assessed in a murine SD model. Results demonstrated a significant 12h delay in epithelial wound closure in the SD group compared to the NC group (Figure 2A). This finding is consistent with previous

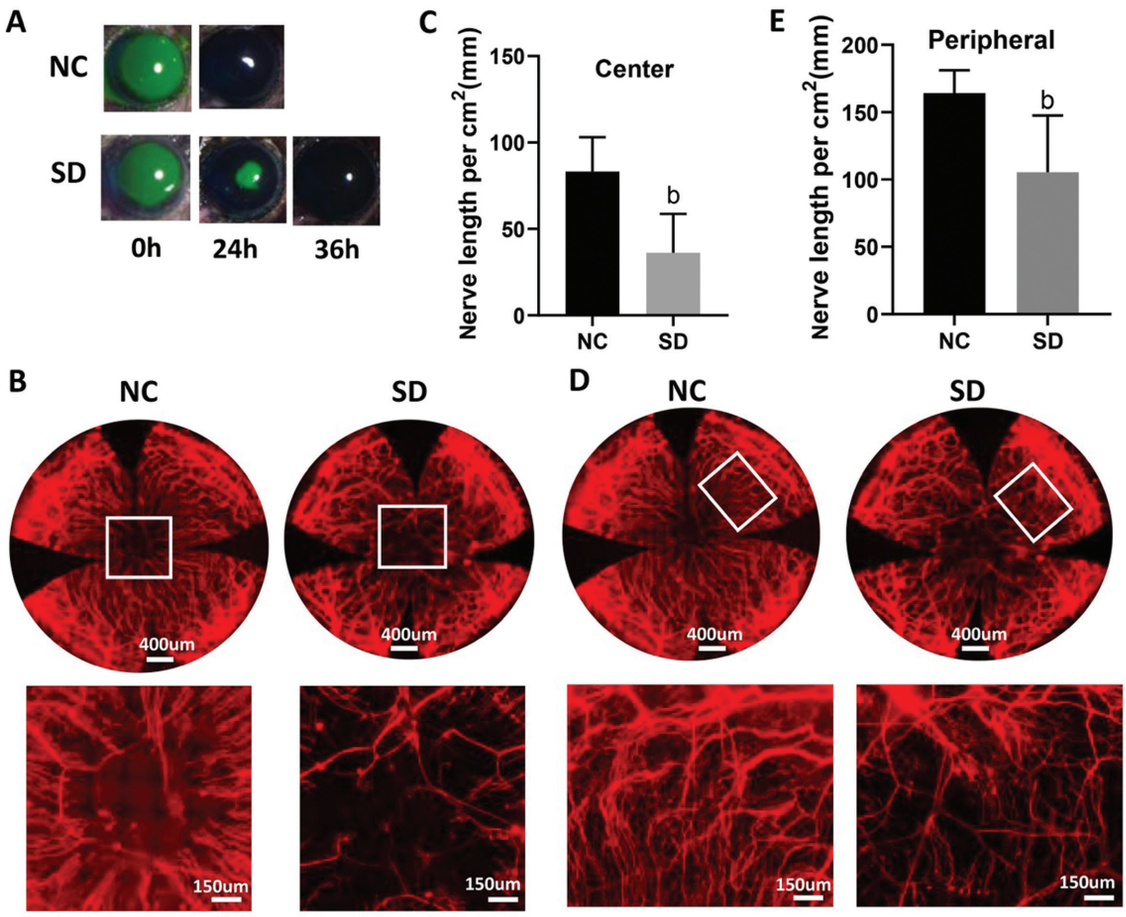


Figure 2 Immunofluorescence analysis of corneal nerve regeneration 6d after corneal injury A: The repair process of corneal epithelial injury in both the normal control (NC) and sleep deprivation (SD) group was visualized using sodium fluorescein staining; B: Corneal tissues stained with β-tubulin III antibody, representing the NC group and the SD group; C: Changes in the density of nerve fibers in the central cornea 6d after corneal injury. A significant difference is observed between the NC and SD groups (^bP<0.01); D: Immunofluorescence images exhibit the distribution of nerves in the peripheral cornea; E: Differences in the density of nerve fibers in the peripheral cornea between the two groups. The SD group shows a significant decrease in nerve fiber density compared to the NC group (^bP<0.01).

Table 1 Sequences of the primers *n*=3

Gene	Primer sequence (5′–3′)	Temperature (°C)	Length (bp)
NGF			261
Forward	5′-GAAGCTAGAGAAAAGGAATCA-3′	54	
Reverse	5′-GCAGAACTGGGTGTATAAAAG-3′	54	
GAPDH			151
Forward	5′-CAAGGACACTGAGCAAGAG-3′	57	
Reverse	5′-TGCAGCGAACTTTATTGATG-3′	57	

NGF: Nerve growth factor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

results[13], further supporting the conclusion that SD impairs corneal epithelial regenerative capacity in mice.

To further assess corneal nerve regeneration after injury, corneal tissues were collected from NC and SD groups 6d post-injury and analyzed by immunofluorescence staining. At this time point, central corneal nerve fiber density in SD mice was significantly lower compared to the NC group (Figure 2B–2C). Similarly, peripheral corneal nerve fiber density in SD mice was also significantly lower than that in the NC group (Figure 2D–2E). These findings suggest that SD decreases corneal nerve fiber density, thereby impairing normal corneal function.

Sleep Deprivation Impairs the Corneal Sensory Function Following Nerve Injury in Mice Corneal sensitivity was assessed using a Cochet–Bonnet esthesiometer after corneal injury. At 6d after corneal injury, corneal sensitivity was significantly lower in SD mice compared with NC mice (Figure 3A). NGF expression was analyzed by qPCR and immunofluorescence staining during corneal repair. qPCR analysis showed that NGF mRNA expression was lower in corneal tissues from SD mice at 24h after injury (Figure 3B). Consistently, immunofluorescence staining revealed lower NGF signal intensity in the corneas of SD mice compared

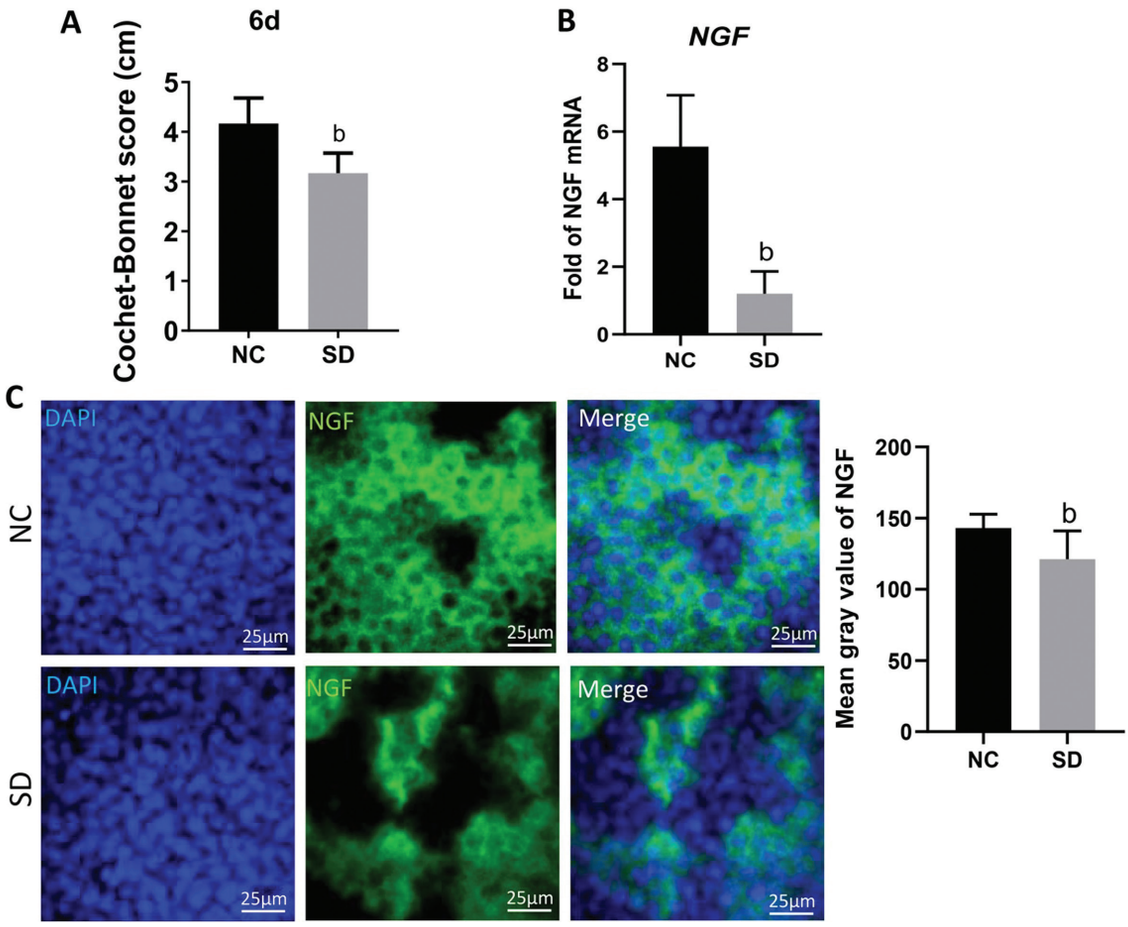


Figure 3 SD reduces corneal sensitivity and NGF expression after corneal injury A: Quantification of corneal sensitivity measured using a Cochet–Bonnet esthesiometer at 6d after corneal injury. Corneal sensitivity was significantly lower in the SD group compared with the NC group; B: qPCR analysis of NGF mRNA expression in mouse corneal tissues at 24h after corneal injury. NGF expression was significantly decreased in the SD group compared with the NC group; C: Representative immunofluorescence images showing NGF expression in corneal tissues from NC and SD mice. Lower NGF immunofluorescence signal was observed in the SD group. Data are presented as mean±standard deviation. ^{b}P<0.01. NGF: Nerve growth factor; SD: Sleep deprivation; NC: Noramal control; qPCR: Quantitative real-time polymerase chain reaction; DAPI: 4′,6-diamidino-2-phenylindole.

with NC mice (Figure 3C). Together, these results showed that SD was associated with lower corneal sensitivity and NGF expression after corneal injury.

Sleep Deprivation Alters the Corneal Transcriptome Following Corneal Epithelial Injury in Mice Transcriptomic profiling of corneal tissues from SD and NC mice identified differentially expressed genes associated with corneal nerve repair after injury. Principal component analysis (PCA) revealed distinct clustering between NC and SD groups, with significant separation in a two-dimensional space, indicating global transcriptomic remodeling in SD-treated corneas (Figure 4A). Differential expression analysis identified 1205 differentially expressed genes (|logFC|≥1, Q<0.05), including 380 upregulated and 825 downregulated genes. Notably, most neuro-related genes were significantly downregulated (Figure 4B–4C). These findings suggest substantial transcriptomic alterations in corneal tissues under SD conditions.

Sleep Deprivation Alters Neural Gene Expression and Functional Pathways in the Corneal Transcriptome A total of 163 differentially expressed genes associated with corneal nerve repair were identified (Figure 5A). Gene ontology enrichment analysis using the DAVID database revealed that these neuro-related differentially expressed genes were significantly enriched in key biological processes, including neurotransmitter secretion, axon guidance, and nervous system development (Figure 5B). To explore the synergistic mechanisms among these pathways, a multidimensional interaction network model was constructed using a Chord diagram, visualizing gene–pathway crosstalk (Figure 5C). Collectively, these findings highlight the mechanistic disruption of corneal regeneration by SD through neural signaling pathway dysregulation.

Gene Set Enrichment Analysis Validates SD-induced Alterations in Corneal Neural Transcription Gene set enrichment analysis (GSEA) further supported a coordinated

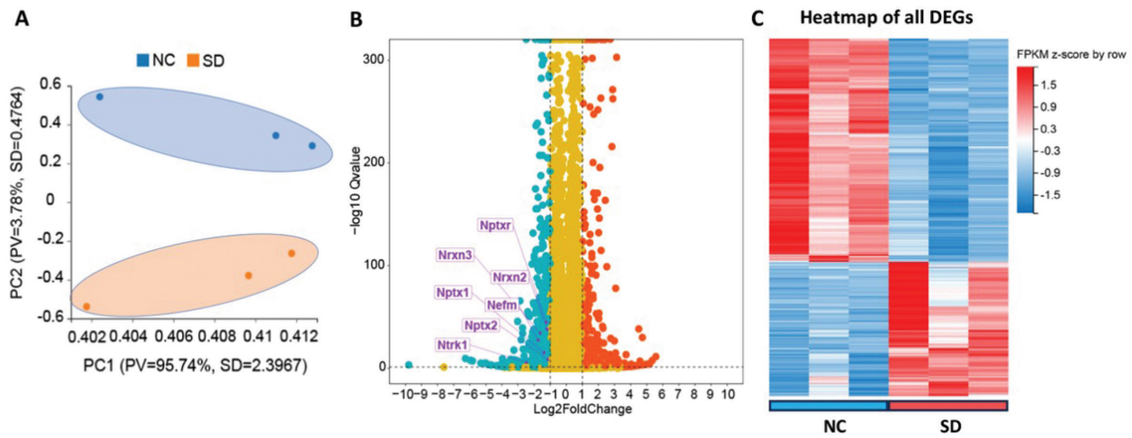


Figure 4 Effects of SD on corneal transcriptome A: PCA analysis illustrates separation between NC and SD groups, with PC1 accounting for 95.74% and PC2 for 3.78% of variability; B: Volcano plot displays DEGs, with blue for downregulation and red for upregulation, highlighting genes related to nerve repair; C: Heatmap presents FPKM Z-scores of DEGs, indicating expression differences between NC and SD groups. RNA-seq analysis was performed using 3 biological replicates per group. PCA: Principal component analysis; NC: Normal control; SD: Sleep deprivation; PC: Principal component; PV: Percent variation; DEGs: Differentially expressed genes; FPKM: Fragments per kilobase of transcript per million mapped reads.

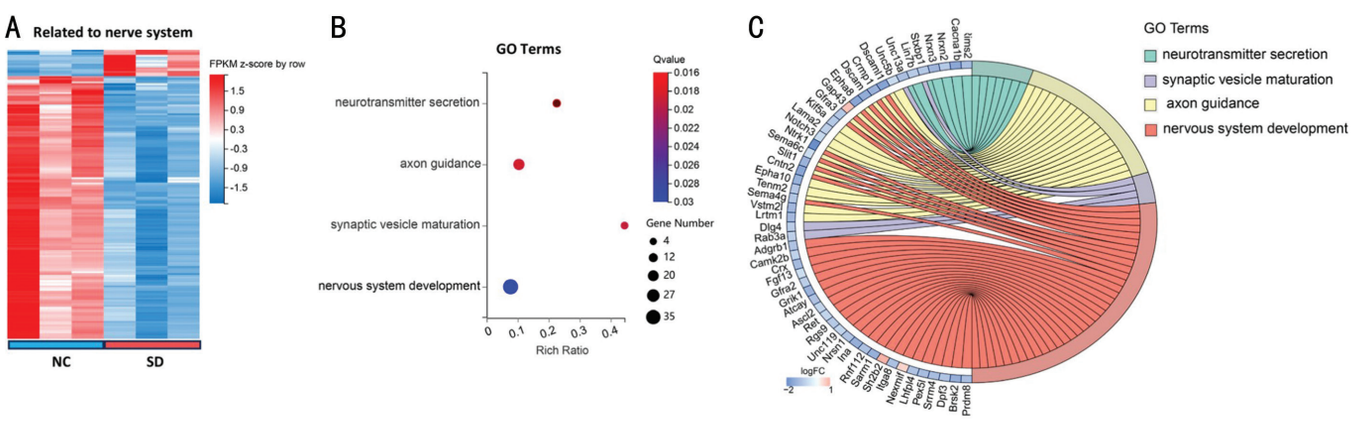


Figure 5 The impact of SD on corneal nerve-related transcriptome 18h after injury A: The heatmap displays differentially expressed genes related to the nervous system between the SD group and the normal control (NC) group. Gene expression changes are represented by FPKM Z-scores, with negative values indicating downregulation in the SD group; B: Gene Ontology (GO) enrichment analysis was performed on differential genes to identify biological processes related to the nervous system, including neurotransmitter secretion, synaptic vesicle maturation, and axon guidance; C: A Chord diagram illustrates the relationships between genes and biological processes identified in the GO enrichment analysis. Each node in the diagram represents a gene, and the connections between nodes indicate the association between genes and biological processes. The rich ratio and *Q*-value are used to assess the enrichment and statistical significance of the GO terms. FPKM: Fragments Per Kilobase of transcript per Million mapped reads; DEG: Differentially expressed genes; SD: Sleep deprivation.

suppression of neural repair-related transcriptional programs in the SD group. Multiple gene sets associated with axonal regeneration and neuronal differentiation, including axon guidance, axonogenesis, nervous system development, neuron differentiation, and synapse organization, showed negative enrichment in SD corneas. In addition, pathways related to neural communication, including neuroactive ligand–receptor interaction, neurotransmitter secretion, and neurotransmitter transport, were also downregulated. These results indicate that SD broadly disrupts molecular programs involved in corneal nerve regeneration and sensory functional recovery (Figure 6).

DISCUSSION

This study successfully established an SD mouse model by modifying the multi-platform water environment method and investigated its effect on corneal nerve repair following injury, elucidating the underlying molecular mechanisms. The SD paradigm used in this study was associated with impaired corneal nerve repair, as evidenced by decreased corneal nerve fiber density, reduced corneal sensitivity, downregulation of the neurotrophic factor NGF, and transcriptomic alterations in corneal nerves.

Previous studies have mainly focused on clinical corneal nerve alterations under established ocular conditions. For example,

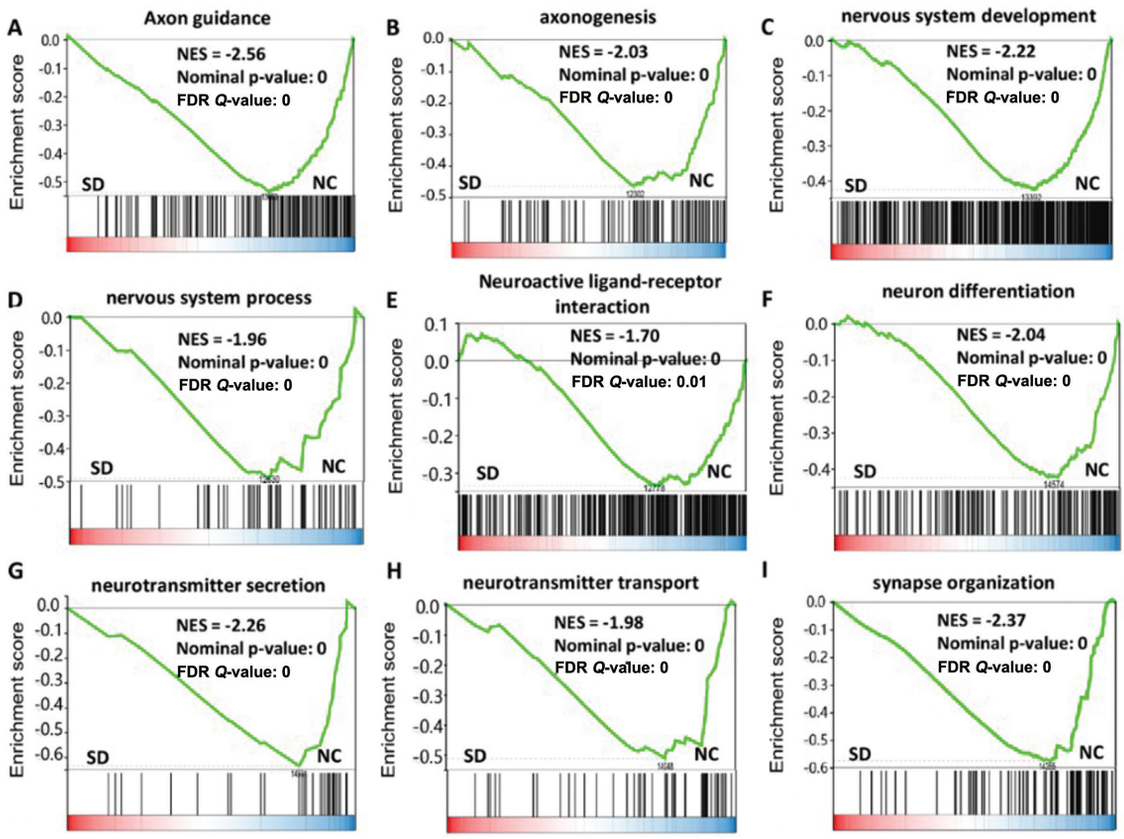


Figure 6 GSEA reveals coordinated suppression of neural repair-related transcriptional programs in SD-treated corneas GSEA was performed to evaluate pathway-level changes in the corneal transcriptome between the NC and SD groups. Representative GSEA plots are shown for gene sets related to axonal regeneration and neuronal differentiation, including axon guidance, axonogenesis, nervous system development, nervous system process, neuron differentiation, and synapse organization, as well as neural communication-related pathways, including neuroactive ligand–receptor interaction, neurotransmitter secretion, and neurotransmitter transport. These pathways displayed negative enrichment in the SD group, indicating broad suppression of molecular programs associated with corneal nerve repair. GSEA: Gene set enrichment analysis; SD: Sleep deprivation; NC: Normal control; NES: Normalized Enrichment Score. FDR *Q*-value: False discovery rate *Q*-value.

recent studies investigated corneal nerve structural changes associated with anti-glaucoma medications using *in vivo* confocal microscopy and identified clinical factors associated with ocular pain in dry eye disease[21-22]. In contrast, the present study uses a mouse corneal injury model to examine corneal nerve repair under an SD model, while integrating nerve fiber density, corneal sensitivity, NGF expression, and transcriptomic pathway analyses. Although this model may also involve procedure-associated factors such as ocular surface exposure, our findings provide experimental evidence that this condition is associated with impaired corneal nerve regeneration after injury.

Corneal nerves may represent a peripheral window into systemic neural integrity beyond their local role in maintaining ocular surface homeostasis. Increasing evidence from corneal confocal microscopy studies indicates that alterations in corneal nerve parameters are associated with peripheral and central neurological disorders, including Parkinson's disease, multiple sclerosis, and other neurodegenerative conditions[23-24]. In parallel, sleep disruption has been implicated in the development and progression of neurological disorders through mechanisms involving neuroinflammation, oxidative stress, and impaired neural homeostasis[25]. Therefore, our findings suggest that corneal nerves may represent a potential sensitive peripheral neural structure responsive to systemic physiological stress. However, whether SD-induced corneal nerve impairment shares common mechanisms with neurodegenerative diseases requires further investigation.

Sleep is essential for the body's tissue repair mechanisms[26]. Previous studies[27-28] have demonstrated that SD induces neurobehavioral alterations in humans and contributes to the pathogenesis of various ocular disorders, including limbal stem cell deficiency and an elevated risk of dry eye disease. This study delineates the impact of SD on corneal nerve repair and the underlying molecular mechanisms. These findings hold significant implications for understanding the pathophysiology of corneal disorders in individuals with sleep disturbances. Moreover, the SD-related corneal nerve impairments observed in mice may parallel similar changes in humans and help explain the increased prevalence of dry eye and related corneal

conditions in chronically SD populations[29-30]. Chronic SD has been demonstrated to impair nervous system function by inhibiting axonal growth and reducing synapsin expression in the rat brain[31]. These neural disruptions interfere with the repair process following injury. This study demonstrated the downregulation of genes related to corneal nerve repair in SD mice, accompanied by the inhibition of key repair pathways, indicating that SD severely compromises corneal nerve regeneration. Furthermore, the cornea is an avascular tissue, and corneal nerve fibers provide essential trophic support for maintaining normal physiological functions. Nerve fiber density serves as a key indicator of corneal nerve integrity[15,32-33]. This study's findings demonstrated a significant reduction in central and peripheral corneal nerve fiber density in SD mice, providing further evidence of the detrimental impact of SD on corneal nerve repair.

Corneal sensory function, assessed using the Cochet–Bonnet esthesiometer, was considerably impaired in SD mice, suggesting that sleep loss may compromise corneal nerve function and thereby disrupt overall corneal health. Additionally, reduced expression of the neurotrophic factor NGF during corneal epithelial wound healing in SD mice may be linked to the observed decline in corneal nerve fiber density. Neurotrophic factors are crucial for neuronal survival and promote the growth and differentiation of nascent neurons and synapses[34-35]. In the corneal epithelium, neurotrophic factors such as NGF and brain-derived neurotrophic factor (BDNF) bind to their specific receptors and activate signaling pathways that facilitate corneal epithelial wound healing and nerve regeneration. SD-induced downregulation of neurotrophic factors may disrupt these signaling pathways, thereby impairing corneal nerve repair and regeneration[36].

Although this study preliminarily identified the detrimental effects of SD on corneal nerve repair, the underlying mechanisms require further investigation. Specifically, the influence of SD on different stages of corneal nerve regeneration—including initiation, axonal extension, and functional recovery—at cellular and molecular levels remains unclear[37]. Moreover, the specific roles of signaling pathways[13], cytokines, and neurotrophic factors in this process remain insufficiently defined[38]. Notably, the light-dark cycle plays a critical role in maintaining normal corneal growth and development[39]. Sleep loss can disrupt circadian rhythms, impair corneal homeostasis, and increase susceptibility to lesions[8,40-41]. Given the strong association between corneal nerve repair and multiple ocular diseases[42-43], our findings may provide novel insights into the prevention and treatment of these conditions. For instance, enhancing sleep quality or targeting relevant signaling pathways may help mitigate SD-induced corneal nerve damage and inform the development of novel clinical interventions. Future studies should further investigate the causal relationship between SD and ocular disorders and explore innovative therapeutic strategies that target nerve repair mechanisms to enhance treatment outcomes for affected individuals.

This study has several limitations. The current SD model cannot completely exclude procedure-associated factors, including ocular surface exposure and systemic stress responses. In addition, autonomic regulation, particularly SNS activity, was not directly evaluated and requires further investigation. Furthermore, only female mice were used, and the model represents acute/subacute rather than chronic sleep restriction. Finally, transcriptomic findings require future validation through targeted interventions. In summary, this study establishes a preliminary framework for investigating the effect of SD on corneal nerve repair. Nevertheless, research in this domain remains nascent. Advancing comprehensive insights into the underlying complex mechanisms requires future interdisciplinary collaborations to strengthen the foundation for clinical translation.

This study developed a murine SD model and systematically examined its effects on corneal nerve injury repair to elucidate the underlying molecular mechanisms. Mice subjected to the SD paradigm exhibited impaired corneal nerve repair, evidenced by lower nerve fiber density, diminished corneal sensitivity, downregulation of the neurotrophic factor NGF, and substantial alterations in the corneal nerve transcriptomic profile. The model established in this study provides a novel framework for exploring the relationship between sleep and corneal nerve regeneration. Transcriptomic analysis indicated that SD may disrupt corneal nerve repair by affecting neurotrophic signaling pathways, thereby providing a theoretical foundation for clinical interventions targeting sleep-associated corneal diseases.

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Authors' Contributions: Yu Q and Xue YX conceived and designed the study; Hu Y, Huang LJ, and Liu ST performed the experiments and acquired the data; Jing YX, Wu JX, and Yan RY conducted the bioinformatics analysis and data visualization; Xiang JY contributed to the sample collection and animal model maintenance; Hu Y and Huang LJ drafted the manuscript; Guo YL and Li ZJ critically revised the manuscript for important intellectual content and supervised the project. All authors have read and approved the final manuscript.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request. All other data generated or analyzed during this study are included in this published article.

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