

Animal Study



Transient Compression Injury Triggers Neuroinflammation in a New Rat Model of Acute Peripheral Neuropathic Pain

Wenkai Wang, MM¹, Xinle Huang, MM¹, Yang Zhang, BS¹, Junlong Wu, MD^{4,5}, Yan Wang, MD, PhD^{2,3}, Lanlan Li, PhD², Jianxiang Zhang, PhD², and Yue Zhou, MD, PhD¹

From: ¹Department of Orthopedics, Xinqiao Hospital, Third Military Medical University (Army Medical University), Chongqing, China; ²Department of Pharmaceutics, College of Pharmacy, Third Military Medical University (Army Medical University), Chongqing, China; ³War Trauma Medical Center, State Key Laboratory of Trauma, Burns and Combined Injury, Army Medical Center, Daping Hospital, Third Military Medical University (Army Medical University), Chongqing, China; ⁴Department of Orthopedics, The Hospital of People's Liberation Army Hong Kong Garrison, Hong Kong, China; ⁵Department of Orthopedics, The 941 Hospital of Chinese People's Liberation Army, Xining, China

Address Correspondence: Yue Zhou, MD, PhD
Xinqiao Hospital
Third Military Medical University
(Army Medical University)
Chongqing 400037, China
E-mail: happyzhou@vip.163.com

Disclaimer: Wenkai Wang and Xinle Huang contributed equally to this work. See page E144 for funding information.

Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 05-23-2023
Revised manuscript received: 08-01-2023
Accepted for publication: 08-10-2023

Free full manuscript:
www.painphysicianjournal.com

Background: Peripheral neuropathic pain (NeP), induced by surgical intervention, is a well-known complication or sequela that remains a clinical challenge with few effective treatments. Ideal animal models that can recapitulate surgery-associated NeP remain to be established for both mechanistic studies and drug discovery.

Objectives: We aimed to establish a new rat model of postsurgical NeP and describe its characteristics, as well as screen-promising therapeutic analgesics.

Study Design: Experimental research in rats.

Setting: The research took place in the laboratory of Xinqiao Hospital of the Third Military Medical University.

Methods: To mimic the surgical procedure associated with peripheral nerve injury (PNI), we established a transient compression injury (TCI) in the sciatic nerve. Behavioral tests of nociception were used to confirm the effect and the time course of this pain model. Histological assessments (transmission electron microscopy evaluation and immunohistochemistry) were performed to observe the neuropathological and immunological features. RNA sequencing (RNA-seq) of injured nerves and dorsal root ganglia (DRGs) was conducted to reveal the underlying mechanism in the newly established animal model and screen promising therapeutic targets.

Results: We established a rat model of TCI of the PN and detected nociceptive hypersensitivity of the injured (ipsilateral) nerve by behavioral tests. This animal model of NeP was further confirmed by observing time-dependent changes in mechanical allodynia and thermal hyperalgesia, as well as by examining the activation of microglia in the ipsilateral spinal dorsal horn. Pathophysiologically, TCI induced macroscopic nerve swelling and demyelination, which resulted in inflammatory responses in ipsilateral nerves. We also found inflammatory cell infiltration in the ipsilateral nerve that was sustained for several weeks, which further exacerbated local inflammation and oxidative stress. Moreover, RNA-seq revealed remarkably upregulated inflammatory reactions in PNs and the DRGs. Notably, the overexpression of inflammatory mediators and the infiltration of macrophages and microglia triggered remote immune responses in DRGs. Based on the RNA-seq results, we also confirmed that gabapentin (GBP) exerts therapeutic effects in TCI-induced NeP by regulating $\alpha 2\delta$ -1.

Limitations: We did not compare the new rat model with the classical pain model (like chronic constriction injury or spared nerve injury) in histology or transcriptomics.

Conclusions: We established a new rat model of NeP and thoroughly characterized neuroinflammation in the injured nerve and DRGs. Based on the upregulated genes in DRGs in this model, we screened a promising analgesic (GBP) capable of reducing pain hypersensitivity in surgery-associated NeP.

Key words: Peripheral neuropathic pain, transient compression injury, hypersensitivity, neuroinflammation, dorsal root ganglion, RNA sequencing, Ca²⁺ channel subunit, gabapentin

Pain Physician 2024; 27:E131-E145

Neuropathic pain (NeP) remains a challenging clinical problem (1). It can result from trauma, surgery, and chronic diseases with painful polyneuropathy (e.g., diabetes, multiple sclerosis, and autoimmune diseases) (2-5). Among cases of chronic pain, peripheral nerve injury (PNI) during surgery accounts for approximately 25% to 33% (6), and the incidence of persistent postoperative NeP following spine surgery is 5% to 36% (2). The typical clinical symptoms of peripheral NeP following surgery are often characterized by abnormal sensation, sensory deficit, and maximum pain in one skin area (7,8). These symptoms often evolve into chronic pain conditions with a major impact on the quality of life of patients for several months or years. As a severe complication in orthopedic surgery and neurosurgery, postoperative NeP remains a pressing clinical problem with few effective treatments (9,10). Therefore, it is imperative that we understand the pathogenesis of postoperative PNI and develop targeted therapies for it.

Investigating the underlying mechanisms of peripheral NeP, postoperation depends on the availability of appropriate animal models capable of mimicking surgical procedures and clinical symptoms. As an acute and temporary process, PNI following surgery is often caused by stretching or clamping of nerves during surgical procedures (9-11). These temporary interventions generally last for seconds or minutes and do not sever the nerves. Hence, full recovery from symptoms, such as NeP or paralysis, is possible (10). However, the present rodent models of NeP reported in early studies, such as the chronic constriction injury (CCI) model (12,44), spared nerve injury (SNI) model (13), and spinal nerve ligation (SNL) model (14), cannot accurately mimic surgery-associated PNI.

A standardized animal model is crucial to exploit mechanism-based therapeutics for analgesic effects (3). Peripheral NeP induced by postoperative PNI is still a challenging clinical problem with few effective therapeutics (3,7,9,11). Therefore, by identifying the neuropathological changes in animal models of postoperative PNI, targeted therapeutics with the maximum analgesic effect can be screened. Considering the procedure of PNI during surgery, our study proposed an idea about transient compression injury (TCI) in the sciatic nerve. Thus, this study aimed to establish a new rat model of peripheral NeP resulting from TCI and investigate the pathophysiological and transcriptomic characteristics. By screening the differentially expressed genes (DEGs) in the dorsal root ganglia (DRGs), we found a

mechanism-based therapeutic drug for postoperative analgesia. Our findings extend the mechanistic understanding of surgery-associated NeP and suggest an effective therapeutic strategy.

METHODS

Animals

All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Third Military Medical University Ethical Committee for Animal Research. A total of 236 male and female Sprague-Dawley rats (200-250 g) were used as indicated.

Surgical Procedure for TCI

The animals were randomized to the TCI and sham groups. For rats in the TCI group, a small incision was made at the right hip, and the sciatic nerve was exposed. Then, the sciatic nerve was clipped with 4 vascular clamps (1.5 cm in length and 2 mm in width) and the compression was maintained for 10 minutes. The compression injury developed by vascular clamps ran 8 mm in the entire width. After that, the clamps were removed and the wound was closed with sutures. The surgical procedure is described in detail in the supplementary information.

Behavioral Assessments of Nociception

The tests of mechanical nociceptive threshold in the rats were conducted with calibrated von Frey monofilaments. We tested the thermal sensitivity with the Hargreaves method using a plantar test apparatus. All behavioral experiments were performed in a blinded manner. Details are provided in the supplementary information.

Histological Assessments and Immunohistochemistry

Sciatic nerves, DRGs, and spinal cord were harvested and embedded in an optimal cutting temperature compound for frozen sections. The procedures were conducted according to the manufacturer's instructions for Masson trichrome (Masson) staining, toluidine blue (TB) staining, and dihydroethidium (DHE) staining. For the preparation of ultrathin sections for the transmission electron microscope (TEM), the tissues were embedded in resin, cut into ultrathin sections (50 nm), and stained with gadolinium acetate and lead citrate. All experiments were repeated 3 times. See the supplementary information for details.

Quantitative Real-time Polymerase Chain Reaction (qPCR) Analysis

Total RNA of sciatic nerves and DRGs from rats was isolated and qPCR was performed with the SYBR® Green technique. The primer sequences were shown in supplemental materials (Suppl Table 2).

RNA Sequencing (RNA-seq) Analysis

Total RNAs from sciatic nerves and DRGs were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). Following the vendor's recommended protocol, RNA-seq analysis was performed on an Illumina Novaseq™ 6000 (LC-Bio Technology Co., Ltd, China). A summary of the RNA-seq analysis and some of the most significantly expressed transcripts are shown in the supplemental materials (Suppl Tables 3-5).

Enzyme-Linked Immunosorbent Assay (ELISA)

The sciatic nerve tissues were collected and homogenized in radioimmunoprecipitation assay lysis buffer. ELISA testing was performed according to the manufacturer's instructions. See the supplementary information for details.

Western Blotting Analysis

Western blotting using spinal cord tissues was performed using standard methods described in the supplementary information.

Statistical Analysis

Results are expressed as means $\pm$ SD. Two-tailed Student's *t* test and one-way analysis of variance followed by Tukey's honestly significant difference test were used for comparisons between the 2 groups. A difference was considered statistically significant if the *P* value was < 0.05 . See the supplementary information for details.

RESULTS

TCI-Induced NeP

To mimic the surgical procedure associated with PNI, we used vascular clamps to induce a compression injury in the sciatic nerve in minutes (Fig. 1a, 1b). Mechanical hypersensitivity was observed in the ipsilateral hindlimb of rats 3 days after TCI surgery (Fig. 1c), which revealed that TCI could induce NeP. Behavioral testing demonstrated the time course of changes in mechanical allodynia and thermal hyperalgesia in the TCI model (Fig. 1c, 1d). NeP presented 3 days after TCI, peaked at

7 to 10 days postoperatively, gradually remitted from day 14, and eventually recovered by day 28. Immunohistochemistry confirmed that the numbers of IBA1⁺ microglia and GFAP⁺ astrocytes increased in the ipsilateral spinal dorsal horn (SDH) after TCI (Fig. 1e-h). As glial cells (microglia and astrocytes) in the SDH are activated rapidly when NeP occurs, these data (15,16) suggested that rats with TCI surgery developed NeP.

Neuropathological Assessment of the Injured Sciatic Nerve

We next evaluated the characteristics of the injured PN after TCI surgery at different time points. After TCI, the PN exhibited hemorrhage and swelling, which developed until 7 days after surgery (Fig. 2a). Masson staining showed the histological variation of PN fibers (Fig. 2b). Injured nerve fibers developed macroscopic swelling, structural disturbance, and demyelination. Fourteen days after TCI, disturbed nerve fibers underwent regeneration and rearrangement, although scars and atrophy were apparent. By 28 days postsurgery, axons had recovered continuity. To visualize myelin sheath regeneration, we used TEM to evaluate the thickness and density of the myelin sheath (Fig. 2c). TCI induced demyelination of myelinated nerve fibers and disturbed the structural integrity of myelin. The injured myelin sheath and axons gradually regenerated 14 days after TCI; whereas, the thickness and density of the myelin sheath decreased obviously. In addition, to assess the regeneration of injured axons, we performed immunostaining of superior cervical ganglion 10, a marker of axons (17). The integrity of axons was remarkably reduced by TCI surgery, but it recovered when NeP was absent (30 days postinjury) (Suppl. Fig. S1).

TCI-Induced Increases in Inflammatory Cells, Cytokines, and Reactive Oxygen Species (ROS) in the Local Areas of Injured Nerves

Injury and repair of tissues always involve inflammation (18,19). We then investigated the inflammatory response in the local area of injured nerves. qPCR and Luminex liquid suspension chip detection were performed to examine the expression of inflammatory cytokines and receptors (Fig. 3a, 3b). These tests showed that proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) and inflammatory chemokines (C-C Motif Chemokine Ligands 2 [CCL2], IL-2, MIP-1 α , RANTES, and TGF- β) were upregulated in injured nerves within 3 days after TCI surgery. Next, we used ELISA to measure the expression of proinflammatory cytokines over time fol-

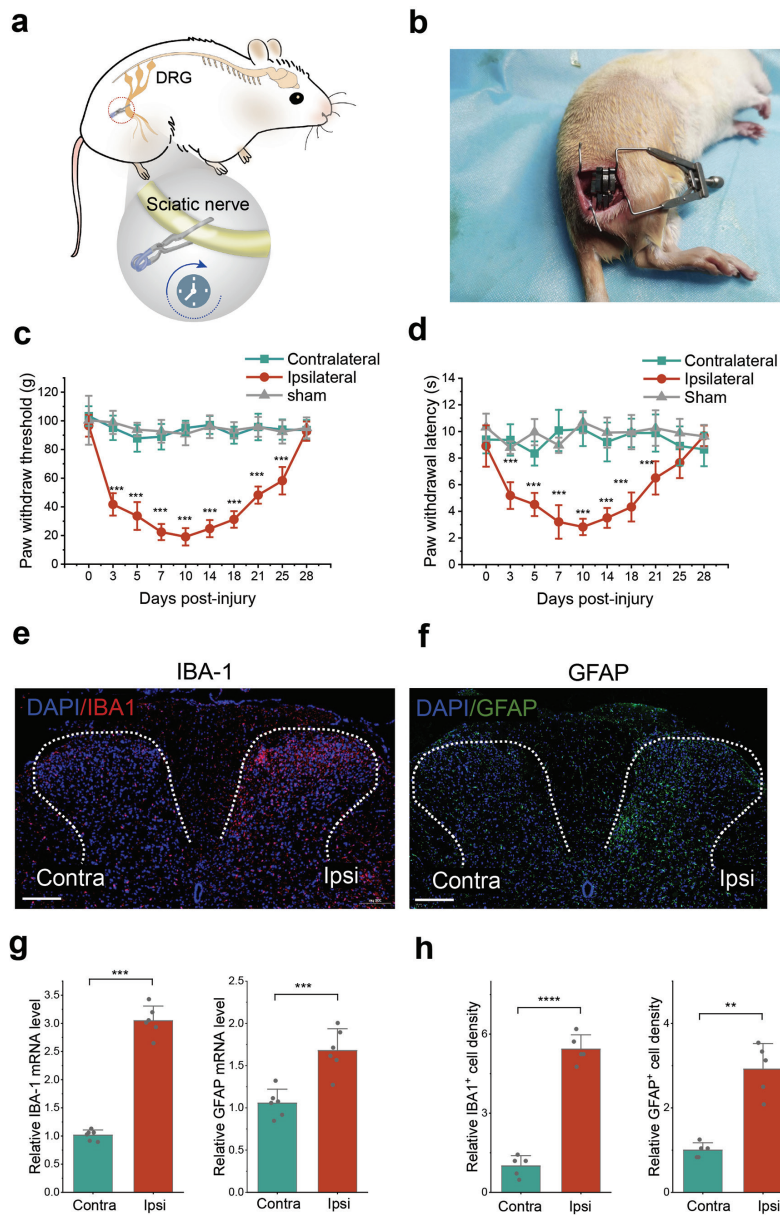


Fig. 1. The rat model of transient compression injury (TCI) induces neuropathic pain. (a) Schematic showing the procedure of TCI surgery. The sciatic nerve was clipped with a vascular clamp, and compression was maintained for 10 minutes. (b) A photograph of the surgical procedure in the TCI model. (c) Mechanical allodynia was tested using von Frey filaments from 3 days to 28 days after TCI surgery. (d) Thermal hyperalgesia was determined using plantar tests from 3 days to 28 days after TCI surgery. (e, f) Immunofluorescence intensity analysis shows that the immunoreactivity of IBA1 (e) and GFAP (f) in the ipsilateral spinal dorsal horn (SDH) was increased compared with that on the contralateral side in TCI rats. Contra, the contralateral uninjured side; Ipsi, the ipsilateral injured side. Scale bars: 200 μ m. (g) qPCR analysis showed the expression levels of IBA1 and GFAP in the ipsilateral and contralateral SDH in TCI rats. (h) Quantification of IBA1⁺ and GFAP⁺ cells in the ipsilateral and contralateral SDH. The positive cells in 10 random fields (magnification 400 $\times$) were counted with ImageJ analysis (V1.8, NIH). All experiments were repeated 3 times. The data are expressed as the mean $\pm$ SD ($n = 8$ rats/group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

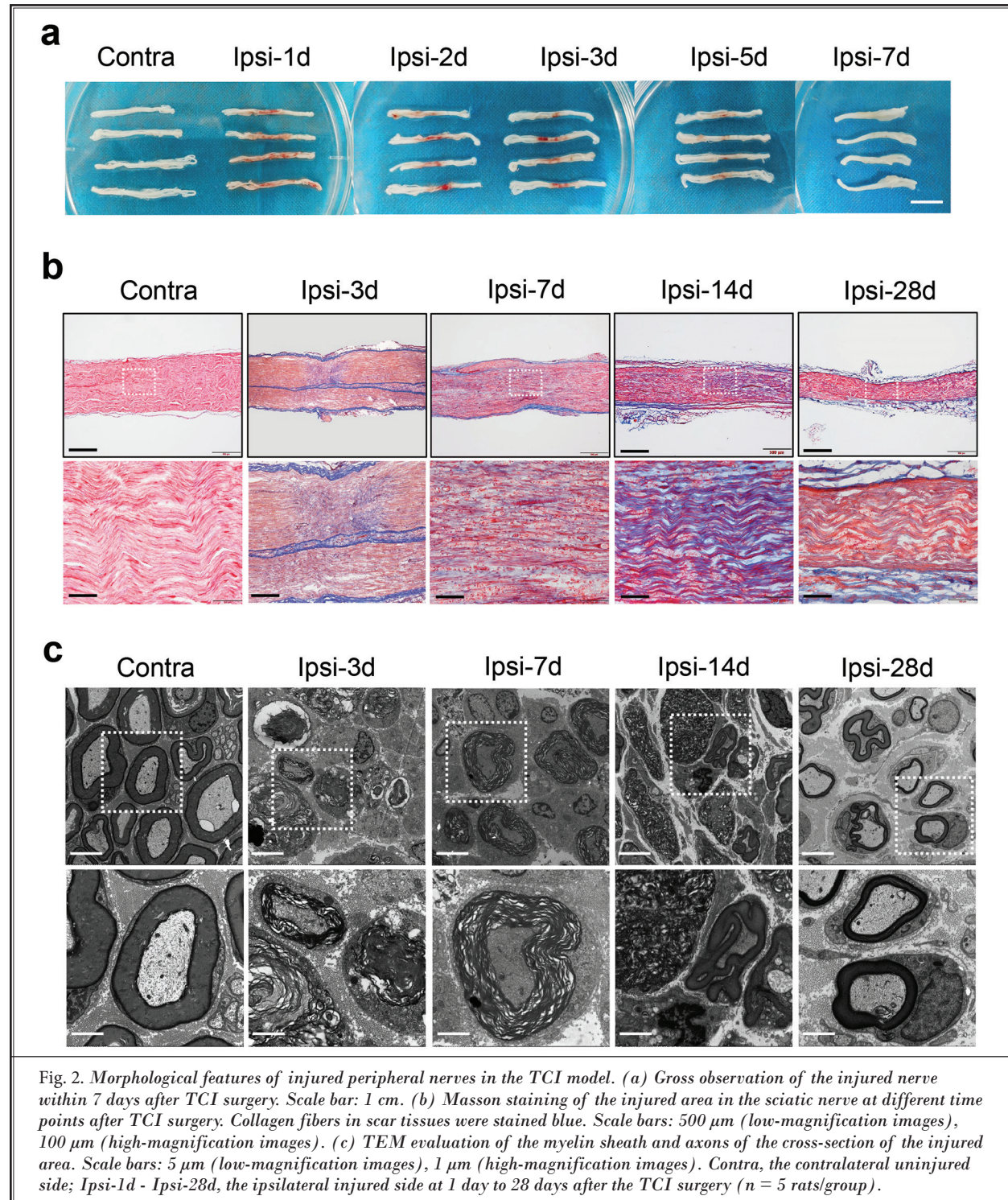
lowing surgery (Fig. 3c-3f), which revealed that neuroinflammation induced by TCI peaked at 3 days after surgery. Since inflammation is intimately linked to oxidative stress (20-22), we tested oxidative stress-associated molecular mediators (H_2O_2) and performed DHE staining assays of the injured nerve (Fig. 3g-3i). Notably, the injured nerve produced numerous ROS within 3 days after TCI surgery. Moreover, to examine the invaded immune cells of the injured nerves, we stained mast cells, neutrophils, and macrophages. Toluidine blue O staining showed that more mast cells accumulated in the injured nerves than in the contralateral nerves (Fig. 4a, 4b). Immunostaining also showed an increased intensity of the neutrophil marker myeloperoxidase and macrophage marker CD68 in the ipsilateral injured nerve 2 days after TCI (Fig. 4c-4f). Finally, we stained the macrophages in the ipsilateral sciatic nerve at different days postinjury. The results revealed that macrophages infiltrated the injured nerve from 2 days postinjury; the infiltration peaked at 14 days and existed for several weeks (Fig. 5). Collectively, these data suggested that TCI resulted in acute neuroinflammation in the local area of injured nerves.

Transcripts Regulated in the Injured Sciatic Nerve and DRGs After TCI Surgery

To gain mechanistic insight into the pathogenesis of TCI-induced NeP, we performed RNA-seq to compare the PN and DRG transcriptomes between the ipsilateral (injured) and contralateral sides at 7 days after TCI, when

pain was triggered. The count matrix was transformed using a regularized logarithm implemented in DESeq2 (Fig. 6a). Gene ontology and Kyoto Encyclopedia of

Genes and Genomes analyses were performed for functional classification of DEGs (log 2-fold change) > 2, $P < 0.05$). Among 2,858 significant DEGs of the injured



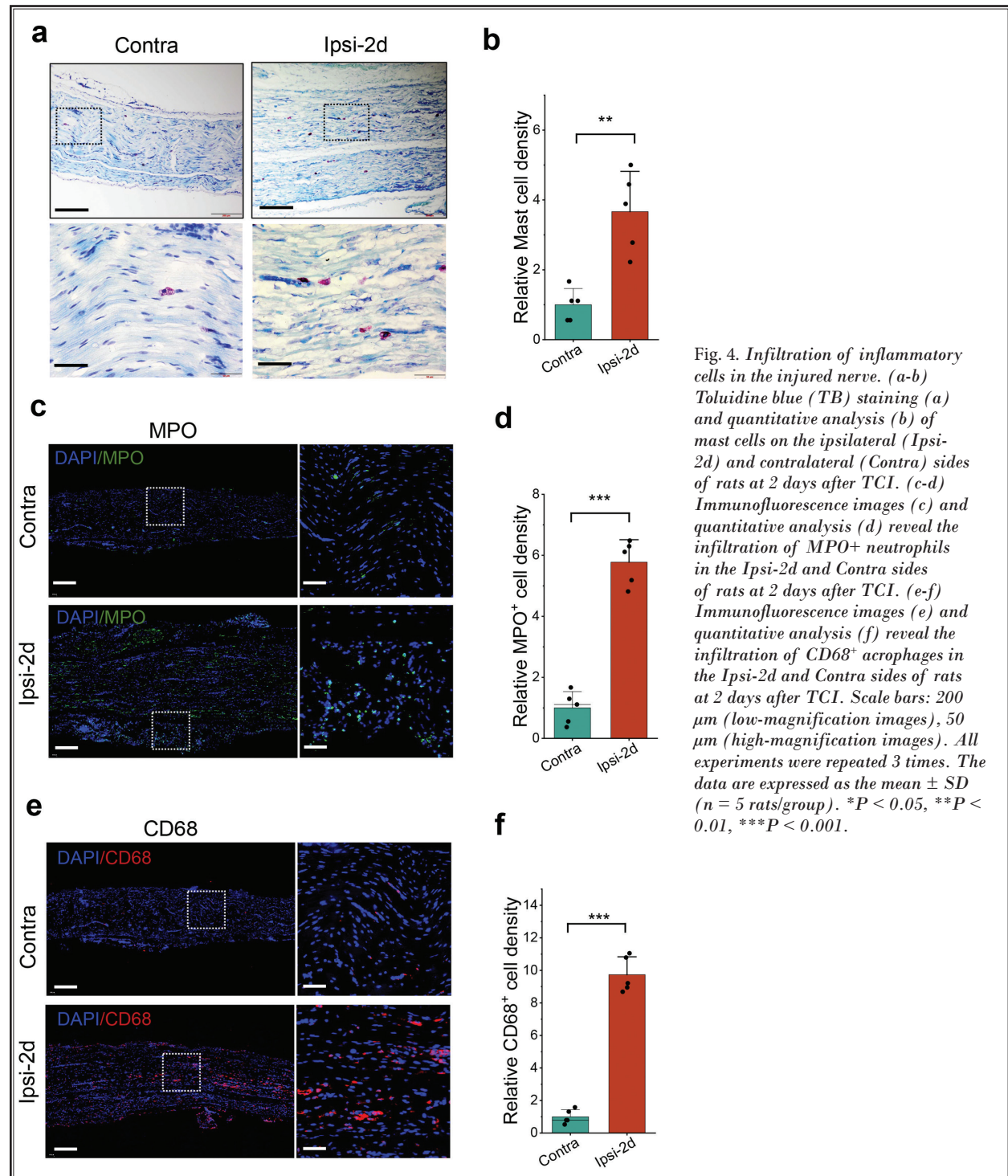
and *Cxcl10*, which are attractants of immune cells, as well as IL-6 and IL18, all important proinflammatory cytokines (23,24). *Csf1r*, *Cd68*, *Arg1*, and *Abca1*, typical macrophage-associated genes (25,26), were also highly upregulated in the injured nerve. Of note, these differ-

Fig. 3. Overproduced inflammatory mediators and reactive oxygen species (ROS) in the local areas of injured nerves caused by TCI. (a) qPCR analysis of the mRNA expression levels of inflammatory cytokines and receptors in contralateral and ipsilateral sciatic nerves. The data are presented as the fold change, and GAPDH was used as an internal control. (b) Luminex liquid suspension chip detection of the inflammatory cytokines and receptors. (c-f) The levels of IL1 β (c), IL-6 (d), TNF- α (e), and TGF- β (f) in the injured nerve at different time points within 10 days postsurgery. Contra, the contralateral uninjured side; 1 d - 10 d, the ipsilateral injured side at 1 day to 10 days after the TCI surgery. (g) The level of H₂O₂ in the injured nerve at different time points. Contra, the contralateral uninjured side; 1d - 10d, the ipsilateral injured side at 1 day to 10 days after TCI surgery. (h-i) Fluorescence images (h) and quantitative analysis (i) of DHE-stained sciatic nerve cryosections on the ipsilateral (Ipsi-3d) and contralateral (Contra) sides of rats at 3 days after TCI. DHE staining was quantified with ImageJ analysis (V1.8, NIH). Scale bar: 50 μ m. All experiments were repeated 3 times. The data are expressed as the mean $\pm$ SD (n = 5 rats/group). *P < 0.05, **P < 0.01, ***P < 0.001.

entially upregulated genes are closely correlated with the pathogenesis of inflammatory reactions. Similarly, gene set enrichment analysis revealed that the immune

response-associated terms and pathways were highly enriched in the injured nerve after TCI (Fig. 6e).

Additionally, RNA-seq of the DRGs screened 419

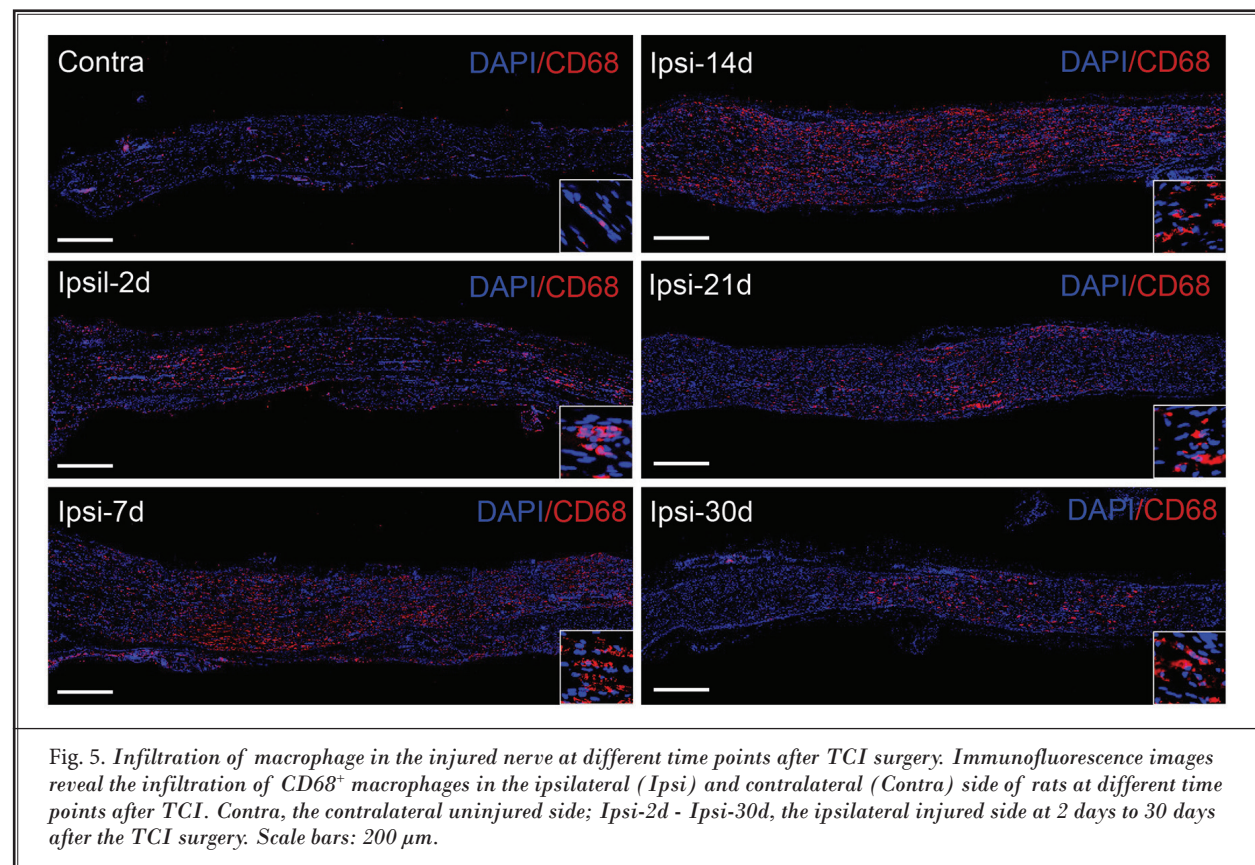


significant DEGs between the injured (ipsilateral) and contralateral sides, including 332 upregulated genes (79.2%) and 87 downregulated genes (20.8%) (Fig. 6f, 6g). Among the upregulated DEGs of the ipsilateral DRGs, there were neuropeptides linked with NeP, such as *Gal*, *Vip*, *Vgf*, and *Npy* (Fig. 6h, 6i). These neuropeptides have been reported to be closely correlated with the onset of NeP (27-30). *Gabar5*, *Npy2r*, and *Bdkrb2*, which are receptors mediating the production and development of NeP (31-34), were also upregulated significantly (Fig. 6j, 6k). These data agreed with previous reports in which neuropeptides and their receptors were expressed at a high level when NeP occurred in early studies. Interestingly, inflammation-related signaling pathways in the ipsilateral DRGs were robustly upregulated (Fig. 6k). These were encoding proinflammatory cytokines and chemokines genes (*Il6*, *Ccl2*, *Csf1*, and *Cxcl13*) and macrophage-associated genes (*Arg1* and *Csf1r*) (35). This may account for the activation of microglia (16). In addition, gene sets related to the postsynaptic density and the response to elevated cytosolic calcium were upregulated in the ipsilateral DRGs,

suggesting that sensory neurons respond to NeP (Fig. 6l, 6m). Collectively, the evidence indicated that TCI surgery not only induced an inflammatory response in the local area of the injured nerve, but also triggered a remote immune response in DRGs.

Inflammatory Response Activated by TCI Surgery

According to the RNA-seq results, we then performed qPCR and immunostaining to verify the neuroinflammatory processes in the DRGs. As a result, *Il6*, *Il18*, *Ccl2*, and *Csf1* were highly expressed in the ipsilateral DRGs compared with the contralateral DRGs (Fig. 7a). Recent studies (16,36) have reported that central neuroinflammation following PNI is triggered by microglia, which can be activated by CSF1 and CCL2 from the central terminals of injured afferents. Moreover, the macrophage-associated genes, *Cd68*, *Csf1r*, and *Arg1*, were also remarkably upregulated in the ipsilateral DRGs (Fig. 7a). *Arg1* is commonly used as a marker of M2-type of macrophages, which are increasingly appreciated for inhibiting sensitization and promoting



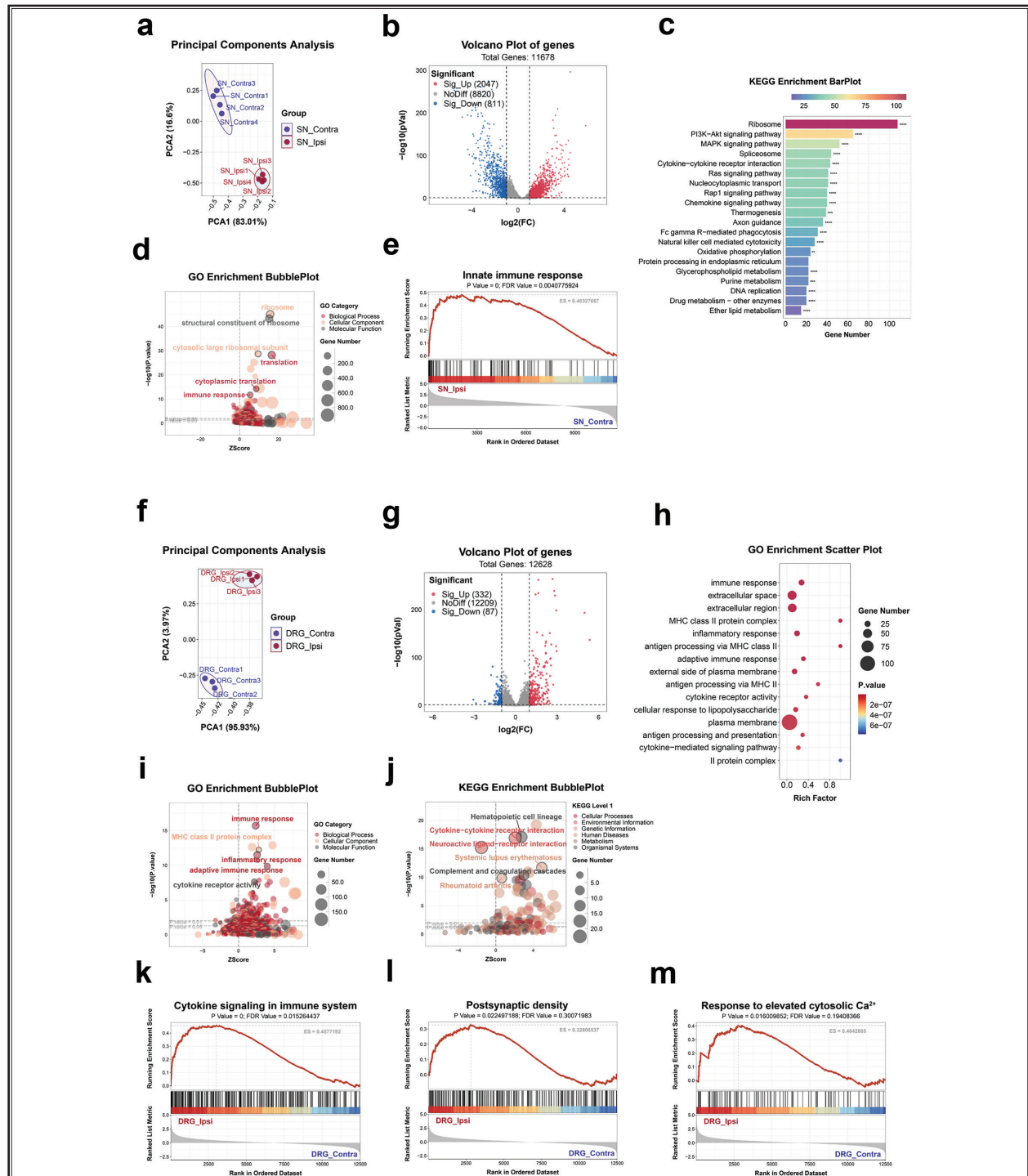


Fig. 6. Transcriptomics analysis of injured sciatic nerves and DRGs after TCI surgery. (a-e) RNA-seq results of the sciatic nerve between the ipsilateral and contralateral sides of TCI rats. (a) Principal component analysis (PCA) of the sciatic nerve groups. (b) The volcano plot shows 2,858 significantly differentially expressed ($P < 0.05$ and $|\log_2FC| > 2$) genes (DEGs) with 811 downregulated and 2,047 upregulated in the ipsilateral sciatic nerve (SN-ipsi) group. FC, fold change. (c) KEGG analysis. (d) GO analysis. (e) The enrichment plot of signaling gene sets of the innate immune response in GSEA. (f-m) RNA-seq results of DRGs between the ipsilateral and contralateral sides of TCI rats. (f) PCA of the DRG groups. (g) The volcano plot shows 419 significantly differentially expressed ($P < 0.05$ and $|\log_2FC| > 2$) genes (DEGs), with 87 downregulated and 332 upregulated in the ipsilateral DRG (DRG-ipsi) group. FC, fold change. (h) GO analysis. (i-j) Enrichment bubble plot of the GO and KEGG analysis results. (k-m) GSEA enrichment gene sets of significantly upregulated genes in the DRG-ipsi group.

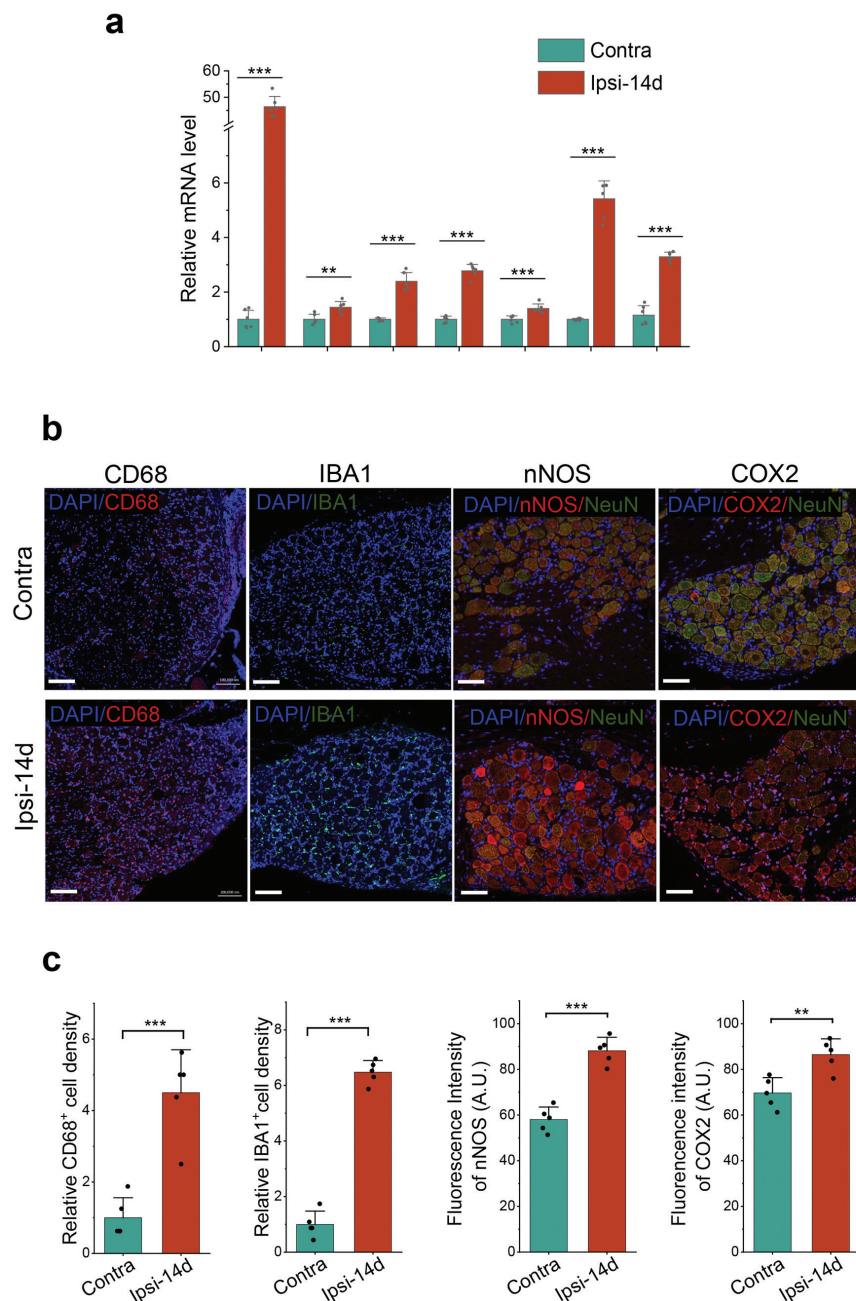


Fig. 7. Verification of inflammatory reactions in DRGs 14 days after TCI surgery. (a) qPCR analysis of the mRNA expression levels of inflammatory mediators in ipsilateral and contralateral DRGs. The data are presented as the fold change, and GAPDH was used as an internal control. (b) Immunofluorescence images of inflammatory cells (CD68⁺ and IBA1⁺ macrophages) and mediators (COX2 and nNOS) in DRGs at 14 days after TCI. Staining of anti-NeuN (green) indicates neurons. Contra, the contralateral DRGs from the uninjured side; Ipsi-14d, the ipsilateral DRGs from the injured side at 14 days after TCI surgery. Scale bar: 200 μ m. (c) Quantification of inflammatory cell (macrophage and microglial) intensity and mediator (COX2 and nNOS) fluorescence intensity in the DRGs. The positive cells in 10 random fields (magnification 400 $\times$) and mean fluorescence intensity were quantified with ImageJ analysis (V1.8, NIH). All experiments were repeated 3 times. The data are expressed as the mean $\pm$ SD ($n = 5$ rats/group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

healing (25,26). Similarly, immunostaining showed an increased intensity of macrophages (CD68⁺ cells and IBA1⁺ cells) in the ipsilateral DRGs (Fig. 7b-7c). Finally, we also investigated the expression of cyclooxygenase-2 (COX-2) and neuronal nitric oxide synthase (nNOS) in the DRGs (Fig. 7b-7c). As inflammatory mediators can significantly enhance nociceptive transduction in the NeP states (35,37,38), the upregulation of COX-2 and nNOS in injured sciatic nerve DRGs confirmed the link between neuroinflammation and NeP induced by TCI surgery. Taken together, TCI surgery not only resulted in pain sensitization, but also facilitated the activation of inflammatory reactions in DRGs.

GBP Identified as an Effective Analgesic for TCI-Induced NeP

RNA-seq of DRGs revealed the DEGs of injured nerve DRGs, which may include targets for the treatment of TCI-induced NeP. RNA-seq of DRGs revealed several upregulated receptors orchestrating nociceptive transduction in the NeP state (Gabra5, Cacna2d1, Npy2, Cckbr, Drd2, and Bdkrb2) (Fig. 8a). Among those genes, Cacna2d1 was one of the most distinct upregulated genes, and encoding voltage-activated Ca²⁺ channel subunit $\alpha 2\delta$ -1, which is the target of analgesic gabapentin (GBP) (39-41). Therefore, we focused on Cacna2d1 in the therapeutic experiments. The data from qPCR analysis showed that Cacna2d1 was upregulated in ipsilateral DRGs of the TCI rats (Fig. 8b). Immunostaining showed the intensity of $\alpha 2\delta$ -1 was increased in the ipsilateral DRGs and SDH of the TCI rats (Fig. 8c-f). These findings suggested that TCI surgery increased the expression of Cacna2d1, which may be a therapeutic target for analgesic treatment. Thus, we intraperitoneally injected GBP into TCI rats 3 days after surgery to treat NeP. Consequently, intraperitoneal injection of 100 mg/kg GBP for 7 days significantly increased the mechanical and thermal threshold (Fig. 9a, 9b). Immunoblotting showed that TCI increased the protein level of $\alpha 2\delta$ -1 in the spinal cord, but not in sham rats (Fig. 9c, 9d). Treatment with GBP normalized the protein levels of $\alpha 2\delta$ -1 in the spinal cord of the TCI rats. These data demonstrated that GBP is an effective analgesic for the treatment of NeP generated by TCI.

DISCUSSION

Peripheral NeP following surgery is a challenging medical problem that remains difficult to treat (2,9,10,42,43). Rapid PNI, one of the leading factors accounting for postoperative NeP, is often caused by

stretching or clamping of nerves during surgical procedures (11). As a temporary type of damage, rapid PNI does not sever the nerves but disturbs the transmission function, and full recovery is possible. Patients with postoperative NeP present transient dysesthesia for several weeks but without motor weakness (43).

The classical rodent model of peripheral NeP is mainly employed in 2 approaches. One involves compressing the PN chronically, such as in the CCI model (12,44). As CCI is a chronic injury pattern, 10 to 30 days are required for mechanical allodynia to emerge in the CCI model. Of note, the appearance time and severity of mechanical hypersensitivity are connected with the technique of experimenters, such as the tension of ligatures and the thickness of threads (45). Therefore, there is a distinct time course of changes in mechanical allodynia in CCI models of different studies, which may reduce the repeatability of this animal model. The other approach involves transecting the distal segment of the PN, such as in the SNI model or the SNL model (13,14). Owing to the severe damage to the axon, these approaches produce hypersensitivity promptly but persistently, which may lead to a ceiling effect (3). Furthermore, a complete transection of nerve fibers can result in the loss of conduction function and paralysis of muscle tissue (45).

Hence, effective animal models simulating PNI during surgery are imperative for the prevention and treatment of surgery-related NeP. In view of the advantages and disadvantages of early rodent models, we propose establishment of TCI in the PN via vascular clamps, which simulates the surgical process associated with NeP. Surgery-associated PNI is mainly caused by transient clipping or stretching, which enlightens experimenters a transient compression process by vascular clamps. To uniform a standard protocol, we choose 10 minutes as the time length of clipping after assessing the pain hypersensitivity in different clipping times (shorter or longer than 10 minutes). Of note, behavioral tests suggested that the 10-minute compression by 4 vascular clamps induces neuropathic allodynia 3 days postoperation, which is an ideal animal model of surgery-associated NeP.

Compared with early surgical animal models of NeP, the TCI model has many benefits. First, the injury mode of the TCI model is short-term compression, in accordance with the damage process of PNI during surgery. Second, the NeP induced by the TCI model is self-limited. Previous surgical models of NeP have mostly induced persistent peripheral NeP, regardless of wheth-

er CCI or SNI was used. In our study, the mechanical nociceptive threshold of TCI rats recovered to normal in a month. Finally, the TCI model is easily reproducible. A prevalent animal model should be easily performed and repeated. Compared with the CCI model, the TCI model uses an objective method for compression injury, avoiding the systematic deviation generated by different experimenters. Moreover, compared with the SNL model, the TCI model does not isolate the paraverte-

bral muscle, which decreases the additional soft tissue damage and time consumption (Suppl. Table 1).

The underlying mechanism of chronic NeP resulting from PNI involves a variety of factors. Most studies have reported that the ectopic impulses generated at the site of nerve injury or the DRGs played a crucial role in peripheral NeP (3). Because of the disturbance of the structural integrity in the somatosensory nervous system, PNI alters the transmission of sensory signals

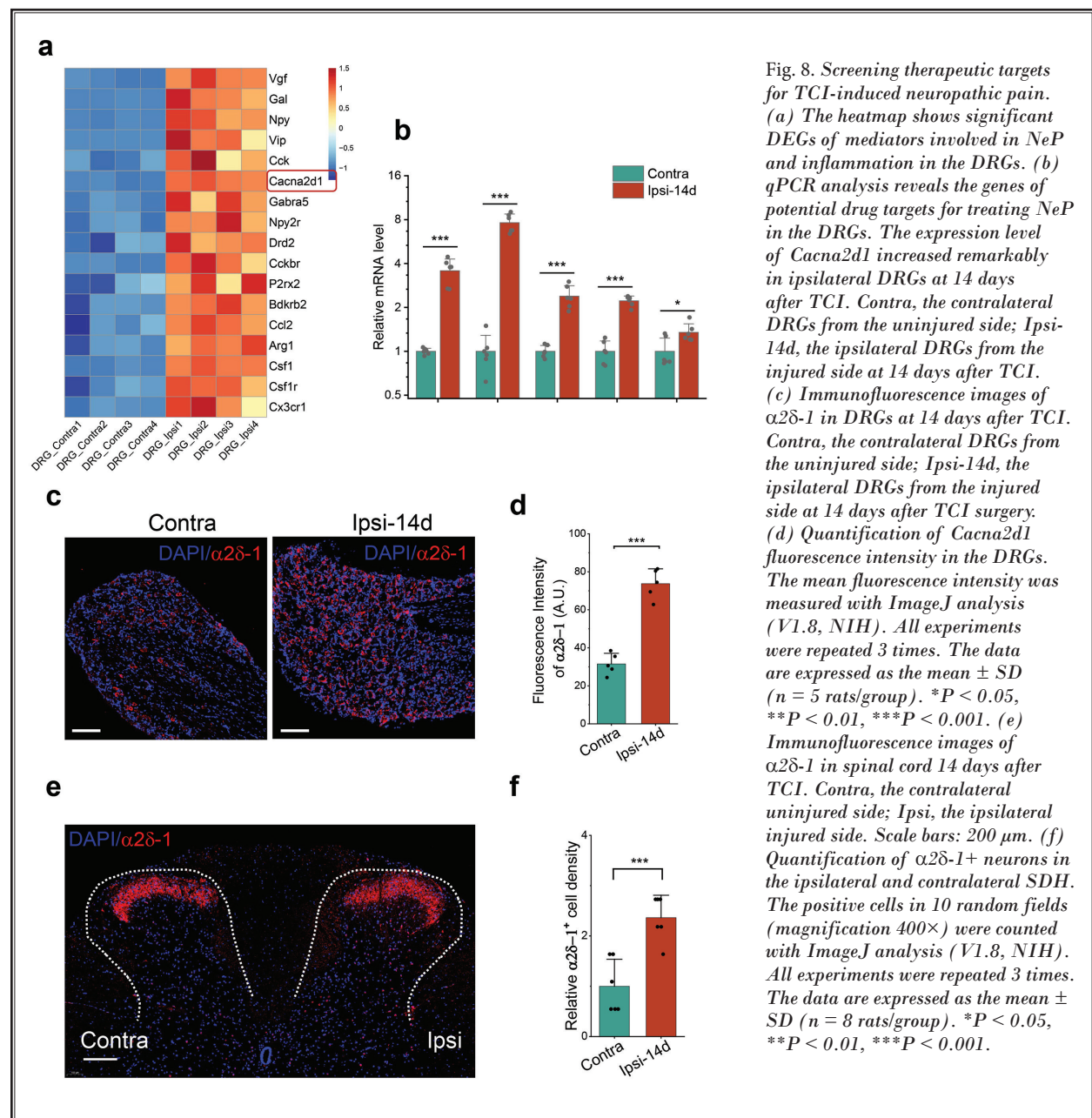
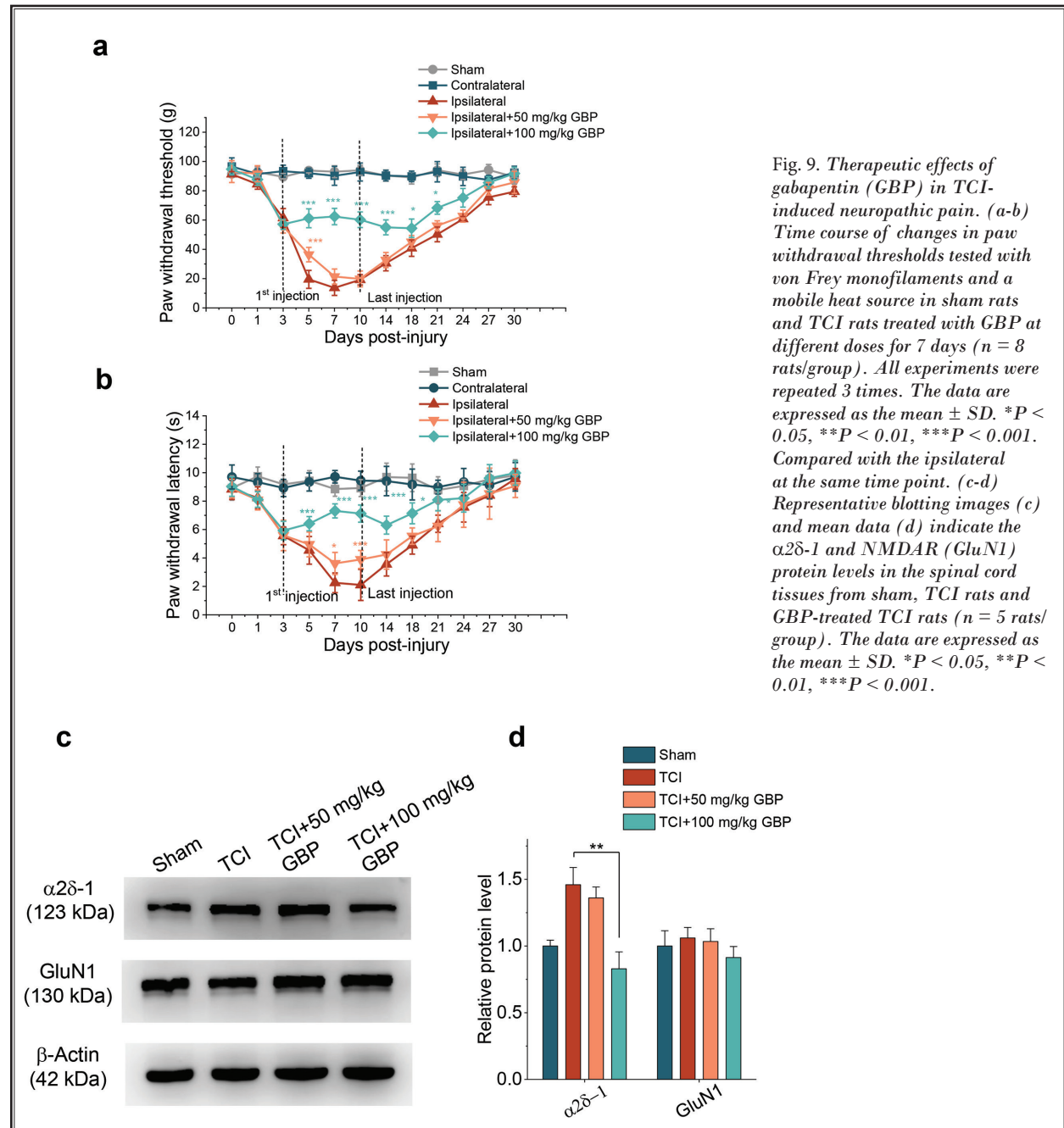


Fig. 8. Screening therapeutic targets for TCI-induced neuropathic pain. (a) The heatmap shows significant DEGs of mediators involved in NeP and inflammation in the DRGs. (b) qPCR analysis reveals the genes of potential drug targets for treating NeP in the DRGs. The expression level of *Cacna2d1* increased remarkably in ipsilateral DRGs at 14 days after TCI. Contra, the contralateral DRGs from the uninjured side; Ipsi-14d, the ipsilateral DRGs from the injured side at 14 days after TCI. (c) Immunofluorescence images of α2δ-1 in DRGs at 14 days after TCI. Contra, the contralateral DRGs from the uninjured side; Ipsi-14d, the ipsilateral DRGs from the injured side at 14 days after TCI surgery. (d) Quantification of *Cacna2d1* fluorescence intensity in the DRGs. The mean fluorescence intensity was measured with ImageJ analysis (V1.8, NIH). All experiments were repeated 3 times. The data are expressed as the mean ± SD (n = 5 rats/group). *P < 0.05, **P < 0.01, ***P < 0.001. (e) Immunofluorescence images of α2δ-1 in spinal cord 14 days after TCI. Contra, the contralateral uninjured side; Ipsi, the ipsilateral injured side. Scale bars: 200 μm. (f) Quantification of α2δ-1+ neurons in the ipsilateral and contralateral SDH. The positive cells in 10 random fields (magnification 400×) were counted with ImageJ analysis (V1.8, NIH). All experiments were repeated 3 times. The data are expressed as the mean ± SD (n = 8 rats/group). *P < 0.05, **P < 0.01, ***P < 0.001.

into the SDH. According to the different outcomes during the injury, sensory loss and ectopic activity can be induced by disconnection or neuroma, resulting in pain in the numb area (46). These changes are attributed to alterations in ion channels, such as the upregulation of sodium channels (Nav1.3, Nav1.7, and Nav1.8) and the loss of potassium channels. Additionally, nociceptive

sensitization mediated by central neural plasticity also contributes to NeP, especially orchestrating the chronicity of pain (47,48).

In our study, NeP following TCI surgery was also mediated by PNI and central neural plasticity. At first, when PNI occurred after TCI surgery, PN fibers showed swelling, structural disturbance, and demyelination.



Disruption of the somatosensory nervous system induced abnormal transmission signals, which generated NeP within 3 days postsurgery. Interestingly, the time course of changes in mechanical hypersensitivity is in accordance with the regeneration of injured nerve fibers, which suggests that recovering the integrity of the PN as early as possible may be a therapeutic strategy for treating surgery-associated NeP. Then, the activation of microglia and astrocytes in the ipsilateral SDH mediates the hyperexcitability of neurons, leading to chronic mechanical hypersensitivity for 30 days. Moreover, the inflammatory response in the injured nerve also plays a role in the generation of NeP. Early studies (35,49,50) have demonstrated that proinflammatory cytokines are associated with pain after PNI. TCI surgery in this study induced a Wallerian degeneration, which had apparent inflammation and immune cell infiltration. In particular, the invasion of macrophages did not completely disappear when NeP had recovered. The inflammatory reaction of the injured PN may be a promising target for NeP treatment.

RNA-seq of DRGs in TCI models also provided therapeutic strategies for treating NeP following surgery. In early studies (29,51), different NeP animal models showed distinct transcriptional signatures of DRGs, which suggests that different pathogeneses determine individual therapeutic targets. In the present study, we screened upregulated mediators involved in NeP in the ipsilateral DRGs and chose the most DEG, *Cacna2d1*, as the therapeutic target. *Cacna2d1* is the coding gene of voltage-gated calcium channel subtype $\alpha 2\delta$ -1, a target of the analgesic GBP (40,52). Specifically,

GBP treatment can effectively decrease the mechanical hypersensitivity of TCI rats. Of note, the upregulated DEGs in ipsilateral DRGs included several genes that are potential targets for analgesia, such as *Drd2*, *Cckrb*, *Npy2r*, and *P2rx2*. However, this study did not test the probable analgesic effects of these new drug targets. In the future, it will be essential to explore new analgesics approved for treating postoperative NeP.

CONCLUSIONS

We have established a rat model of surgery-associated NeP induced by TCI. This model presents prominent neuropathological changes in PNI and inflammation. By examining inflammatory cytokines and immune cells, we investigated the cycle of change during nerve injury and regeneration. Notably, we evaluated the transcriptomics of the injured nerves and DRGs, which identified the activation of inflammatory reactions in DRGs. Finally, we screened voltage-gated calcium channel subtype $\alpha 2\delta$ -1 as a therapeutic target and confirmed the effect of the analgesic GBP. Thus, our findings extend the mechanistic understanding of surgery-associated NeP and suggest an effective therapeutic strategy.

Funding

This project was supported by the National Natural Science Foundation of China (Grant Nos 82072495 and 82102548), the Chongqing Natural Science Foundation Postdoctoral Science Foundation Project (cstc2021jcyj-bshX0163), and the Basic Science and Innovation Project for graduate students of Chongqing (2021).

REFERENCES

1. Cohen SP, Vase L, Hooten WM. Chronic pain: An update on burden, best practices, and new advances. *Lancet* 2021; 397:2082-2097.
2. Haroutiunian S, Nikolajsen L, Finnerup NB, et al. The neuropathic component in persistent postsurgical pain: A systematic literature review. *Pain* 2013; 154:95-102.
3. Finnerup NB, Kuner R, Jensen TS. Neuropathic pain: From mechanisms to treatment. *Physiol Rev* 2021; 101:259-301.
4. Alles SRA, Smith PA. Etiology and pharmacology of neuropathic pain. *Pharmacol Rev* 2018; 70:315-347.
5. Cohen SP, Mao J. Neuropathic pain: Mechanisms and their clinical implications. *BMJ* 2014; 348:f7656.
6. Glare P, Aubrey KR, Myles PS. Transition from acute to chronic pain after surgery. *Lancet* 2019; 393:1537-1546.
7. Jensen RK, Kongsted A, Kjaer P, et al. Diagnosis and treatment of sciatica. *BMJ* 2019; 367:l6273.
8. Jensen TS, Finnerup NB. Allodynia and hyperalgesia in neuropathic pain: Clinical manifestations and mechanisms. *The Lancet Neurology* 2014; 13:924-935.
9. Arcidiacono UA, Armocida D, Pesce A, et al. Complex regional pain syndrome after spine surgery: A rare complication in mini-invasive lumbar spine surgery: An updated comprehensive review. *J Clin Med* 2022; 11:7409.
10. Joaquim AF, Makhni MC, Riew KD. Post-Operative nerve injuries after cervical spine surgery. *Int Orthop* 2019; 43:791-795.
11. Mahan MA. Nerve stretching: A history of tension. *J Neurosurg* 2019; 132:252-259.
12. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988; 33:87-107.
13. Decosterd I, Woolf CJ. Spared nerve injury: An animal model of persistent peripheral neuropathic pain. *Pain* 2000; 87:149-158.
14. Røytta M, Wei H, Pertovaara A. Spinal

- nerve ligation-induced neuropathy in the rat: Sensory disorders and correlation between histology of the peripheral nerves. *Pain* 1999; 80:161-170.
15. Li T, Chen X, Zhang C, et al. An update on reactive astrocytes in chronic pain. *J Neuroinflammation* 2019; 16:140.
16. Inoue K, Tsuda M. Microglia in neuropathic pain: Cellular and molecular mechanisms and therapeutic potential. *Nat Rev Neurosci* 2018; 19:138-152.
17. Westerlund N, Zdrojewska J, Padzik A, et al. Phosphorylation of SCG10/stathmin-2 determines multipolar stage exit and neuronal migration rate. *Nat Neurosci* 2011; 14:305-313.
18. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science* 2017; 356:1026-1030.
19. Medzhitov R. The spectrum of inflammatory responses. *Science* 2021; 374:1070-1075.
20. Mittal M, Siddiqui MR, Tran K, et al. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014; 20:1126-1167.
21. Fialkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radic Biol Med* 2007; 42:153-164.
22. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid Med Cell Longev* 2016; 2016:5698931.
23. Réaux-Le Goazigo A, Van Steenwinckel J, Rostène W, et al. Current status of chemokines in the adult CNS. *Prog Neurobiol* 2013; 104:67-92.
24. Murakami M, Kamimura D, Hirano T. Pleiotropy and specificity: Insights from the Interleukin 6 family of cytokines. *Immunity* 2019; 50:812-831.
25. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* 2016; 44:450-462.
26. Zigmund RE, Echevarria FD. Macrophage biology in the peripheral nervous system after injury. *Prog Neurobiol* 2019; 173:102-121.
27. Sah DW, Ossipo MH, Porreca F. Neurotrophic factors as novel therapeutics for neuropathic pain. *Nat Rev Drug Discov* 2003; 2:460-472.
28. Skorput AGJ, Zhang X, Waataja JJ, et al. Involvement of the VGF-derived peptide TLQP-62 in nerve injury-induced hypersensitivity and spinal neuroplasticity. *Pain* 2018; 159:1802-1813.
29. Sun W, Kou D, Yu Z, et al. A transcriptomic analysis of neuropathic pain in rat dorsal root ganglia following peripheral nerve injury. *Neuromolecular Med* 2020; 22:250-263.
30. Dickinson T, Fleetwood-Walker SM. VIP and PACAP: Very important in pain? *Trends Pharmacol Sci* 1999; 20:324-329.
31. Ralvenius WT, Benke D, Acuña MA, et al. Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. *Nat Commun* 2015; 6:6803.
32. Arcourt A, Gorham L, Dhandapani R, et al. Touch receptor-derived sensory information alleviates acute pain signaling and fine-tunes nociceptive reflex coordination. *Neuron* 2017; 93:179-193.
33. Dubin AE, Schmidt M, Mathur J, et al. Inflammatory signals enhance piezo2-mediated mechanosensitive currents. *Cell Rep* 2012; 2:511-517.
34. Petho G, Reeh PW. Sensory and signaling mechanisms of bradykinin, eicosanoids, platelet-activating factor, and nitric oxide in peripheral nociceptors. *Physiol Rev* 2012; 92:1699-1775.
35. Sommer C, Leinders M, Uceyler N. Inflammation in the pathophysiology of neuropathic pain. *Pain* 2018; 159:595-602.
36. Chen G, Zhang YQ, Qadri YJ, et al. Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain. *Neuron* 2018; 100:1292-1311.
37. Liang HY, Chen ZJ, Xiao H, et al. nNOS-Expressing neurons in the vmPFC transform pPVT-derived chronic pain signals into anxiety behaviors. *Nat Commun* 2020; 11:2501.
38. Scholz J, Woolf CJ. The neuropathic pain triad: Neurons, immune cells and glia. *Nat Neurosci* 2007; 10:1361-1368.
39. Li L, Chen SR, Zhou MH, et al. α 2 δ -1 switches the phenotype of synaptic AMPA receptors by physically disrupting heteromeric subunit assembly. *Cell Rep* 2021; 36:109396.
40. Deng M, Chen SR, Chen H, et al. α 2 δ -1-Bound N-Methyl-D-aspartate receptors mediate morphine-induced hyperalgesia and analgesic tolerance by potentiating glutamatergic input in rodents. *Anesthesiology* 2019; 130:804-819.
41. Cassidy JS, Ferron L, Kadurin I, et al. Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary α 2 δ -1 subunits. *Proc Natl Acad Sci U S A* 2014; 111:8979-8984.
42. Wang H, Zhou Y, Zhang Z. Postoperative dysesthesia in minimally invasive transforaminal lumbar interbody fusion: A report of five cases. *Eur Spine J* 2016; 25:1595-1600.
43. Zhang Z, Wang H, Zheng W. Postoperative dysesthesia in lumbar three-column resection osteotomies. *Eur Spine J* 2016; 25:2622-2628.
44. Okamoto K, Martin DP, Schmelzer JD, et al. Pro- and anti-inflammatory cytokine gene expression in rat sciatic nerve chronic constriction injury model of neuropathic pain. *Exp Neurol* 2001; 169:386-391.
45. Challa SR. Surgical animal models of neuropathic pain: Pros and cons. *Int J Neurosci* 2015; 125:170-174.
46. Rotshenker S. Wallerian degeneration: The innate-immune response to traumatic nerve injury. *J Neuroinflammation* 2011; 8:109.
47. Woolf CJ, Salter MW. Neuronal plasticity: Increasing the gain in pain. *Science* 2000; 288:1765-1769.
48. Todd AJ. Neuronal circuitry for pain processing in the dorsal horn. *Nat Rev Neurosci* 2010; 11:823-836.
49. Ellis A, Bennett DL. Neuroinflammation and the generation of neuropathic pain. *Br J Anaesth* 2013; 111:26-37.
50. Msheik Z, El Massry M, Rovini A, et al. The macrophage: A key player in the pathophysiology of peripheral neuropathies. *J Neuroinflammation* 2022; 19:97.
51. Cuevas-Diaz Duran R, Li Y, Garza Carbajal A, et al. Major differences in transcriptional alterations in dorsal root ganglia between spinal cord injury and peripheral neuropathic pain models. *J Neurotrauma* 2023; 40:883-900.
52. Chen J, Li L, Chen SR, et al. The α 2 δ -1-NMDA receptor complex is critically involved in neuropathic pain development and gabapentin therapeutic actions. *Cell Rep* 2018; 22:2307-2321.

SUPPLEMENTAL MATERIAL

METHODS

Animals

A total of 236 male and female Sprague-Dawley rats (200 to 250 g; Experimental Animal Center, Third Military Medical University, Chongqing, China) were used as indicated. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Third Military Medical University Ethical Committee for Animal Research. Animals were housed 3 per cage with food, watered at a temperature of 20-25°C, and kept on a 12-h light/dark cycle.

Surgical Procedure for TCI of the Peripheral Nerve

TCI surgery on rats was performed following the ethical guidelines of the International Association for the Study of Pain (IASP) regarding the use of laboratory animals. The animals were randomized to the TCI and sham groups. For rats in the TCI group, after receiving anesthesia with 1% pentobarbital sodium (4 mL/kg, intraperitoneal), the animal was placed in a lateral position. A small incision was made at the right hip, and the sciatic nerve was exposed. Then, the sciatic nerve was clipped with 4 vascular clamps (1.5 cm in length and 2 mm in width) and the compression was maintained for 10 minutes. The compression injury developed by vascular clamps ran 8 mm in the entire width. After that, the clamps were removed and the wound was closed with sutures. For sham surgery rats, the sciatic nerves were exposed without clipping. The animals were allowed to recover for at least 3 days before the behavioral tests were performed.

Gabapentin (GBP) Treatment and Drug Delivery

GBP was obtained from MCE (USA). After being dissolved in saline (25 mg/mL), GBP was injected intraperitoneally at a dose of 50 mg/kg or 100 mg/kg in TCI rats per day for 7 days ($n = 8$ rats). Meanwhile, an equal dose of saline was injected into the control rats.

Behavioral Assessments of Nociception

The tests of mechanical nociceptive threshold in the rats were conducted with calibrated von Frey monofilaments on the hind paw plantar surface (Electronic von Frey Anesthesiometer, IITC Life Sciences, USA). Briefly, all rats ($n = 160$) were adapted to the testing equipment for 30 minutes before baseline measurement. The experimenter evoked hind paw withdrawal by applying the handheld force transducer with a 0.5 mm² probe to the hind paw with a gradual increase in pressure. The intensity of the applied pressure was measured and displayed automatically, with the paw withdrawal threshold being evaluated from the average of 3 measurements. We tested the thermal sensitivity with the Hargreaves method using a Plantar Test Apparatus (IITC Life Sciences, USA). The rat was placed on the glass surface (maintained at 30°C), and its hindpaw was irradiated by a mobile radiant heat source under the glass. The paw withdrawal latency was recorded when the rat briskly lifted its foot. All behavioral experiments were performed in a blinded manner.

Histological Assessments

For the acquisition of tissue sections, animals ($n = 58$) were anesthetized with 1% pentobarbital sodium and perfused with phosphate-buffered saline. Then, the sciatic nerves were fixed in 4% paraformaldehyde overnight and embedded in optimal cutting temperature compound (OTC) for frozen sections. Then, 15 μ m-thick longitudinal sections were mounted on slides. The procedures were conducted according to the manufacturer's instructions for Masson-trichrome (Masson) staining and toluidine blue (TB) staining. For dihydroethidium (DHE) staining, sciatic nerve cryosections were incubated with DHE (1:200) at 37°C in the dark for 30 min.

Transmission Electron Microscopy (TEM) Evaluation

For the preparation of ultrathin sections for TEM, sciatic nerves from rats ($n = 20$) were fixed with 2.5% glutaraldehyde and postfixed with 1% osmium. Then the tissues were embedded in resin, cut into ultrathin sections (50 nm), and stained with gadolinium acetate and lead citrate. The TEM images were visualized and acquired using a Zeiss LIBRA 200 electron microscope.

Immunohistochemistry

Sections (15 μ m) were obtained from fresh frozen L5 and L6 spinal cords and DRGs using a cryostat (Leica CM1950,

UK). The sections were incubated with mouse anti-rat IBA1 (1:200, GT10312, GeneTex), mouse anti-GFAP (1:200, UM500005, Origene, USA), rabbit anti-NeuN (1:200, 26975-1-AP, Proteintech, China), rabbit anti-CD68 (1:200, 25747-1-AP, Proteintech, China), rabbit anti-MPO (1:300, 22225-1-AP, Proteintech, China), rabbit anti-COX2 (1:200, 66351-1-Ig, Proteintech, China), rabbit anti-nNOS (1:200, 18984-1-AP, Proteintech, China), rabbit anti-Cacna2d1 (1:100, 27453-1-AP, Proteintech, China), and rabbit anti-SCG10 (1:200, 10586-1-AP, Proteintech, China) antibodies, followed by Alexa Fluor 555-labeled Donkey Anti-Rabbit IgG (1:500, A0453, Beyotime) and Alexa Fluor 488-labeled Goat Anti-Mouse IgG (1:500, A0428, Beyotime, China). DAPI (1:500, ab104139, Abcam, Cambridge, UK) was used to counterstain nuclei. Immunoreactivity was visualized using a Zeiss LSM880 confocal microscope, and images were acquired using the LSM software (Zeiss, UK). The positive cells were quantified by counting the cells in 10 random fields (magnification 400×) with ImageJ analysis (V1.8, NIH). All experiments were repeated 3 times.

Quantitative Real-time Polymerase Chain Reaction (qPCR) Analysis

Total RNA of sciatic nerves and DRGs from rats ($n = 20$) was isolated by RNAiso Plus (9108, TaKaRa, Japan) following the manufacturer's instructions. cDNA was acquired by reverse transcription using RT Master Mix for qPCR II (gDNA digester plus) (HY-K0511A, MCE, USA). qPCR was performed with SYBR Green qPCR Master Mix (HY-K0501A, MCE, USA) in a CFX96 Touch Real-Time PCR Detection System (Bio-rad, USA). The primer sequences were shown in supplemental materials (Suppl. Table 1).

RNA Sequencing (RNA-seq) Analysis

Total RNA from sciatic nerves and DRGs from TCI rats ($n = 16$) was isolated using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then the total RNA was quantified using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA) and assessed with a Bioanalyzer 2100 (Agilent, CA, USA) with RIN > 7.0 . The poly (A) RNA was purified from 1 μ g of total RNA using Dynabeads Oligo (25-61005, Thermo Fisher, USA) and fragmented using Magnesium RNA Fragmentation Module (NEB, cat.e6150, USA). The cDNA was obtained by SuperScript™ II Reverse Transcriptase (Invitrogen, cat. 1896649, USA). Then the final cDNA library with an average insert size of 300 ± 50 bp was created. After that, 2×150 bp paired-end sequencing (PE150) on an Illumina Novaseq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) was performed following the vendor's recommended protocol. The significantly DEGs were filtered with thresholds of a $|\text{Log}_2(\text{fold change, FC})| > 2$ and P value < 0.05 with the R package DESeq2. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs were performed in the OmicStudio tools at <https://www.omicstudio.cn>. A summary of the RNA sequencing analysis and some of the most significantly expressed transcripts are shown in the supplemental materials (Suppl. Table 2-4).

Enzyme-linked Immunosorbent Assay (ELISA)

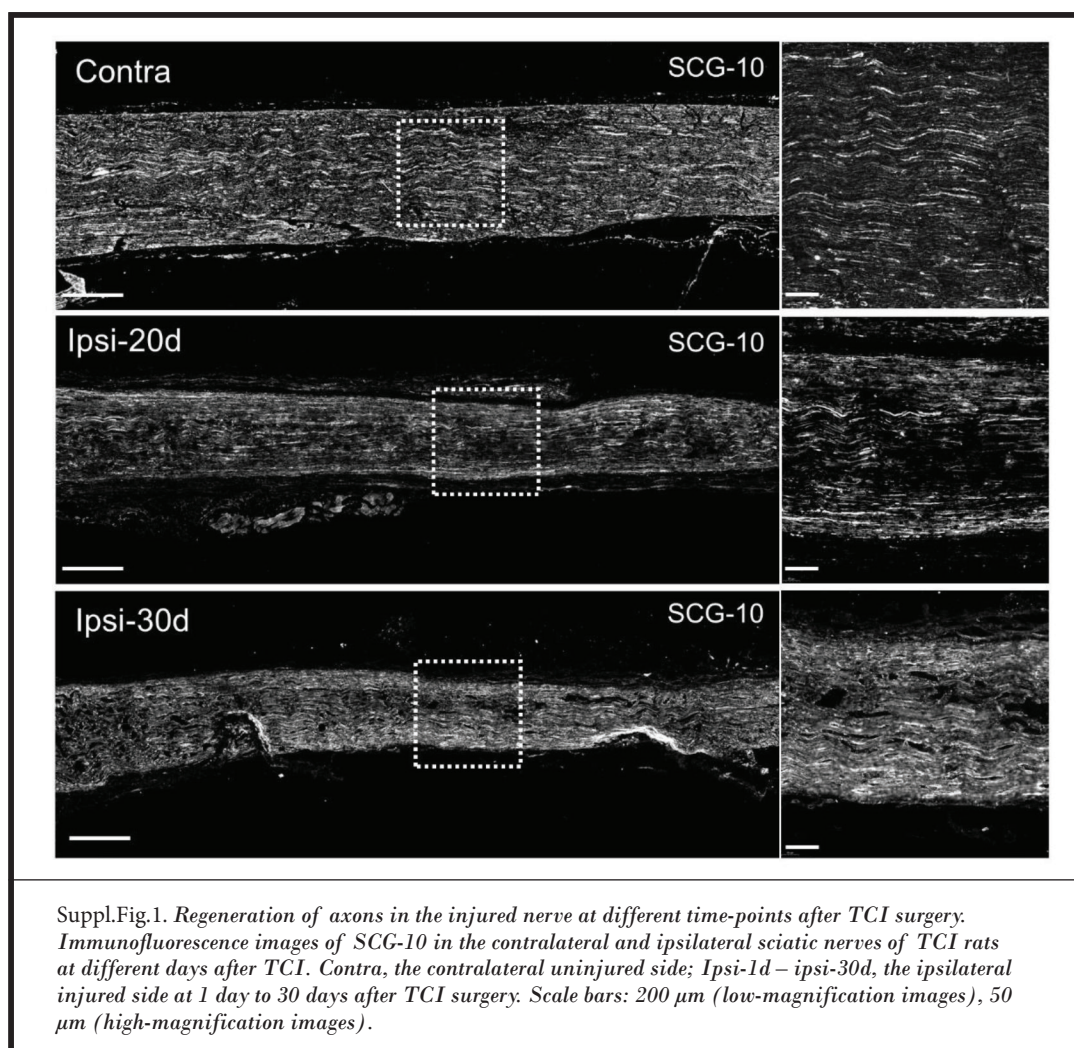
The sciatic nerve tissues were collected and homogenized in RIPA lysis buffer (P0013, Beyotime, China) for 30 minutes. Then the sample was centrifuged at 12,000 rpm for 10 minutes at 4°C, and the supernatant was acquired. The protein concentration was tested using the bicinchoninic acid (BCA) method (BCA Protein Assay Kit, P0012S, Beyotime, China). ELISA testing of IL-1 β , IL-6, TNF α , and TGF- β was performed according to the manufacturer's instructions (Rat IL-6 ELISA Kit, EK0412; Rat IL-1 α ELISA Kit, EK0390; Rat TNF α ELISA Kit, EK0526; Rat TGF- β 1 ELISA Kit, EK0514; Boster, China).

Western Blotting Analysis

Ca²⁺ channel subunit ($\alpha 2\delta$ -1) and NMDARs subunit (GluN1) protein levels in the spinal cord were quantified by immunoblotting. Spinal cord tissues from rat were collected and homogenized in ice-cold RIPA lysis buffer (P0013, Beyotime, China). Samples were then centrifuged at 12,000 rpm for 20 min at 4°C, and the supernatant was acquired. Thirty μ g of total proteins from each samples were loaded to 7.5% Tris-HCl SDS-PAGE (Bio-rad, TGX Stain-Free™ FastCast™ Acrylamide Kit) and transferred onto 0.45 μ m PVDF membranes. The membrane was incubated with the primary antibodies: anti- $\alpha 2\delta$ -1 (1:1000, sc-271697, Santa Cruz Biotechnology, USA), anti-GluN1 (1:500, 27676-1-AP, Proteintech, China) or anti- β -Actin (1:1000, BM3873, Boster, China). The membrane then incubated with anti-HRP-conjugated secondary antibody: anti-rabbit IgG (1:3000, A0208, Beyotime, China) or anti-mouse IgG (1:3000, A0216, Beyotime, China). The target protein bands were visualized by immobilon western HRP substrate (WBKLS0100, Mippipore) and quantified using the ImageJ analysis (V1.8, NIH). Relative protein levels were normalized to the β -Actin level on the same blot.

Statistical Analysis

Statistical analysis was performed with Origin 2021 (OriginLab, USA), and all quantitative data are expressed as the mean $\pm$ SD. Two-tailed Student's t test and one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test were used for comparisons between 2 groups. Two-way ANOVA followed by Tukey's honestly significant difference test was used to compare the behavioral data at different time points in more than 2 groups. A difference was considered statistically significant if the P value was < 0.05 .



Suppl. Table 1. *Comparison of different animal models of neuropathic pain.*

	CCI (Chronic Constriction Injury)	SNI (Spared Nerve Injury)	TCI (Transient Compression Injury)
Surgical Site	Sciatic nerve	Tibial nerve	Sciatic nerve
Surgical Tool	Ligature	Ligature, scissor	Vascular clamp
Surgical Procedure	Isolating the sciatic nerve and placed 4 loose ligature (5-0 chromic catgut) with 1-mm spacing.	Tightly ligating the tibial and common peroneal nerves and removing the 2-3 mm of the nerves distal to the ligation.	Isolating the sciatic nerve and clip it with 4 vascular clamps (1.5 cm in length and 2 mm in width) for 10 minutes.
Symptom	Mechanical allodynia Thermal hyperalgesia	Mechanical allodynia	Mechanical allodynia Thermal hyperalgesia
Symptom Duration	One month, reach maximum at 10 to 14 days after surgery.	One month to 15 months in mouse or rat animal models.	Twenty-eight days, reach maximum at 7 to 10 days postoperatively.

Suppl Table 2. *Specific primer sequences*

Gene	Primer	Sequence
IL-1 β	Forward	5'-GAAGTCAAGACCAAAGTGG-3'
	Reverse	5'-TGAAGTCAACTATGTCCCG-3'
TNF- α	Forward	5'-CAGCCAGGAGGGAGAAC-3'
	Reverse	5'-GTATGAGAGGGACGGAACC-3'
IL-6	Forward	5'-CACCAGGAACGAAAGTCAA-3'
	Reverse	5'-CAACAACATCAGTCCCAAGA-3'
TGF- β 1	Forward	5'-CCTACATTTGGAGCCTGGA-3'
	Reverse	5'-CCGGGTTGTGTGGTTG-3'
NLRP3	Forward	5'-TGTTGTCAGGATCTCGCA-3'
	Reverse	5'-AGTGAAGTAAGGCCGAAT-3'
CSF1 {AU: "[Csfl]"?}	Forward	5'-TGA CTAGGGGAAGGGAGAG-3'
	Reverse	5'-CTGGCAAGGGACTGGAG-3'
CSF1R {AU: "[Csflr]"?}	Forward	5'-GCCTTTGGTCTGGGCAAA-3'
	Reverse	5'-AGCCGTGGACTTGAGCATCT-3'
Cybb	Forward	5'-TTTTGAGTGGTTCGCAGAC-3'
	Reverse	5'-TCATCCCAGCCAGTAAGG-3'
NOX4	Forward	5'-CTTTCCGTCCCAAGCAC-3'
	Reverse	5'-CAGCCAAACAAGCAGACA-3'
Arg1	Forward	5'-CAGTATTCACCCCGGCTA-3'
	Reverse	5'-CCTCTGGTGTCTTCCCA-3'
Inos	Forward	5'-CGGAGAACAGCAGAGTTGG-3'
	Reverse	5'-GGAATAGCACCTGGGGTTT-3'
CCL2 {AU: "[Ccl2]"?}	Forward	5'-CAGGTCTCTGTACGCTTC-3'
	Reverse	5'-AGTTCTCCAGCCGACTCA-3'
IL-18	Forward	5'-GGACTGGCTGTGACCCTATC-3'
	Reverse	5'-TGTCCTGGCACACGTTTCTG-3'
Nnos	Forward	5'-GACTCACCCCGTCCTTTG-3'
	Reverse	5'-GCCTGCCCCATTAGCTT-3'
Gabar5	Forward	5'-GCTTGAGGACTTCCCGAT-3'
	Reverse	5'-TCTGGAGCCATCTTCTGC-3'
Cacna2d1	Forward	5'-AAAGCAAGGAGCAGGACATCG-3'
	Reverse	5'-GCCTCAAGGAGCCGTGGA-3'
Npy2r	Forward	5'-GGGGGATGTTAGCAATTTTC-3'
	Reverse	5'-TCAGCATCCAGAGAAGTGCA-3'
Drd2	Forward	5'-GGCCACACTGGTAATGCCGTGG-3'
	Reverse	5'-TGCTGATGGCACACAGGTTCAAG-3'
Cckbr	Forward	5'-CGCCATCTGCCGACCACTG-3'
	Reverse	5'-CCACACCCGGGATGAAGAAC-3'

Suppl Table 3. *Summary of RNA-seq analysis of the sciatic nerve and DRGs from the ipsilateral and contralateral side of TCI rats.*

Group		Ipsilateral Sciatic Nerve vs Contralateral Sciatic Nerve	Ipsilateral DRGs vs Contralateral DRGs
No of DEGs	Upregulated	3,255	765
	Downregulated	1,467	276
No of differentially expressed transcripts	Upregulated	3,052	877
	Downregulated	2,041	434

Suppl Table 4. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral sciatic nerve compared with the contralateral.*

Sequence Number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
1	ENSRNOT00000066896	Tnnt3	20.72	up	yes
2	ENSRNOT00000082304	Lgals3	18.90	up	yes
3	ENSRNOT00000082871	Myh4	18.58	up	yes
4	ENSRNOT00000088387	Ahnak	-18.45	down	yes
5	ENSRNOT00000012577	Serpina1	18.01	up	yes
6	ENSRNOT00000004174	Gc	17.54	up	yes
7	ENSRNOT00000042860	Rup2	17.50	up	yes
8	ENSRNOT00000058422	AABR07034632	17.47	up	yes
9	ENSRNOT00000051028	Mpdz	-17.46	down	yes
10	ENSRNOT00000076906	RGD1566134	17.43	up	yes
11	ENSRNOT00000075494	C3	17.09	up	yes
12	ENSRNOT00000089382	Mup5	17.03	up	yes
13	ENSRNOT00000012293	LOC259244	16.99	up	yes
14	ENSRNOT00000008840	Fabp1	16.97	up	yes
15	ENSRNOT00000038898	Lilrb4	16.76	up	yes
16	ENSRNOT00000031935	Hk3	16.71	up	yes
17	ENSRNOT00000091038	AABR07007032	-16.69	down	yes
18	ENSRNOT00000086554	Zfp385a	16.68	up	yes
19	ENSRNOT00000009248	Ambp	16.58	up	yes
20	ENSRNOT00000091468	Ppt2	16.44	up	yes
21	ENSRNOT00000088905	Vps29	16.40	up	yes
22	ENSRNOT00000090488	AABR07030791	16.39	up	yes
23	ENSRNOT00000082271	Plec	16.34	up	yes
24	ENSRNOT00000048739	Fetub	16.31	up	yes
25	ENSRNOT00000064572	Agfg2	-16.31	down	yes
26	ENSRNOT00000046857	LOC259244	16.30	up	yes
27	ENSRNOT00000012075	Ass1	16.30	up	yes
28	ENSRNOT00000086806	Cdk1	16.22	up	yes
29	ENSRNOT00000013896	Serpina3c	16.21	up	yes
30	ENSRNOT00000035875	Ahctf1	-16.21	down	yes
31	ENSRNOT00000004662	Apoa2	16.20	up	yes
32	ENSRNOT00000091389	Fmo2	-16.10	down	yes
33	ENSRNOT00000068043	LOC100360095	16.01	up	yes
34	ENSRNOT00000087017	LOC299282	15.96	up	yes
35	ENSRNOT00000009111	Aldob	15.87	up	yes

Suppl Table 4 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral sciatic nerve compared with the contralateral.*

Sequence Number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
36	ENSRNOT00000007903	LOC680406	15.87	up	yes
37	ENSRNOT00000046456	Mta1	15.83	up	yes
38	ENSRNOT00000031889	Chodl	-15.80	down	yes
39	ENSRNOT00000006218	Rhot1	-15.75	down	yes
40	ENSRNOT00000087067	Me3	-15.72	down	yes
41	ENSRNOT00000090638	AABR07001382	-15.69	down	yes
42	ENSRNOT00000016424	Oplah	15.53	up	yes
43	ENSRNOT00000077967	AABR07035437	-15.50	down	yes
44	ENSRNOT00000085690	Ash1l	-15.48	down	yes
45	ENSRNOT00000081786	Btaf1	-15.36	down	yes
46	ENSRNOT00000067149	Plce1	-15.32	down	yes
47	ENSRNOT00000084350	Sec24c	-15.32	down	yes
48	ENSRNOT00000064091	Fga	15.20	up	yes
49	ENSRNOT00000073404	Tmem45a1	14.99	up	yes
50	ENSRNOT00000014041	Mmp7	14.95	up	yes
51	ENSRNOT00000071633	Cyp2c13	14.92	up	yes
52	ENSRNOT00000086488	AABR07004549	14.84	up	yes
53	ENSRNOT00000082336	Slc4a5	14.84	up	yes
54	ENSRNOT00000047595	LOC681544	14.80	up	yes
55	ENSRNOT00000067689	AABR07017902	14.78	up	yes
56	ENSRNOT00000093708	Mpl	-14.66	down	yes
57	ENSRNOT00000022721	Tat	14.59	up	yes
58	ENSRNOT00000003951	Ren	14.56	up	yes
59	ENSRNOT00000086599	Myh4	14.55	up	yes
60	ENSRNOT00000008319	RGD1565355	14.54	up	yes
61	ENSRNOT00000015190	Mat1a	14.52	up	yes
62	ENSRNOT00000023890	Neddl4l	-14.51	down	yes
63	ENSRNOT00000080658	Apoa4	14.50	up	yes
64	ENSRNOT00000005665	Abca8a	-14.50	down	yes
65	ENSRNOT00000075167	Plaur	-14.46	down	yes
66	ENSRNOT00000086363	LOC690045	14.43	up	yes
67	ENSRNOT00000047243	Dync1i2	-14.42	down	yes
68	ENSRNOT00000081488	Mug1	14.39	up	yes
69	ENSRNOT00000013045	Serpina4	14.34	up	yes
70	ENSRNOT00000080132	Hps5	14.32	up	yes
71	ENSRNOT00000021653	Cyp1a2	14.30	up	yes
72	ENSRNOT00000058497	Fcgr2b	14.29	up	yes
73	ENSRNOT00000012545	Clasp2	-14.29	down	yes
74	ENSRNOT00000077285	Arpc2	14.28	up	yes
75	ENSRNOT00000091503	AABR07070161	-14.25	down	yes
76	ENSRNOT00000080065	Dact2	14.25	up	yes
77	ENSRNOT00000091757	Mcpt1	14.20	up	yes

Suppl Table 4 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral sciatic nerve compared with the contralateral.*

Sequence Number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
78	ENSRNOT00000019023	Cps1	14.19	up	yes
79	ENSRNOT00000084867	Knq1	14.18	up	yes
80	ENSRNOT00000057472	Slc11a1	14.17	up	yes
81	ENSRNOT00000044944	Gsta5	14.17	up	yes
82	ENSRNOT00000060370	AABR07035479	-14.15	down	yes
83	ENSRNOT00000012092	Apcs	14.15	up	yes
84	ENSRNOT00000060250	AABR07017902	14.11	up	yes
85	ENSRNOT00000040325	Cyp2c7	14.11	up	yes
86	ENSRNOT00000093188	RGD735065	14.11	up	yes
87	ENSRNOT00000017840	Cyp2c23	14.11	up	yes
88	ENSRNOT00000076813	AABR07035374	14.06	up	yes
89	ENSRNOT00000031653	Mcemp1	14.02	up	yes
90	ENSRNOT00000080965	Hrob	13.99	up	yes
91	ENSRNOT00000093679	Fhod3	-13.96	down	yes
92	ENSRNOT00000079704	AABR07062170	13.90	up	yes
93	ENSRNOT00000044520	AABR07044383	-13.88	down	yes
94	ENSRNOT00000052369	Ugt2b15	13.85	up	yes
95	ENSRNOT0000006991	Tfap2c	13.83	up	yes
96	ENSRNOT00000024750	Kcnp2	-13.83	down	yes
97	ENSRNOT00000055810	Pced1a	13.81	up	yes
98	ENSRNOT00000024622	LOC501233	13.76	up	yes
99	ENSRNOT00000088923	Krt75	13.72	up	yes
100	ENSRNOT00000083068	Clta	-13.70	down	yes
101	ENSRNOT00000087664	Pigr	13.68	up	yes
102	ENSRNOT00000092079	Cd99l2	-13.67	down	yes
103	ENSRNOT00000078265	Angel1	13.66	up	yes
104	ENSRNOT00000011801	Nav1	-13.60	down	yes
105	ENSRNOT00000017834	Scd4	-13.60	down	yes
106	ENSRNOT00000082868	AABR07070161	-13.54	down	yes
107	ENSRNOT00000071432	Tspan10	13.51	up	yes
108	ENSRNOT00000061417	Dsc2	13.50	up	yes
109	ENSRNOT00000039417	AABR07073350	13.49	up	yes
110	ENSRNOT00000018545	C9	13.49	up	yes
111	ENSRNOT00000039864	Drp2	-13.46	down	yes
112	ENSRNOT00000044408	Mug1	13.46	up	yes
113	ENSRNOT00000081015	Cchcr1	13.45	up	yes
114	ENSRNOT00000014248	Fgl1	13.43	up	yes
115	ENSRNOT00000081848	Mrap2	13.42	up	yes
116	ENSRNOT00000087240	Myog	13.42	up	yes
117	ENSRNOT00000019969	Mug1	13.36	up	yes
118	ENSRNOT00000076653	Tp53i3	13.32	up	yes
119	ENSRNOT0000003088	Lnx1	-13.30	down	yes

Suppl Table 4 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral sciatic nerve compared with the contralateral.*

Sequence Number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
120	ENSRNOT00000048344	Itih4	13.28	up	yes
121	ENSRNOT00000022702	Gulo	13.28	up	yes
122	ENSRNOT00000090143	Tacc2	-13.26	down	yes
123	ENSRNOT00000087257	Cyp3a23/3a1	13.25	up	yes
124	ENSRNOT00000079797	Cadm1	13.24	up	yes
125	ENSRNOT00000011486	Angptl3	13.20	up	yes
126	ENSRNOT00000081399	Gata6	13.19	p	yes
127	ENSRNOT00000054724	Cyp2c22	13.18	up	yes
128	ENSRNOT00000066586	F12	13.17	up	yes
129	ENSRNOT00000093297	Mycbp2	-13.17	down	yes
130	ENSRNOT00000052315	Efemp2	13.17	up	yes
131	ENSRNOT00000037974	Map2	13.17	up	yes
132	ENSRNOT00000014909	Cpb2	13.15	up	yes
133	ENSRNOT00000068697	Calr4	13.15	up	yes
134	ENSRNOT00000013175	Serpina3m	13.08	up	yes
135	ENSRNOT00000001285	Cyp3a18	13.04	up	yes
136	ENSRNOT00000006249	Parpbp	13.03	up	yes
137	ENSRNOT00000060535	Afm	13.00	up	yes
138	ENSRNOT00000054742	Cyp2c6v1	12.97	up	yes
139	ENSRNOT00000052006	Stk32b	-12.97	down	yes
140	ENSRNOT00000012500	Serpina6	12.96	up	yes
141	ENSRNOT00000028249	Cyp2a1	12.96	up	yes
142	ENSRNOT00000081484	Mat1a	12.94	up	yes
143	ENSRNOT00000059468	Timd2	12.89	up	yes
144	ENSRNOT00000041130	Itpr1	-12.86	down	yes
145	ENSRNOT00000042251	Slc8a1	12.86	up	yes
146	ENSRNOT00000084826	Kcnh2	-12.85	down	yes
147	ENSRNOT00000011727	Mmp12	12.85	up	yes
148	ENSRNOT00000050551	Nlrp6	12.85	up	yes
149	ENSRNOT00000065145	Sdk2	-12.82	down	yes
150	ENSRNOT00000076973	Unc45b	12.82	up	yes
151	ENSRNOT00000084973	Serpind1	12.82	up	yes
152	ENSRNOT00000004559	F9	12.79	up	yes
153	ENSRNOT00000036876	Rnf144b	-12.77	down	yes
154	ENSRNOT00000073636	Bdnf	12.73	up	yes
155	ENSRNOT00000050149	Kcna2	-12.70	down	yes
156	ENSRNOT00000019596	Proc	12.70	up	yes
157	ENSRNOT00000019808	Nlrp6	12.66	up	yes
158	ENSRNOT00000092985	Cyp2c6v1	12.63	up	yes
159	ENSRNOT00000085349	Lipc	12.59	up	yes
160	ENSRNOT00000091575	LOC100911374	-12.57	down	yes
161	ENSRNOT00000079192	Mug1	12.51	up	yes

Suppl Table 4 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral sciatic nerve compared with the contralateral.*

Sequence Number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
162	ENSRNOT00000067650	Noa1	12.46	up	yes
163	ENSRNOT00000050760	Igf2	-12.44	down	yes
164	ENSRNOT00000032229	Bcl2l14	12.43	up	yes
165	ENSRNOT00000022270	Dsg2	12.41	up	yes
166	ENSRNOT00000074103	Cyp2c6v1	12.41	up	yes
167	ENSRNOT00000038817	Itih2	12.38	up	yes
168	ENSRNOT00000065601	Tmc5	12.31	up	yes
169	ENSRNOT00000056364	Il4i1	12.30	up	yes
170	ENSRNOT00000075680	LOC100911660	12.30	up	yes
171	ENSRNOT00000073466	Lmod2	12.26	up	yes
172	ENSRNOT00000008461	Kcnk16	-12.20	down	yes
173	ENSRNOT00000079754	Hsd3b5	12.18	up	yes
174	ENSRNOT00000010100	C8b	12.18	up	yes
175	ENSRNOT00000067850	C8a	12.17	up	yes
176	ENSRNOT00000078993	Adora1	-12.17	down	yes
177	ENSRNOT00000041569	Hdc	-12.16	down	yes
178	ENSRNOT0000007371	Apob	12.09	up	yes
179	ENSRNOT00000044131	Lepr	-12.09	down	yes
180	ENSRNOT00000018628	Igsf10	12.02	up	yes
181	ENSRNOT00000089093	Dnmt3a	12.01	up	yes
182	ENSRNOT00000034642	Nipal1	-11.99	down	yes
183	ENSRNOT00000041924	Nhs	-11.97	down	yes
184	ENSRNOT00000020414	Bard1	11.96	up	yes
185	ENSRNOT00000072396	LOC108348114	11.96	up	yes
186	ENSRNOT00000003921	Alb	11.95	up	yes
187	ENSRNOT00000049681	Acan	11.94	up	yes
188	ENSRNOT00000003498	Hsd17b6	11.87	up	yes
189	ENSRNOT00000080918	LOC102546889	-11.87	down	yes
190	ENSRNOT00000086342	Zfp438	-11.82	down	yes
191	ENSRNOT00000092736	C5	11.74	up	yes
192	ENSRNOT00000089334	Sorcs2	11.70	up	yes
193	ENSRNOT00000047472	AABR07001382	-11.69	down	yes
194	ENSRNOT00000050330	Unc5c	-11.69	down	yes
195	ENSRNOT00000013182	Acod1	11.67	up	yes
196	ENSRNOT00000043197	Prr36	-11.52	down	yes
197	ENSRNOT00000074209	Etnppl	11.41	up	yes
198	ENSRNOT00000026054	Kif27	-11.36	down	yes
199	ENSRNOT00000076699	Yipf6	-11.14	down	yes
200	ENSRNOT00000003255	Golgb1	-11.12	down	yes

Suppl Table 5. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral DRGs compared with the contralateral.*

Sequence number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
1	ENSRNOT00000040232	Gabbr1	-19.27	down	yes
2	ENSRNOT00000025477	Vip	17.96	up	yes
3	ENSRNOT00000056485	Lce1f	17.37	up	yes
4	ENSRNOT00000012577	Serpina1	-17.21	down	yes
5	ENSRNOT00000012174	Sprr1a	17.16	up	yes
6	ENSRNOT00000007103	Myef2	17.12	up	yes
7	ENSRNOT00000018163	Crisp3	17.04	up	yes
8	ENSRNOT00000078969	Reep2	-16.94	down	yes
9	ENSRNOT00000088721	Flot2	-16.72	down	yes
10	ENSRNOT00000079672	Ube2e3	16.07	up	yes
11	ENSRNOT00000076906	RGD1566134	-16.06	down	yes
12	ENSRNOT00000004174	Gc	-15.97	down	yes
13	ENSRNOT00000042860	Rup2	-15.87	down	yes
14	ENSRNOT00000058422	AABR07034632	-15.85	down	yes
15	ENSRNOT00000008037	Ucn	15.84	up	yes
16	ENSRNOT00000067655	Mknk1	15.60	up	yes
17	ENSRNOT00000074942	Apoc3	-15.48	down	yes
18	ENSRNOT00000046857	LOC259244	-15.47	down	yes
19	ENSRNOT00000008840	Fabp1	-15.43	down	yes
20	ENSRNOT00000048752	Brd8	15.31	up	yes
21	ENSRNOT00000092791	Vip	15.25	up	yes
22	ENSRNOT00000009813	Fgb	-15.22	down	yes
23	ENSRNOT00000078034	Ahctf1	-15.19	down	yes
24	ENSRNOT00000084776	Ncaph2	15.14	up	yes
25	ENSRNOT00000005467	Cep170	-15.08	down	yes
26	ENSRNOT00000009248	Ambp	-15.07	down	yes
27	ENSRNOT00000088740	AABR07044837	14.98	up	yes
28	ENSRNOT00000075987	Bud31	-14.88	down	yes
29	ENSRNOT00000051835	Kcnd3	-14.71	down	yes
30	ENSRNOT00000013896	Serpina3c	-14.67	down	yes
31	ENSRNOT00000013732	Il6	14.63	up	yes
32	ENSRNOT00000087017	LOC299282	-14.62	down	yes
33	ENSRNOT00000083007	En1	14.58	up	yes
34	ENSRNOT00000016883	Cyp2e1	-14.53	down	yes
35	ENSRNOT00000046456	Mta1	-14.52	down	yes
36	ENSRNOT00000041430	Gzmb12	14.50	up	yes
37	ENSRNOT00000007903	LOC680406	-14.45	down	yes
38	ENSRNOT00000018934	Acat2l1	14.43	up	yes
39	ENSRNOT00000064905	Scml4	-14.39	down	yes
40	ENSRNOT00000082161	Cacna2d1	14.36	up	yes
41	ENSRNOT00000059660	Gzmb	14.36	up	yes
42	ENSRNOT00000068043	LOC100360095	-14.28	down	yes
43	ENSRNOT00000046676	LOC681364	14.05	up	yes
44	ENSRNOT00000090727	Lmo7	13.92	up	yes
45	ENSRNOT00000030036	Ptk2b	-13.91	down	yes

Suppl Table 5 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral DRGs compared with the contralateral.*

Sequence number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
46	ENSRNOT00000064091	Fga	-13.84	down	yes
47	ENSRNOT00000088853	Cyp2c11	-13.69	down	yes
48	ENSRNOT00000087162	Bst2	13.54	up	yes
49	ENSRNOT00000086488	AABR07004549	-13.53	down	yes
50	ENSRNOT00000020566	Vtn1	13.51	up	yes
51	ENSRNOT00000088065	Tctn3	13.44	up	yes
52	ENSRNOT00000086096	Slc16a3	13.42	up	yes
53	ENSRNOT00000077967	AABR07035437	13.38	up	yes
54	ENSRNOT00000021653	Cyp1a2	-13.37	down	yes
55	ENSRNOT00000068410	Adam22	13.35	up	yes
56	ENSRNOT00000081798	Fut7	-13.34	down	yes
57	ENSRNOT00000071633	Cyp2c13	-13.33	down	yes
58	ENSRNOT00000064168	Ildr2	13.30	up	yes
59	ENSRNOT00000075078	Shank2	13.26	up	yes
60	ENSRNOT00000064951	Pds5b	13.24	up	yes
61	ENSRNOT00000067585	AABR07066510	13.21	up	yes
62	ENSRNOT00000011727	Mmp12	13.17	up	yes
63	ENSRNOT00000080686	Tprg1l	13.15	up	yes
64	ENSRNOT00000066798	Synj2	13.15	up	yes
65	ENSRNOT00000012310	Mmp3	13.15	up	yes
66	ENSRNOT00000040325	Cyp2c7	-13.09	down	yes
67	ENSRNOT00000085277	Ager	-13.09	down	yes
68	ENSRNOT00000081488	Mug1	-13.08	down	yes
69	ENSRNOT00000020002	Anpep	-13.05	down	yes
70	ENSRNOT00000022721	Tat	-13.02	down	yes
71	ENSRNOT00000091652	Ablim2	-12.96	down	yes
72	ENSRNOT00000076111	Pappa1	-12.96	down	yes
73	ENSRNOT00000015190	Mat1a	-12.94	down	yes
74	ENSRNOT00000022809	Hfe	12.86	up	yes
75	ENSRNOT00000017840	Cyp2c23	-12.80	down	yes
76	ENSRNOT00000052369	Ugt2b15	-12.73	down	yes
77	ENSRNOT00000013045	Serpina4	-12.71	down	yes
78	ENSRNOT00000047595	LOC681544	-12.68	down	yes
79	ENSRNOT00000080132	Hps5	-12.65	down	yes
80	ENSRNOT00000084867	Kng1	-12.64	down	yes
81	ENSRNOT00000002680	Sult1e1	-12.62	down	yes
82	ENSRNOT00000092044	Nccrp1	12.58	up	yes
83	ENSRNOT00000088981	Tep1	-12.58	down	yes
84	ENSRNOT00000018795	Hsd17b2	-12.47	down	yes
85	ENSRNOT00000087091	Fam161a	12.42	up	yes
86	ENSRNOT00000090644	Fgl1	-12.40	down	yes
87	ENSRNOT00000086554	Zfp385a	12.34	up	yes
88	ENSRNOT00000019969	Mug1	-12.34	down	yes
89	ENSRNOT00000018668	Tbc1d12	12.29	up	yes
90	ENSRNOT00000007671	AABR07058423	12.15	up	yes

Suppl Table 5 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral DRGs compared with the contralateral.*

Sequence number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
91	ENSRNOT00000084339	Chrna4	12.13	up	yes
92	ENSRNOT00000041569	Hdc	12.11	up	yes
93	ENSRNOT00000033151	Impad1	-12.05	down	yes
94	ENSRNOT00000000142	Dnajb5	12.01	up	yes
95	ENSRNOT00000051751	Itih1	-11.98	down	yes
96	ENSRNOT00000025119	Sncaip	-11.93	down	yes
97	ENSRNOT00000024622	LOC501233	-11.87	down	yes
98	ENSRNOT00000044257	Shank1	-11.86	down	yes
99	ENSRNOT00000086712	AABR07016865	-11.82	down	yes
100	ENSRNOT0000004559	F9	-11.79	down	yes
101	ENSRNOT00000005461	C4bpa	-11.70	down	yes
102	ENSRNOT00000066855	Stac2	11.64	up	yes
103	ENSRNOT00000081570	Rgs12	11.63	up	yes
104	ENSRNOT00000001493	Nos1	11.62	up	yes
105	ENSRNOT00000077458	Ltbp2	11.60	up	yes
106	ENSRNOT00000022702	Gulo	-11.49	down	yes
107	ENSRNOT00000076725	Phka1	-11.49	down	yes
108	ENSRNOT00000044408	Mug1	-11.39	down	yes
109	ENSRNOT00000091561	Optn	-11.22	down	yes
110	ENSRNOT00000089332	Pappa2	11.21	up	yes
111	ENSRNOT00000088734	Agpat1	11.04	up	yes
112	ENSRNOT00000078405	Lipn	10.98	up	yes
113	ENSRNOT00000010118	Akap9	-10.97	down	yes
114	ENSRNOT00000068238	Slc4a10	-10.70	down	yes
115	ENSRNOT00000092327	Shank1	-10.66	down	yes
116	ENSRNOT00000088078	Glmn	-9.65	down	yes
117	ENSRNOT00000003921	Alb	-9.53	down	yes
118	ENSRNOT00000078972	RT1-A1	9.17	up	yes
119	ENSRNOT00000089382	Mup5	-8.51	down	yes
120	ENSRNOT00000079093	Me2	-8.44	down	yes
121	ENSRNOT00000084053	Lss	-8.24	down	yes
122	ENSRNOT00000081200	AABR07069821	8.15	up	yes
123	ENSRNOT00000027315	Tgm1	8.05	up	yes
124	ENSRNOT00000061687	RGD1565355	-7.83	down	yes
125	ENSRNOT00000002003	Cldn4	7.75	up	yes
126	ENSRNOT00000019311	Csrp3	7.69	up	yes
127	ENSRNOT00000074412	Igsf23	7.68	up	yes
128	ENSRNOT00000019555	Rnf4	-7.64	down	yes
129	ENSRNOT00000072179	Rack1	-7.60	down	yes
130	ENSRNOT00000092211	Csrp3	7.52	up	yes
131	ENSRNOT00000080266	Pacsin3	7.46	up	yes
132	ENSRNOT00000066486	Arhgap29	7.43	up	yes
133	ENSRNOT00000006830	Birc6	7.37	up	yes
134	ENSRNOT00000091978	Stac2	7.35	up	yes
135	ENSRNOT00000035875	Ahctf1	7.19	up	yes

Suppl Table 5 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral DRGs compared with the contralateral.*

Sequence number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
136	ENSRNOT00000003409	Serpinb2	7.15	up	yes
137	ENSRNOT00000079237	Cldn7	7.06	up	yes
138	ENSRNOT00000022233	F2	-7.05	down	yes
139	ENSRNOT00000090125	Ehmt2	-7.00	down	yes
140	ENSRNOT00000006106	Il24	6.96	up	yes
141	ENSRNOT00000051239	Rasgrp3	-6.92	down	yes
142	ENSRNOT00000059823	Lsmem1	6.91	up	yes
143	ENSRNOT00000029706	Fmo9	6.75	up	yes
144	ENSRNOT00000012080	Draxin	6.75	up	yes
145	ENSRNOT00000092372	Rgl2	-6.72	down	yes
146	ENSRNOT00000078469	LOC102554842	6.65	up	yes
147	ENSRNOT00000013145	LOC100912228	6.60	up	yes
148	ENSRNOT00000083342	Optn	-6.54	down	yes
149	ENSRNOT00000067538	Nfib	6.51	up	yes
150	ENSRNOT00000004662	Apoa2	-6.45	down	yes
151	ENSRNOT00000034521	Fgg	-6.43	down	yes
152	ENSRNOT00000074351	Npy	6.43	up	yes
153	ENSRNOT00000025045	Ugt1a6	6.42	up	yes
154	ENSRNOT00000031586	Pck1	-6.38	down	yes
155	ENSRNOT00000090149	Chd2	6.32	up	yes
156	ENSRNOT00000012413	Cyp2d2	-6.26	down	yes
157	ENSRNOT00000025258	Ankrd1	6.20	up	yes
158	ENSRNOT00000015336	Bhmt	-6.08	down	yes
159	ENSRNOT00000008212	Reg3b	6.02	up	yes
160	ENSRNOT00000059763	Srd5a3	5.95	up	yes
161	ENSRNOT00000086425	Aar2	-5.84	down	yes
162	ENSRNOT00000058414	Tbc1d14	-5.80	down	yes
163	ENSRNOT00000077325	Setdb1	-5.80	down	yes
164	ENSRNOT00000022113	Ttr	-5.77	down	yes
165	ENSRNOT00000023829	Htr2b	5.74	up	yes
166	ENSRNOT00000025052	Aars	5.73	up	yes
167	ENSRNOT00000014073	LOC299282	5.67	up	yes
168	ENSRNOT00000088206	AABR07057353	-5.64	down	yes
169	ENSRNOT00000087781	Tnni2	-5.62	down	yes
170	ENSRNOT00000065224	Thbs4	-5.56	down	yes
171	ENSRNOT00000005875	AABR07057436	-5.50	down	yes
172	ENSRNOT00000085979	Nrcam	-5.49	down	yes
173	ENSRNOT00000076737	Tnfsf13b	5.48	up	yes
174	ENSRNOT00000080900	RT1-A1	-5.48	down	yes
175	ENSRNOT00000019023	Cps1	-5.42	down	yes
176	ENSRNOT00000018545	C9	-5.38	down	yes
177	ENSRNOT00000087664	Pigr	-5.37	down	yes
178	ENSRNOT00000017714	Chst10	5.37	up	yes
179	ENSRNOT00000020425	Gal	5.36	up	yes
180	ENSRNOT00000003748	Serpinc1	-5.31	down	yes

Suppl Table 5 cont. *Two hundred transcripts of the most significantly expressed DEGs in the ipsilateral DRGs compared with the contralateral.*

Sequence number	Transcript ID	Gene Name	log2 (fold change)	Regulation	Significant
181	ENSRNOT00000060515	LOC100911576	5.31	up	yes
182	ENSRNOT00000079189	Nosip	-5.31	down	yes
183	ENSRNOT00000076467	Pappa1	5.29	up	yes
184	ENSRNOT00000061027	RT1-CE1	5.28	up	yes
185	ENSRNOT00000047958	Grk6	5.27	up	yes
186	ENSRNOT00000015432	Ankrd44	5.22	up	yes
187	ENSRNOT00000091629	AABR07013477	5.18	up	yes
188	ENSRNOT00000005085	Atf3	5.01	up	yes
189	ENSRNOT00000051135	Xk	5.00	up	yes
190	ENSRNOT00000020141	Wdr63	4.93	up	yes
191	ENSRNOT00000010156	Tacstd2	4.93	up	yes
192	ENSRNOT00000005844	Pah	-4.86	down	yes
193	ENSRNOT00000077793	Clec7a	4.80	up	yes
194	ENSRNOT00000086268	AABR07028998	4.74	up	yes
195	ENSRNOT00000000058	Crp	-4.73	down	yes
196	ENSRNOT00000016637	Zc3h12c	4.62	up	yes
197	ENSRNOT00000019096	Plch2	4.60	up	yes
198	ENSRNOT00000024077	Cckbr	4.51	up	yes
199	ENSRNOT00000085334	Ptprb	4.47	up	yes
200	ENSRNOT00000090039	Flot1	4.46	up	yes