European Journal of Medical Research

RESEARCH

Open Access

# Resolvin D1 accelerates resolution of neuroinflammation by inhibiting microglia activation through the BDNF/TrkB signaling pathway

Cunju Bo[1], Xiaoming Liu[1], Yongjian Liu[1], Lingjun Xu[1] and Qiaodong Huang[1*]

## Abstract

**Background** Neuropathic pain is characterized by hyperalgesia, allodynia, and inflammation and it is often resistant to treatment. The formyl peptide receptor 2 (ALX/FPR2), a G-protein-coupled receptor, has been implicated in resolving inflammation, making its agonist, Resolvin D1 (RvD1), a potential therapeutic agent. Previous studies suggest that RvD1 alleviates neuropathic pain via anti-inflammatory effects, but its mechanisms remain unclear, particularly in relation to microglial activation and the brain-derived neurotrophic factor (BDNF)/TrkB signaling pathway.

**Objective** To investigate the analgesic effects of RvD1 in a spared nerve injury (SNI) model of neuropathic pain and explore its mechanisms through the regulation of neuroinflammation and the BDNF/TrkB signaling pathway.

**Methods** SNI mice received intrathecal RvD1 at varying doses (10–40 ng) to determine its efficacy in reducing mechanical allodynia and thermal sensitivity. The anti-inflammatory effects of RvD1 were assessed using ELISA, immunofluorescence, and western blotting to measure the expression of pro-inflammatory cytokines and BDNF. The involvement of ALX/FPR2 and TrkB receptors was further examined using antagonists Boc2 and K252a.

**Results** RvD1 significantly reduced mechanical and thermal allodynia in SNI mice in a dose-dependent manner. RvD1 also decreased microglial activation and expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and BDNF in both in vivo and in vitro models. These effects were reversed by Boc2 and K252a, confirming that the analgesic actions of RvD1 are mediated via the ALX/FPR2 receptor and inhibition of BDNF/TrkB signaling.

**Conclusion** RvD1 alleviates neuropathic pain by reducing neuroinflammation through the ALX/FPR2 receptor and suppressing BDNF/TrkB signaling. These findings suggest RvD1 as a promising therapeutic agent for neuropathic pain management.

**Keywords** Resolvin D1, Microglia activation, BDNF/TrkB, Neuropathic pain

## Introduction

Neuropathic pain refers to pain caused by injury or illness of the somatosensory nervous system. It is often defined by hyperalgesia and allodynia, as well as burning pain, paresthesia, and dysesthesia [1]. In the general population, neuropathic pain is a significant socioeconomic issue, with a prevalence of 7%–10% and is notoriously resistant to treatment [2]. Effective pharmacotherapies

*Correspondence:
Qiaodong Huang
qdhuang@126.com
[1] Department of Pain Medicine, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou 510317, Guangdong, China

capable of attenuating neuropathic pain are still lacking and require further development.

Resolvin D1 (RvD1), synthesized by 15- and 5-lipoxygenase (LOX) from docosahexaenoic acid (DHA), is a lipid mediator with significant anti-inflammatory properties, which promotes several types of neuroinflammatory resolution [3]. A previous study found that RvD1 attenuated the production of pro-inflammatory cytokines, limited excessive leukocyte infiltration, and regulated macrophage phagocytosis via direct activation of lipoxin A4 receptor/formyl peptide receptor 2 (ALX/FPR2), a G-protein-coupled receptor (GPCR) that plays an important role in host defense and inflammation [4–6]. Recently, experimental evidence has demonstrated that resolvins could [prevent? limit?] hyperalgesia in several pain models. RvD1 reduced neuronal hyperexcitability in spinal trigeminal nucleus caudalis and alleviated inflammation-induced hyperalgesia [7]. Intrathecal injection of RvD1 reversed allodynia in chemotherapy-induced peripheral neuropathy through its anti-inflammatory effects [8]. These results suggested that RvD1 may have the potential to treat different types of neuropathic pain through its anti-inflammatory effects.

Accumulating evidence suggests that neuroinflammation plays a key role in the induction and maintenance of neuropathic pain [9]. Following peripheral nerve injury, activated microglial cells release mediators that influence pain sensitivity [9]. The activated microglia produce proinflammatory mediators, such as TNF-α, IL-1β, and BDNF, which contribute to neuropathic pain development [7]. TNF-α and IL-1β serve as neuromodulators in the spinal cord dorsal horn after peripheral injury and induce/enhance synaptic plasticity (e.g., LTP) and inflammatory and neuropathic pain [10, 11]. Brain-derived neurotrophic factor (BDNF) is an important regulator involved in neuronal survival and synaptic plasticity of the central and peripheral nervous system [12]. In neurons, BDNF binds both TrkB receptors and p75 receptors to mediate neuroplastic effects [13, 14], and cell apoptosis and survival, respectively [13, 15]. Recently, Hu et al. [16] demonstrated that stimulation of microglia via the BDNF–TrkB pathway contributes to generation of dynamic allodynia in an SNI animal model. During neuropathic pain, BDNF/TrkB signaling enhances *N*-methyl-D-aspartate (NMDA) receptor activity in primary afferent neurons of the spinal cord's dorsal horn. Systemic administration of anti-BDNF or anti-TrkB neutralizing antibodies, or BDNF scavenger TrkB/Fc, can inhibit behavioral pain hypersensitivity [17]. Moreover, in models of inflammation-induced hyperalgesia, blocking BDNF/TrkB signaling or specifically inhibiting NMDA receptor activity restores normal physiological states and mitigates the progression of neuroinflammation [18]. BDNF signaling is significantly affected by neuroinflammatory processes, which can have both protective and detrimental effects on neuronal function. Under pathological conditions, pro-inflammatory cytokines may disrupt BDNF signaling, impacting neurogenesis and synaptic plasticity [19]. Conversely, BDNF can modulate neuroinflammatory responses, suggesting a complex interplay between BDNF–TrkB signaling and neuroinflammation that may be crucial for understanding the mechanisms underlying various CNS disorders [20]. Recently, RvD1 has been found to reduce BDNF expressed by microglia resulting in decreased neuroinflammation in several chronic pain models; whether RvD1 alleviates peripheral nerve injury-induced neuropathic pain and its underlying mechanisms will require further exploration.

In the present study, we hypothesized that RvD1 could decrease BDNF produced by microglia to reduce neuroinflammation to alleviate neuropathic pain in the SNI model. We examined the analgesic effects of RvD1 in the spared never injury-induced neuropathic pain mice model. Finally, we investigated the inflammatory mediators and BDNF expressed by activated microglia after RvD1 injection in vivo and in vitro.

## Materials and methods

### Animals and pain model

Male C57BL/6 mice (8–10 weeks old, 22 ± 2 g) were sourced from the Animal Experimental Center, South China Agricultural University (Guangzhou, China). All procedures were approved by the Institutional Animal Ethical Care Committee of Southern Medical University Experimental Animal Centre and adhered to the guidelines of the International Association for the Study of Pain (LAEC-2021-158). Food and water were available ad libitum, and the mice were confined in groups of 4–6 per cage under standard conditions with a 12-h light/dark cycle (temperature 26 °C). Prior to experiments, mice were permitted to adjust to the new environment for a minimum of 3 days.

Animals were randomly divided into the following groups: (1) Sham group; (2) SNI + Vehicle group; and (3) SNI + RvD1 group. Spared nerve injury (SNI) was employed to induce a chronic neuropathic pain model in mice [21]. Briefly, anesthesia was achieved via administration of 2% isoflurane, and a skin incision was made on the left hind leg. The muscle was gently separated to expose the left sciatic nerve and its three terminal branches. The main peroneal and sural nerves were bound, transected, and 2 mm segments were excised. The skin was sutured and disinfected with iodophor. For mice in the Sham group, the sciatic nerve was exposed and isolated in the same manner but without any nerve damage.

### Administration of drugs in SNI mice

Intrathecal injection was performed as previously described. Briefly, sevoflurane was used to anesthetize the mouse and fix the mouse position. First, the tip of the sixth lumbar spinous process (the highest position of the spine) was located and a micro syringe was inserted into the fifth intervertebral space (L5–L6). Following observation of tail twists, a 10 μL volume of miRNA agomir solution was delivered into the body. Then, the needle was kept in a specific position for 10 s and pulled out slowly to prevent the injected liquid from flowing out [22].

To determine the optimal dose of RvD1 in SNI mice, SNI + RvD1 groups received 10 ng, 20 ng, and 40 ng RvD1 dissolved in a 1% ethanol vehicle [23] (Cayman Chemical, Ann Arbor, Michigan, USA), respectively. We administered 10 μL of reagents (10, 20, or 40 ng RvD1 or vehicle) via intrathecal injection daily, 30 min before and daily after sham or SNI surgery for 3 consecutive days.

The ALX/FPR2 antagonist Boc-2 (10 μM/600 ng/kg) was administered via intraperitoneal injection, followed by an additional intrathecal injection of 10 μL of RvD1 (40 ng) after 15 min. Injections were administered consecutively for 3 days. K252a, a TrkB antagonist, was first prepared as a 20 mM stock solution in DMSO, then diluted to a working solution (20 μM) with PBS. Thirty minutes before SNI modeling, 10 μL of K252a was intrathecally injected, followed by an additional intrathecal injection of 10 μL of RvD1 (40 ng) after 15 min. Injections were administered consecutively for 3 days.

### Behavioral tests

Von Frey test, Hargreaves test and Open field test were adopted to test the pain threshold and spontaneous motor behavior. To avoid bias, researchers conducting the behavioral tests were blinded to the treatment groups.

#### *von Frey test*

The von Frey filament test was used to assess the onset and maintenance of mechanical allodynia. All mice were tested between 6 a.m. and 6 p.m. For at least 3 days before the baseline test, the animals were placed daily in a transparent plastic box on a grid iron rack (9 × 25 × 25 cm) to habituate to the test environment. The von Frey filaments (bending force ranging from 0.02 to 2 g; Semmes Weinstein) were used to quantify mechanical allodynia by the "up–down" method [24]. The filament was used on the lateral plantar aspect of the hind paw, bending it for at least 5 s while observing the mouse's reaction. A positive response included brisk paw withdrawal, flinching, licking, or shaking. The stimulus producing a 50% likelihood of a positive response was calculated based on the test results. Each stimulation was separated by more than 30 s.

#### *Hargreaves test*

Thermal withdrawal latency was assessed using the Hargreaves' apparatus. Mice were placed in transparent compartments on a 2 mm glass platform and allowed 30 min to habituate in a quiet environment. The Hargreaves device (Ugo Basile, 37450, Italy) was used to create a radiant heat beam focused onto the nerve-injured hind paw. Paw withdrawal latency was measured with a 20 s cutoff to prevent tissue damage. Three trials were performed at 20-min intervals, and the average latency was calculated.

#### *Open field test*

The open field test (OFT) was applied to assess spontaneous motor behavior after SNI and RvD1 treatment. The OFT was conducted in a square white plastic cube (80 cm × 80 cm) with a computer connected to a digital video camera positioned above the box center. Automatic video monitoring and behavioral analysis were implemented through the use of an animal movement tracking system (EthoVision XT). A consistent light intensity, temperature, and humidity were maintained in the experimental environment to prevent interference. The environment was kept silent. The locomotion of the mice was monitored and recorded for a period of 5 min in the center of the cage. The entire distance (cm) traversed by each mouse was recorded and analyzed by the software.

### Cell culture and treatment

The BV2 murine microglial cell line was purchased from iCell Bioscience, Inc. (Shanghai, China). BV2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% streptomycin-penicillin in a 5% $CO_2$ incubator at 37 °C. Cells ($5 \times 10^5$ cells/well or $1 \times 10^4$ cells/well) were seeded in 6-well and 24-well plates, respectively, and after 1 day, changed to serum-free medium and starved for 4 h. The cells were pretreated with 10 nM RvD1 for 30 min and then stimulated with 100 ng/mL LPS for 6 h. To investigate the involvement of ALX/FPR2 and BDNF/TrkB, cells were treated with 10 μM butyloxycarbonyl-Phe-Leu-Phe-Leu-Phe (Boc-2) (GenScript Corporation, Piscataway, NJ, USA) or 30 μM K252a (Sigma-Aldrich, St. Louis, MO, USA) prior to treatment with RvD1 for 30 min.

Mouse neuro-2a neuroblastoma cells (ATCC® CCL-131TM, RRID: CVCL_0470) were cultured in ATCC-formulated Eagle's minimal essential medium (ATCC®, Cat. No. 30–2003, 2019) enriched with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in accordance with the manufacturer's instructions. A maximum

of six cell passages was implemented. The lower compartment of 12-well plates was inoculated with $1.5 \times 10^5$ cells/well of Neuro-2a cells. Then, the N2a cells were divided in a Control group, LPS group, LPS/K252a group, LPS + RvD1 group, and (LPS + RvD1)/K252a group. Prior to treatment with RvD1 for 24 h in a monoculture system, N2a cells in the LPS/K252a and (LPS + RvD1)/K252a groups were treated with 30 μM K252a to block TrkB. Then, all the groups were treated with supernatant from LPS-activated BV2 cells.

#### Immunofluorescence (IF)

Mice were deeply anesthetized with isoflurane and perfused with PBS followed by cold 4% paraformaldehyde (PFA). The L4–L5 spinal cord segments were collected, post-fixed in 4% PFA for 4 h, and immersed in 30% sucrose for 48 h. The tissues were cut into 20 μm sections using a cryostat microtome (Leica CM3050s, Germany) and immunofluorescence was performed. Sections were blocked with 5% donkey serum for 1 h at room temperature (RT) and incubated with the following primary antibodies overnight at 4 °C: Iba1 (1:500, Abcam), BDNF (1:500, Millipore), IL-1β (1:200, Abcam), IL-6 (1:200, Abcam), and TNF-α (1:200, Proteintech). After washing, the sections were incubated with the appropriate secondary antibodies (Alexa Fluor 488, 555, and 647; Life Technologies) for 1 h at RT. Single or double immunofluorescence staining was performed using DAPI (Solarbio Science & Technology Co., Ltd.) for counterstaining. Fluorescent images were obtained using an EVOS FL Imaging System.

#### Western blotting

BV2 cells, N2a neurons, and L4–L5 spinal cord tissues from mice were lysed in RIPA buffer (Beyotime, China). Samples were then separated by 6–12% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene-fluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat milk in Tris-buffered containing 0.1% Tween-20 (TBST) for 1 h, then washed three times with TBST. The samples were incubated overnight at 4 °C with the following primary antibodies: BDNF, Bcl-2, and Bax (Proteintech, 1:1000). After washing, membranes were incubated with the appropriate secondary antibodies and immunoreactive bands were detected using ECL reagents. Protein bands were quantified with ImageJ software (v1.8.0, NIH, Bethesda, MD, USA).

#### Enzyme-linked immunosorbent assay (ELISA)

Following treatment with RvD1 for 24 h, the supernatant of N2a cells was collected. Similarly, L4–L5 spinal cords were lysed following treatment for cytokine detection. Levels of IL-1β, TNF-α, IL-6, and BDNF using ELISA were determined according to the manufacturer's instructions (Proteintech). A microplate reader (Thermo Scientific) was employed to measure the optical density at 450 nm.

#### Statistics

Statistical analyses were performed using SPSS 22.0 Statistics (IBM SPSS Statistics for Version 22.0, IBM Corp., North Castle). All data are expressed as mean ± SEM. For data obtained via behavioral test data, two-way ANOVA with repeated measures followed by Tukey's post hoc test was used to analyze differences between different groups. For data obtained via qRT-PCR, western blotting, ELISA, and immunofluorescence staining, one-way ANOVA followed by Tukey's post hoc test was used for multiple group comparisons. The Shapiro–Wilk normality test indicated that the data have a normal distribution; therefore, comparisons were done using parametric tests. Differences were deemed to be statistically significant at $p < 0.05$.

## Results

#### RvD1 alleviated SNI-induced neuropathic pain behavior

We investigated whether intrathecal injection of RvD1 could alleviate mechanical and thermal allodynia after SNI. The paw withdrawal threshold (PWT) and latency (PWL) in the vehicle largely decreased after SNI compared to the sham group, lasting up to 21 days. Compared with the SNI group, intrathecal injection of RvD1 (10–40 ng) alleviated mechanical allodynia from day 3 to 21 in SNI mice. In a previous study, Xu et al. demonstrated that intrathecal injection of 20 ng RvD1 resulted in profound analgesia in an experimental model of pain at a dose of 20 ng [23]. According to our results, RvD1 exhibits a dose-dependent alleviation of mechanical and thermal allodynia while the PWT and PWL in SNI mice treated with 40 ng/10 μl of RvD1 showed a statistically significant decrease compared with 10 or 20 ng. Thus, we selected 40 ng/10 μl RvD1 as the optimal dosage in the following experiments (Fig. 1A, B). SNI mice treated with the optimal dosage of RvD1 did not exhibit motor dysfunction nor anxiety-like behavior in the open field test (Fig. 1C).

#### RvD1 inhibits the upregulation of inflammatory factors in the spinal cord of SNI mice

Neuroinflammation is an important mechanism of neuropathic pain. Numerous inflammatory mediators are produced and secreted as a result of inflammatory cell infiltration and resident immune cell activation in response to nervous system injury. These mediators can

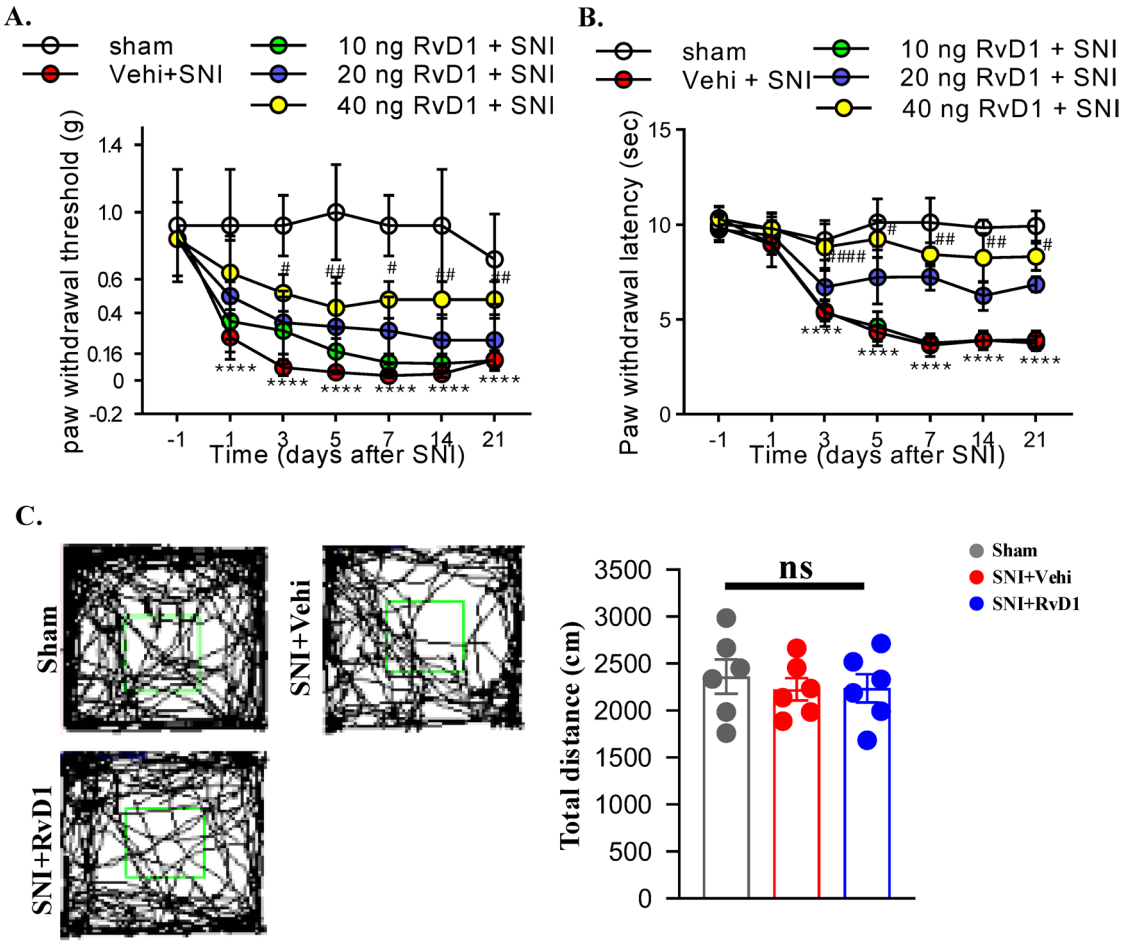


**Fig. 1** RvD1 alleviates SNI-induced neuropathic pain. **A** Threshold of the paw withdrawal (PWT) response to mechanical stimulation in the SNI model after intrathecal injection of different doses of RvD1. **B** Latency of the paw withdrawal (PWL) response to thermal stimulation in the SNI model after intrathecal injection of different doses of RvD1. **C** Typical movement trajectories of mice in the open field test under the SNI model following intrathecal administration of 40 ng RvD1 on day 7. Data are presented as mean ± SEM (n = 5). $^{****}P < 0.0001$ represents the comparison between Vehi + SNI and Sham group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#\#}P < 0.0001$ represents the ratio of RvD1 + SNI group to Vehi + SNI group at different doses

sensitize primary afferent neurons, trigger neuroimmune activation, and heighten pain sensitivity [25]. We used ELISA to determine the protein expression of TNF-α, IL-1β, and IL-6 in the spinal cord to determine the effect of RvD1 after SNI. As shown in Fig. 2A–C, SNI upregulated the expression of TNF-α, IL-1β, and IL-6 in the spinal cord ($P < 0.0001$). RvD1 treatment significantly downregulated TNF-α, IL-1β, and IL-6 expression ($P < 0.001$). Furthermore, proinflammatory cytokines were still upregulated in the ipsilateral spinal cord of the SNI + vehicle group (Fig. 2D–G). However, RvD1 attenuated proinflammatory cytokine expression induced by SNI compared with the SNI + vehicle group. There is evidence that RVD1 may reduce inflammation by preventing pro-inflammatory cytokines from being released following stem cell injection. However, the most pronounced anti-inflammatory effect was observed in the RVD1 group, which may be partly ascribed to its intrinsic anti-inflammatory properties, consistent with prior research findings [23].

### RvD1 suppresses spinal inflammation through the BDNF/TrkB signaling pathway in microglia

Glial cell activation, including microglia and astrocytes, in the spinal cord and brain is closely associated with neuroinflammation [26]. Microglia cells in the spinal cord horn are strongly activated after peripheral nerve injury and produce proinflammatory cytokines such as TNF-α and IL-1β, which act as neuromodulators in the spinal cord dorsal horn and trigger inflammatory and

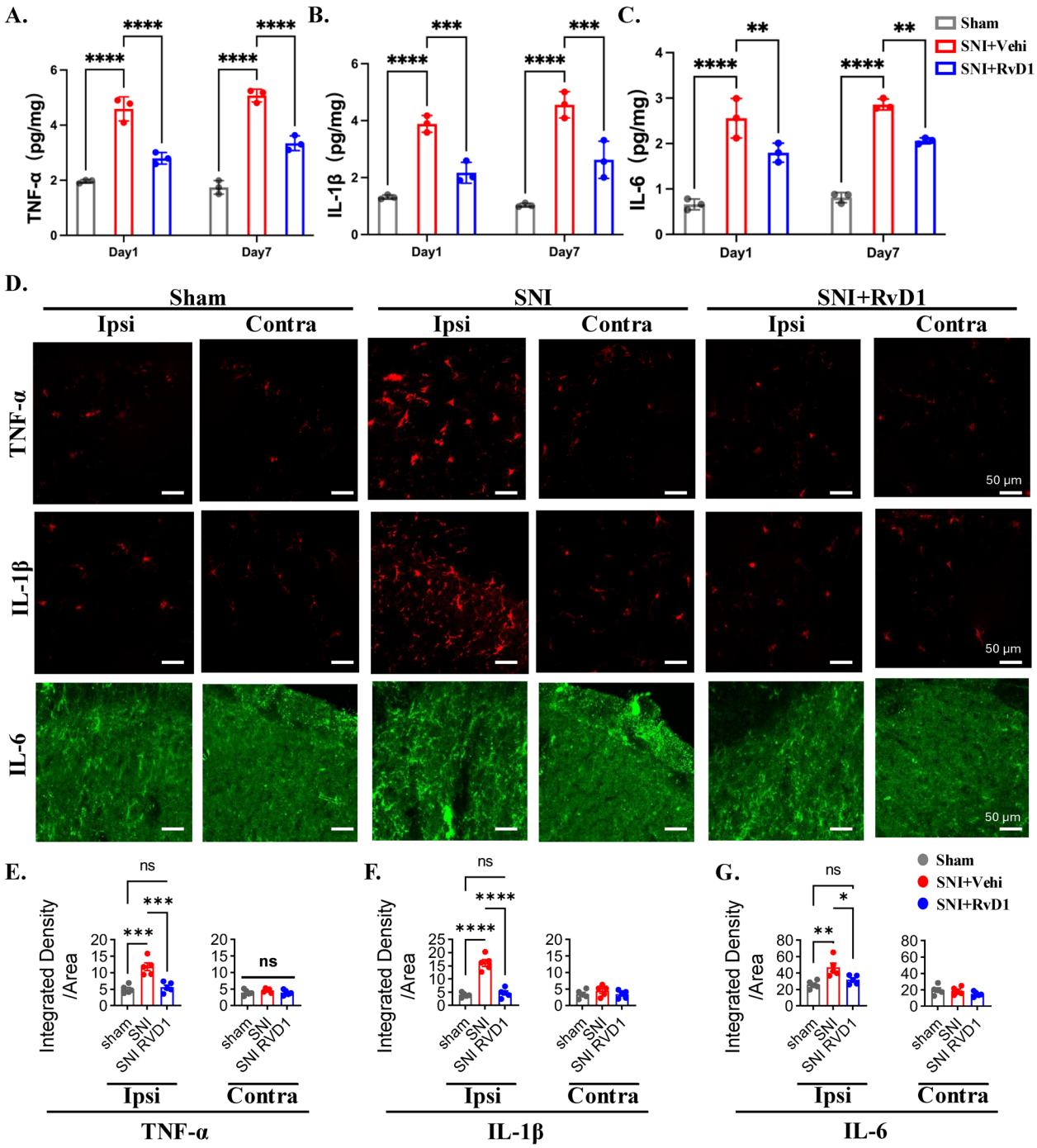


**Fig. 2** RVD1 downregulates pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the spinal cord of SNI mice. **A**–**C** ELISA results of TNF-α, IL-1β, and IL-6 in the spinal cord of SNI mice and after intrathecal injection of RVD1 after SNI. **D** Immunofluorescence revealed TNF-α, IL-1β, and IL-6 expression after intrathecal injection of RVD1 after SNI (scale bar = 20 μm). **E**–**G** Mean fluorescence intensity of TNF-α, IL-1β, and IL-6 following intrathecal injection of RVD1. Samples were obtained from the L4–L6 section of the spinal cord. Data are shown as the mean ± SEM (n = 5). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns, P > 0.05

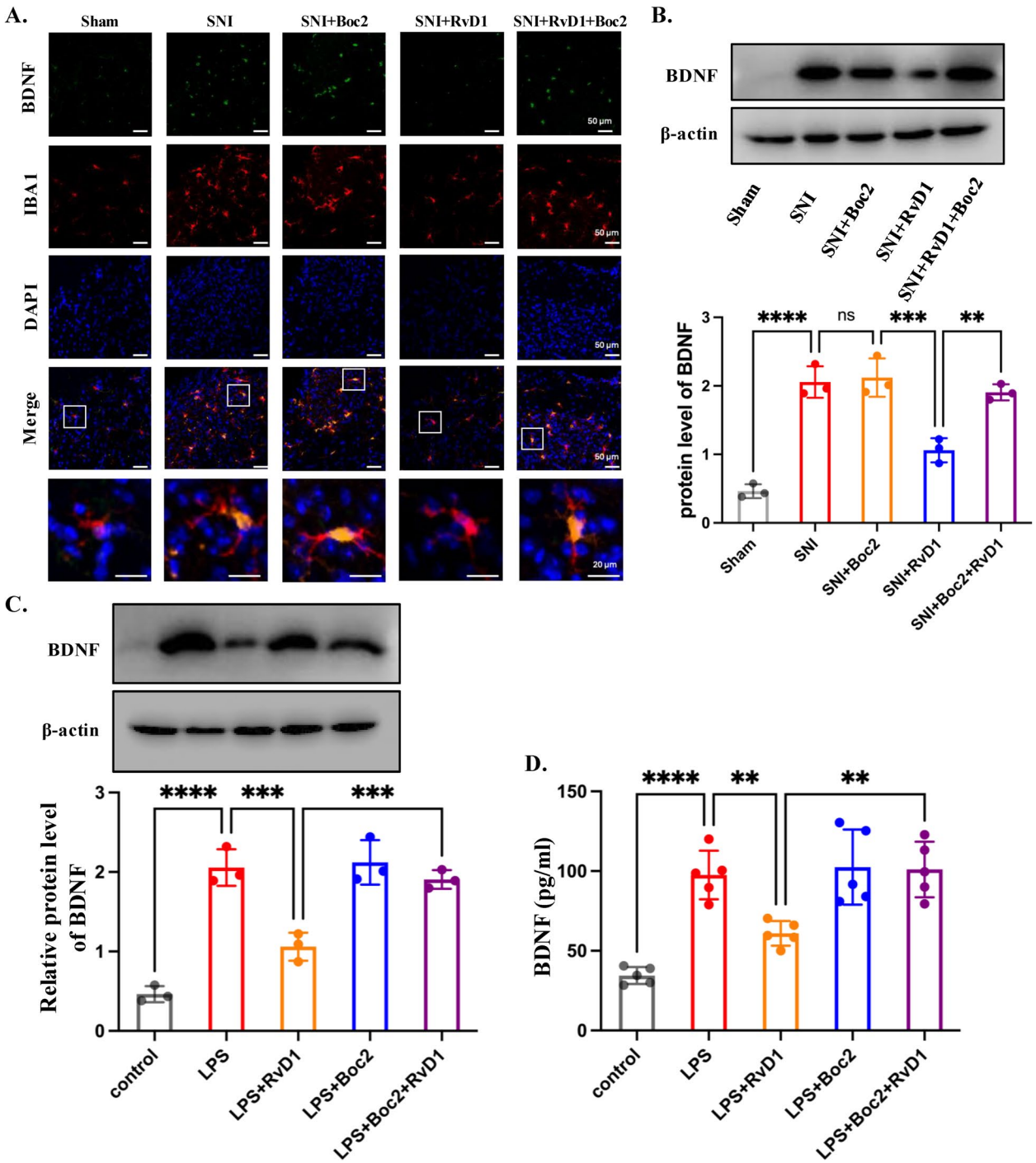


**Fig. 3** RVD1 inhibits upregulation of BDNF in the spinal cord of SNI mice and LPS-induced BV2 microglial cells. **A** Fluorescence of microglia marker Iba-1 and BDNF in the spinal cord of SNI mice after RvD1 or RvD1 + Boc2 treatment (scale bar = 20 μm). **B** Expression of BDNF in the spinal cord of SNI mice with RvD1 or RvD1 + Boc2 treatment. Data are presented as the mean ± SEM (n = 5). **C** Expression of BDNF in BV2 microglial cells following treatment with LPS (1 μg/L) for 24 h with RvD1 or RvD1 + Boc2. **D** ELISA of BDNF in the supernatant of LPS-stimulated BV2 microglial cells (n = 3 of independent cell culture preparations/group for Transwell system coculture). Data are presented as the mean ± SD; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not statistically significant

neuropathic pain [27]. After SNI with RvD1 treatment for 7 days, immunofluorescence staining and western blotting were employed to evaluate the expression of BDNF (Fig. 3A, B). Immunofluorescence showed that $BDNF^+$ cells were co-expressed with $Iba1^+$ cells (Fig. 3A). Expression of BDNF in microglia was significantly increased after SNI whereas RvD1 treatment reversed the upregulation of BDNF expression in the spinal cord (Fig. 3B). However, the effect of RvD1 on BDNF was abrogated by the ALX/FPR2 inhibitor Boc2, indicating that RvD1 is involved in the mechanism of spinal neuroinflammation caused by neuropathic pain through ALX/FPR2. This indicated a link between the inhibitory effect of RvD1 and activation of BDNF/TrkB signaling.

#### RvD1 inhibits upregulated expression of BDNF in BV2 cells

In the central nervous system, microglia are cells that resemble macrophages, and their activation contributes to neuroinflammation during neuropathic pain. Prior research has demonstrated that lipopolysaccharide (LPS) can stimulate brain-resident microglia and BV-2 microglial cells to proliferate and become activated both in vitro and in vivo [28, 29]. Spinal microglia derived BDNF could initiate neuropathic pain [30]. We stimulated BV2 microglia cells with LPS and detected expression of BDNF. While BDNF expression was increased in LPS-induced BV-2 microglia cells, RvD1 treatment significantly reversed LPS-induced BDNF upregulation ($P < 0.001$, Fig. 3C). We also detected BDNF levels in the cell culture supernatant though ELISA. As shown in Fig. 3D, expression of BDNF was significantly increased in the supernatants of LPS-stimulated BV2 microglial cells, while RvD1 treatment significantly decreased expression of BDNF in the supernatants. Moreover, the effect of RvD1 on BDNF was revered by the ALX/FPR2 inhibitor Boc2.These results further indicate RvD1 plays a critical role in neuropathic pain through microglia activation and neuroinflammation.

#### Neuroprotective effect of RvD1 through BDNF/TrkB signaling in N2a neurons stimulated by supernatant from LPS-activated BV2

Numerous studies have highlighted the pro-nociceptive role of BDNF in pain processes in the peripheral and central nervous systems [31]. BDNF plays an important role in neuronal survival and learning and memory by binding its receptor tyrosine protein kinase B (TrkB) [32]. Moreover, BDNF is believed to function as an autocrine or paracrine signal regulating TrkB in sensory neurons [33]. Thus, we postulated that RvD1 plays a role in controlling the BDNF/TrkB signaling pathway during inflammation of neurons. Thus, we stimulated N2a neurons using the supernatant from BV2 cells triggered by LPS and enriched with BDNF. Pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 had considerably higher protein expression in N2a neurons following treatment with supernatant from LPS-activated BV2 cells ($P < 0.01$) compared with the control group (Fig. 4A–C). While RvD1 treatment significantly decreased expression of TNF-α, IL-1β and IL-6 ($P < 0.01$) in N2a neurons. K252a, a TrkB antagonist, upregulated expression of the cytokines in N2a neurons, suggesting K252a could reverse the anti-inflammatory effect of RvD1. The results also suggest RvD1 attenuates inflammation though BDNF/TrkB signaling.

Research has shown that proinflammatory cytokines such as IFN-γ, TNF-α, IL-1β, and IL-6 can trigger autophagic processes [34]. Additional apoptosis processes are initiated by the intrinsic apoptosis pathway, which is mediated by Bax/Bak on the mitochondrial membrane. To further investigate whether RvD1 regulates neuronal apoptosis through Bcl-2 and Bax, supernatant from LPS-activated BV2 cells was used to stimulate N2 neurons, followed by treatment with RvD1. Western blotting was used to assess expression of Bax and Bcl-2 in neurons (Fig. 4D–F). Protein expression of Bax was significantly upregulated in the LPS group and markedly downregulated following RvD1 treatment. K252a led to a significant reduction in Bax expression; however, in the RvD1-treated group, this downregulation was less pronounced compared to that observed in the LPS + RvD1 group ($P < 0.0001$). Concurrently, Bcl-2 expression levels were significantly decreased in the LPS group but showed substantial upregulation with RvD1 treatment. Notably, when further treated with K252a, the upregulation of Bcl-2 was less than that of the LPS + RvD1group (Fig. 4D, $P < 0.05$), indicating K252a could reduce the neuroprotective effect of RvD1. These findings suggest that RvD1 inhibits the BDNF/TrkB pathway to regulate Bcl-2 and Bax in neurons.

#### RVD1 suppresses neuroinflammation through BDNF/TrkB signaling in the spinal cord of SNI mice

To confirm if RvD1 affects the BDNF-TrkB pathway in SNI mice, daily intrathecal infusions of the TrkB antagonist K252a were administered to SNI mice for 3 days consecutively following RvD1 therapy. Immunofluorescent staining was performed to assess TNF-α, IL-1β, and IL-6 following K252a administration in RvD1-treated SNI mice. The decreased mean fluorescence intensity of the cytokines following RvD1 treatment

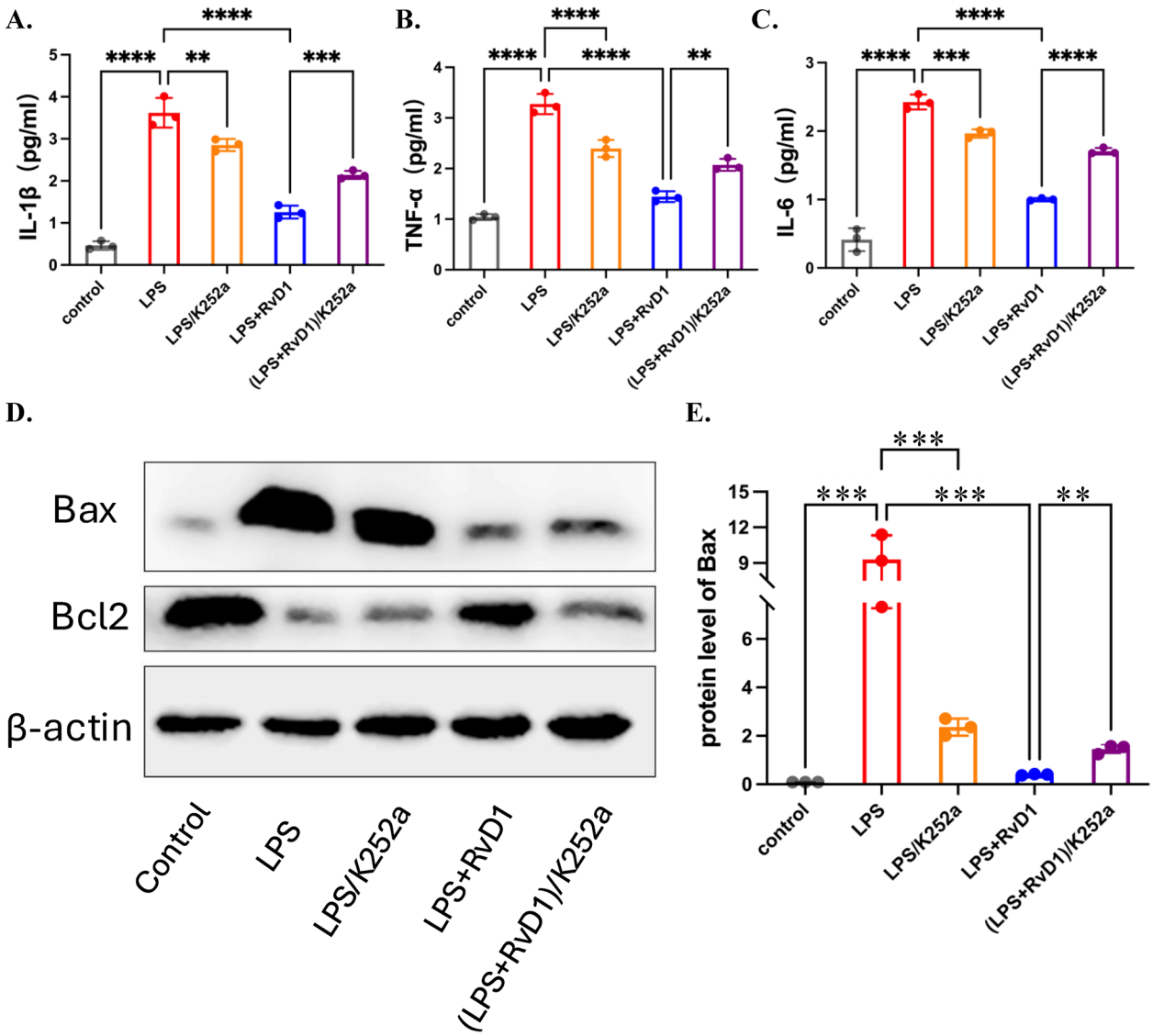


**Fig. 4** RVD1 suppresses inflammation in N2a neurons in vitro through BDNF/TrkB signaling. **A**–**C** ELISA of TNF-α, IL-1β, and IL-6 in neurons stimulated by supernatant from LPS-activated BV2 cells after administration of RVD1 and/or K252a. **D**–**E** Expression of Bax/Bcl2 in N2a neurons stimulated by supernatant from LPS-activated BV2 cells. Data are presented as mean ± SEM (n = 5). ***p < 0.01; ***p < 0.001; ****p < 0.0001; ns, p > 0.05

was obviously reversed by intrathecal infusions with K252a. K252a markedly increased expression of pro-inflammatory cytokines that were suppressed by RvD1 therapy (Fig. 5A–D). These findings suggest RvD1 may exercise its anti-inflammatory impact on SNI mice via modulation of BDNF/TrkB signaling to inhibit inflammatory cytokine production.

## Discussion

The present study demonstrated that Resolvin D1 (RvD1) significantly alleviated neuropathic pain in a spared nerve injury (SNI) model by modulating neuroinflammation through the BDNF/TrkB pathway. Intrathecal administration of RvD1 decreased mechanical sensitivity and thermal allodynia sensitivity in a dose-dependent manner, with the 40 ng/10 μl dose providing the most effective relief. Moreover, RvD1 treatment decreased expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, in the spinal cord, suggesting an anti-inflammatory effect via inhibition of microglial activation. Notably, RvD1 was found to downregulate BDNF expression in microglia through ALX/FPR2 and then reduce the neuroinflammatory response in both in vivo and in vitro models. Additionally, the neuroprotective

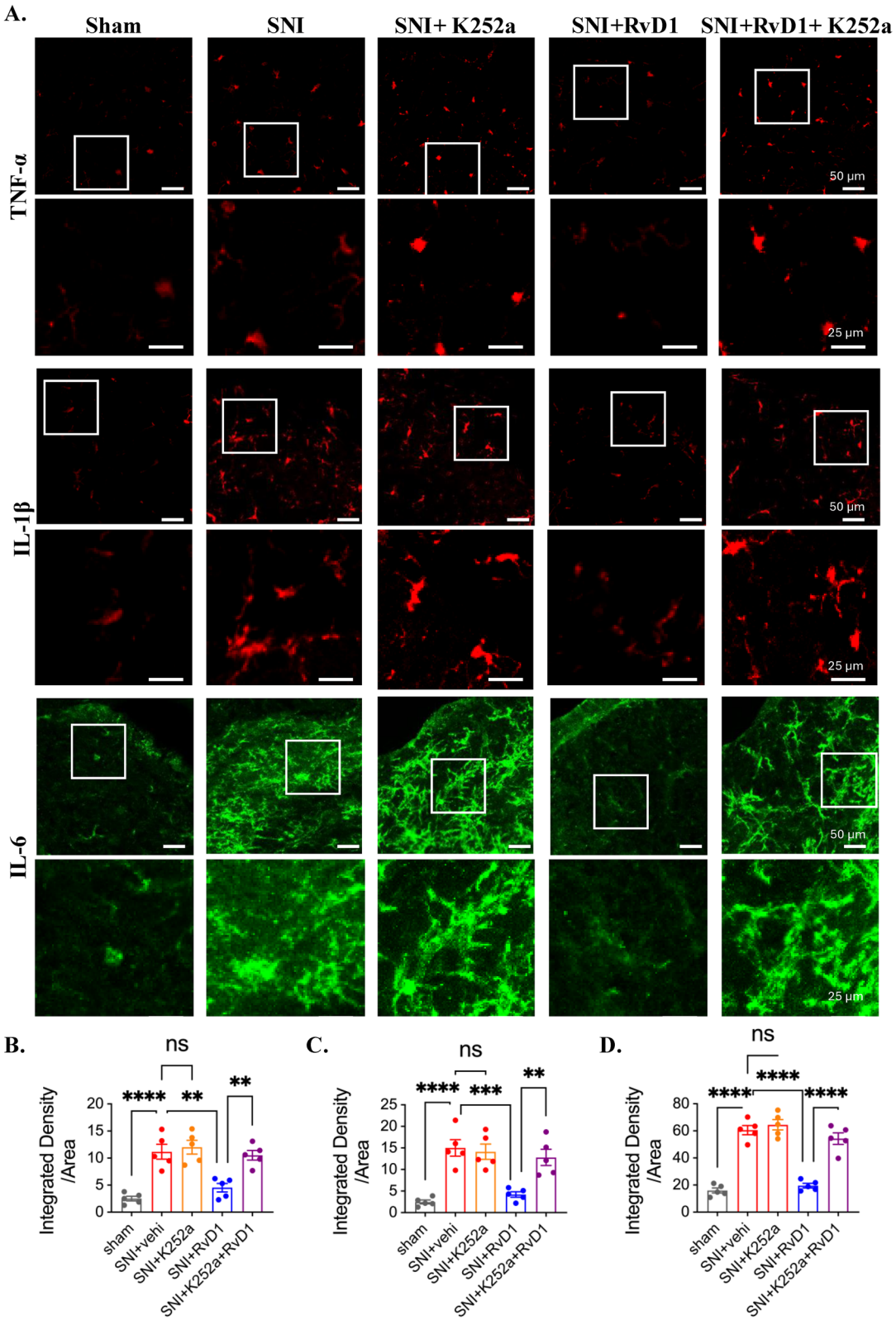


**Fig. 5** RVD1 suppresses spinal neuroinflammation via BDNF/TrkB signaling after SNI. **A** Immunofluorescence of TNF-α, IL-1β, and IL-6 after injection of RVD1 with or without K252a. **B**–**D** Mean fluorescence intensity of TNF-α, IL-1β, and IL-6 after injection of RVD1 with or without K252a. Data are presented as mean ± SEM (n = 5). **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, p > 0.05

and anti-inflammatory effects of RvD1 were reversed by K252a, a TrkB antagonist, further confirming the critical role of BDNF/TrkB signaling in mediating the analgesic effects of RvD1. These findings highlight the potential therapeutic use of RvD1 in treating neuropathic pain through its modulation of neuroinflammation and BDNF signaling.

In the past two decades, several studies have suggested that RvD1 has a promising analgesic effect in different chronic pain models. Xu et al. [23] reported that Resolvins RvE1 and RvD1 could alleviate inflammatory pain through peripheral (intraplantar) or spinal (intrathecal) administration in acute and persistent inflammation mouse models. Post-treatment with RvD1 reduced CFA-induced mechanical hyperalgesia and allodynia by inhibiting TRPA1 [35]. Furthermore, RvD1 (100 ng and 500 ng) resulted in a dose-dependent decrease in mechanical allodynia in an SNI rat model during the late phase (3 weeks). This model resists numerous anti-inflammatory interventions and spinal cord stimulation [36]. RvD1, derived from EPA and DHA, could produce an anti-inflammatory effect at very low concentration (nM) compared with EPA and DHA (lM) and has been considered a promising anti-inflammatory compound in previous reports [37]. In terms of analgesia, post-treatment with DHA intrathecal injection does not reduce neuropathic pain after nerve injury or post-operative pain after bone fracture even at high doses [500 μg, 1000-fold of SPMs (specialized pro-resolving mediators, SPMs)] [38]. However, Zhang et al. adopted a tibial bone fracture mice model and found that intravenous perioperative treatment with DHA (500 μg), RvD1 (500 ng), and MaR1 (500 ng) could effectively prevent and delay postoperative pain. Their results also indicated that RvD1 and other SPMs could alleviate established pain, but the exact mechanism remains elusive [39]. Our results also suggested that intrathecal RvD1 (10–40 ng) exhibited a dose-dependent reduction in mechanical allodynia in SNI-mice. We also found that intrathecal administration of RvD1 could significantly reduce mechanical and thermal allodynia for at least 3 weeks. Resolvin D1 (RvD1) demonstrates significant therapeutic potential in various inflammatory conditions without apparent toxicity. In arthritis models, RvD1 inhibits osteoclast differentiation, reduces bone resorption, and alleviates joint inflammation [40]. RvD1 attenuates doxorubicin-induced cardiotoxicity by reducing inflammation, oxidative stress, and endoplasmic reticulum stress in mice [41]. In hyperoxic acute lung injury, RvD1 treatment decreases oxidative stress, inflammation, and apoptosis, improving lung function [42]. Furthermore, perivascular delivery of RvD1 inhibits neointimal hyperplasia in a rat model of arterial injury by attenuating inflammatory pathways and reducing vascular smooth muscle cell proliferation and migration [43]. Across these studies, RvD1 consistently demonstrates anti-inflammatory, tissue-protective, and resolution-promoting effects without reported toxicity, suggesting its potential as a therapeutic agent for inflammatory conditions. Despite its therapeutic promise, the safety and toxicity profile of RvD1 remains an area of active investigation. Preclinical studies indicate that RvD1 is generally non-toxic at tested doses, exhibiting no adverse effects on cell viability in murine macrophage models and demonstrating a favorable safety profile in vivo during inflammation-related treatments [44]. As RvD1 continues to be explored for its clinical relevance, particularly in chronic inflammatory conditions and potential cancer therapies, understanding its safety profile becomes increasingly critical [45]. Therefore, we also adopted the open field test to evaluate the motor function and mood status of SNI mice. Our results showed that intrathecal administration of RvD1 does not impair the motor function of mice, which suggested that intrathecal injection of 40 ng RvD1 did result in toxicity. Although neuropathic pain is often comorbid with anxiety or depressive-like behaviors [46], our OPT did not found obvious differences among groups, which may be partly because anxiety or depressive-like behaviors are normally exhibited after 3 weeks [47].

Neuroinflammation plays a pivotal role in neuropathic pain [3]. Currently, mounting evidence suggests RvD1 can reduce neuroinflammation to produce an analgesic effect. Wang et al. found that RvD1 in a spinal nerve ligation (SNL)-induced neuropathic pain rat model could decrease inflammatory cytokines (e.g., IL-1β and TNF-α) in the dorsal root ganglion and spinal cord through nod-like receptor protein 3/extracellular signal-related kinase signaling to inhibit NLRP3 inflammasome [48]. Microglia are well known to play a pivotal role in pain modulation and neuroinflammation [49]. Activated microglia display an amoeboid morphology and higher levels of cell surface markers during neuroinflammation. They release pro-inflammatory cytokines (TNF-α, IL-1β, IL-18, IL-6), chemokines, and reactive oxygen species (ROS). These substances can induce pain, disrupt neuronal communication, and cause neuronal injury. Subsequently, pro-inflammatory cytokines can deeper stimulate microglia in neuropathic pain [50]. RvD1 and RvE1 reduced LPS-induced TNF-α, IL-6, and IL-1β expression, indicating their proresolutive activity in microglia [51]. RvD1 can suppress TNF-α and IL-1β release, along with activation of the NF-κB pathway, in LPS-stimulated microglia in vitro [52]. RvD1 has also been shown to augment IL-4-induced anti-inflammatory polarization of the BV2 microglial cell line by boosting nuclear translocation and DNA binding capacity of PPARγ [53]. We also found

that RvD1 decreased TNF-α, IL-1β, and IL-6 in the spinal cord by ELISA and immunofluorescence staining. In addition to inhibiting pro-inflammatory cytokines, RvD1 also facilitates the production of regulatory cytokines, such as transforming growth factor (TGF)-β, which promotes an anti-inflammatory environment and supports resolution of inflammation [44]. The induction of TGF-β by RvD1 has been associated with increased phagocytic activity of macrophages and the promotion of regulatory T cell (Treg) responses, further contributing to inflammation resolution [44]. However, whether this mechanism works in neuropathic situations requires further investigation.

Although previous and our current data suggest that RvD1 might be used as a potentially effective treatment against neuroinflammation to alleviate SNI-induced mechanical allodynia, its underlying mechanism in SNI mice remains unclear. Inflammatory processes are often accompanied by an increased release of BDNF [54]. Microglial-derived BDNF has also been shown to be involved in neuropathic pain [27]. BDNF is also important in mature animals for regulating the mechanical sensitivity of slowly adapting mechanoreceptors, myelinated fibers required for fine tactile discrimination [55]. Co-localization of BDNF with microglia was validated by our immunofluorescence data, which also demonstrated a rise in microglia and BDNF in neuropathic pain generated by SNI, while BDNF levels fell after RvD1 therapy. Similarly, western blotting revealed an upregulation of BDNF in SNI mice, which was reduced by RvD1 treatment. LPS-stimulated BV2 microglial cells are often used as in vitro neuroinflammation models [30]. Therefore, we used BV2 cells to study the effects of RvD1 on BDNF in microglia. Consistent with the in vivo results, RvD1 inhibited expression of BDNF in LPS-induced BV2 microglial cells. Moreover, the effects of RvD1 on SNI mice and LPS-induced BV2 microglial cells were abrogated by the ALX/FPR2 inhibitor Boc2. SPM receptors, such as ALX/FPR2 (resolvin D1 receptor) and GPR18 (resolvin D2 receptor), are also expressed on microglia to regulate neuroinflammation and neuropathic pain [27, 56]. GPR32, although identified as a receptor for RvD1, has a lower affinity ($EC_{50}$ of ~8.8 pM) compared with ALX/FPR2 [53]. In studies involving human polymorphonuclear leukocytes, low concentrations of RvD1 (1 nM) were found to be sensitive to GPR32 blockade, while higher concentrations (10 nM) primarily acted through ALX/FPR2 [53]. Thus, in the present study, we chose ALX/FPR2 as the main target and our SNI model and cell-based studies suggest RvD1 regulates BDNF expression of microglia through ALX/FPR2.

In neuropathic pain models following peripheral nerve and spinal cord injuries, activated microglia secrete BDNF, which binds neuronal TrkB [20, 57–59]. Intracellular signals mediated by BDNF/TrkB are also involved in numerous neuronal aspects, such as neural survival, synaptic plasticity, and neurogenesis-associated cognition in the CNS [60]. Additionally, primary sensory neurons exhibit high TrkB expression [61]. BDNF binds TrkB to participate in nociceptive pain-related signaling of primary sensory neurons [61, 62]. Phan et al. demonstrated that BDNF/TrkB signaling inhibition can effectively suppress reactive astrocytes, resulting in a reduction in allodynia within the PCI model [63]. Recently, Zhao et al. [64] reported that RvD1 could reduce mechanical allodynia associated with burn injuries by suppressing spinal cord glial activation and p38 MAPK signaling in microglia as well as BDNF/TrkB signaling in the spinal dorsal horn. This evidence provoked us to define whether the anti-inflammatory effect of RvD1 on SNI-induced neuroinflammation is associated with BDNF/TrkB signaling. In the present study, immunofluorescence demonstrated an upregulation of BDNF in the microglia of SNI mice, whereas RvD1 treatment significantly reduced BDNF expression. Conversely, intrathecal administration of the TrkB antagonist K252a reversed the anti-inflammatory effects of RvD1 in SNI mice, resulting in a marked increase in proinflammatory cytokines (TNF-α, IL-6, and IL-1β). Furthermore, immunofluorescence revealed that intrathecal administration of K252a alone did not affect the expression of proinflammatory cytokines in SNI mice. These results indicated that K252a may selectively block TrkB receptors in neurons but did not affect microglia activation and BDNF expression.

Microglia interact with nerve cells to alter synaptic transmission and chronic pain conditions by secreting pro-inflammatory mediators, including TNF-α and BDNF [30, 65]. Spinal lamina projection neurons to BDNF induce a depolarizing change in the anion reversal potential, leading to disinhibition of the GABAergic system, a crucial regulation mechanism in chronic pain [66]. Therefore, we used the cultured supernatant from LPS-activated BV2 cells, which is enriched with BDNF, to stimulate neurons. RvD1 treatment significantly decreased expression of proinflammatory cytokines (TNF-α, IL-6 and IL-1β) in neurons treated with supernatant from LPS-activated BV2 cells. The TrkB antagonist K252a reversed the anti-inflammatory effect of RvD1, indicating that RvD1 attenuated inflammation via BDNF/TrkB signaling. Moreover, western blotting showed K252a could reverse Bcl-2 upregulation and Bax downregulation induced by RvD1, indicating RvD1 exerts

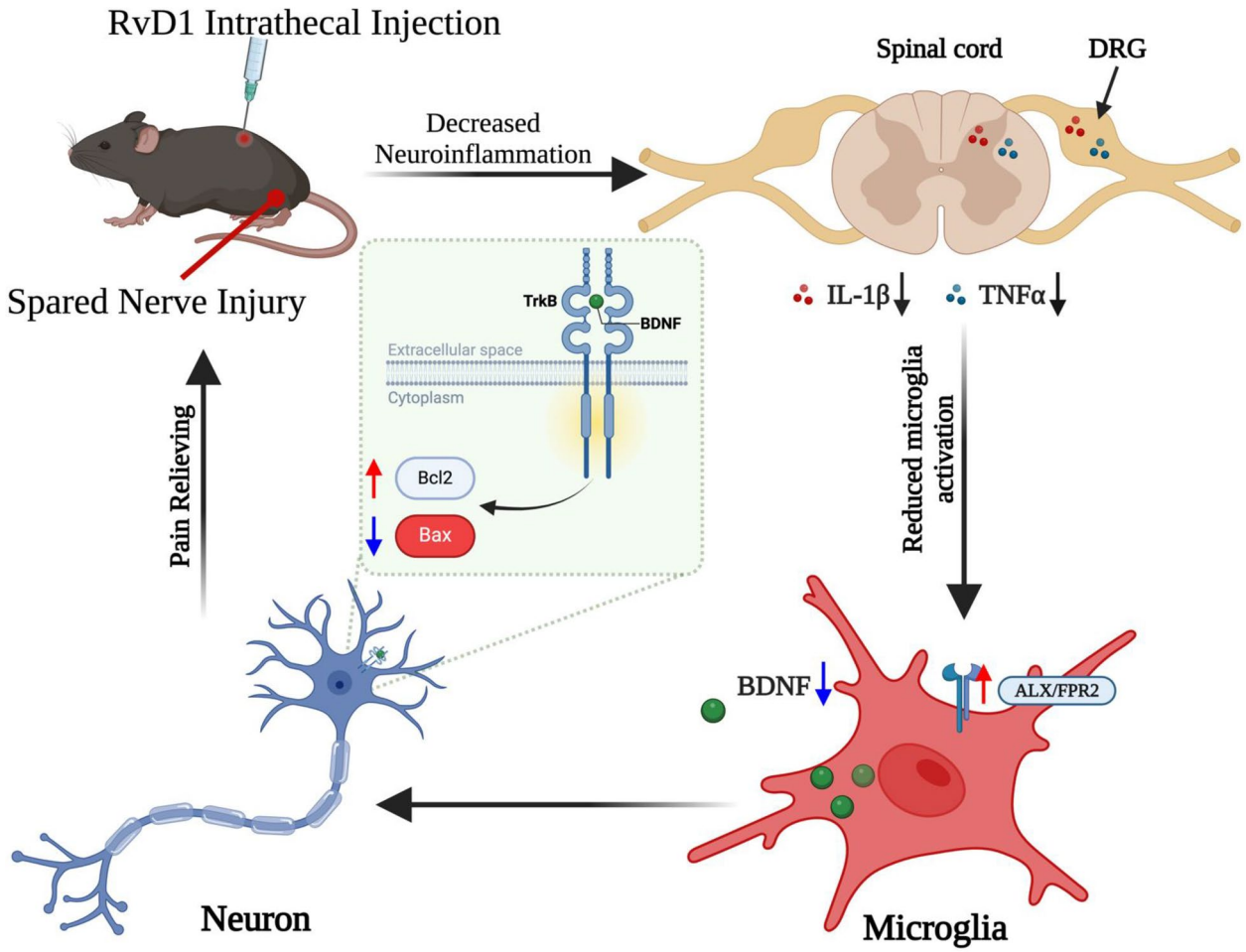


**Fig. 6** Schematic representation of the model by which RvD1 addresses SNI-induced neuropathic pain. Activation of ALX/FPR2 facilitates microglial inhibition and regulates BDNF/TrkB signaling, mitigating SNI-induced neuroinflammation

neuroprotective effects via inhibition of BDNF/TrkB signaling.

The underlying mechanism of the anti-inflammatory effect of RvD1 may involve several cellular signaling pathways, such as NF-κB and p-ERK pathways. Liu et al. confirmed that intrathecal administration of RvD1 (10 or 100 ng) might alleviate neuropathic pain via regulation of inflammatory mediators and NF-κB/p65 and p-ERK pathways in a noncompressive lumbar disk herniation model [67]. Spinal administration of RvD1 and E1 reduced pain behavior and the release of inflammatory cytokines (TNF-α, IL-1β, and IL-6) via inhibition of p-ERK signaling pathway in arthritis rats [23]. Recently, Lv et al. [68] confirmed that RvD1 combined with exercise rehabilitation activates BDNF/TrkB/PI3K/AKT signaling, effectively reduces neuronal apoptosis and inflammatory responses following ICH in mice, and participates in mitochondrial autophagy-related states. Overall, the mechanism of RVD1 in neuropathic pain still requires further research.

In summary, we demonstrated that RvD1 ameliorates neuropathic pain in SNI mice. Our data indicate that the underlying mechanism of RvD1 in relieving neuropathic pain in SNI mice may be mediated by regulation of ALX/FPR2 to alleviate neuroinflammation and decrease BDNF from microglia, which then reduce the activity of TrkB on neurons (Fig. 6). Our results suggest RvD1 has promising therapeutic potential in neuropathic pain. One limitation of this study is that while RvD1 demonstrated significant effects in reduction of neuroinflammation and neuropathic pain through the BDNF/TrkB pathway, the effects of RvD1 treatment were not evaluated beyond 3 weeks. Additionally, specific interactions between microglia and neurons in vivo were not fully explored, limiting our understanding of the broader effects of RvD1 on synaptic plasticity and chronic pain mechanisms. Future studies

should assess the long-term safety and efficacy of RvD1 and further investigate microglia-neuron communication dynamics.

**Abbreviations**

| | |
|---|---|
| BDNF | Brain-derived neurotrophic factor |
| ALX/FPR2 | Lipoxin A4 receptor/formyl peptide receptor 2 |
| OFT | Open field test |
| Iba-1 | Ionized calcium binding adaptor molecule 1 |
| LPS | Lipopolysaccharide |
| RvD1 | Resolvin D1 |
| SDH | Spinal dorsal horn |
| SNI | Spared nerve injury |
| TrkB | Tropomyosin-related kinase B |
| K252a | A specific inhibitor of TrkB |
| Boc2 | Butyloxycarbonyl-Phe-Leu-Phe-Leu-Phe, the ALX/FPR2 antagonist |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02424-7.

Supplementary Material 1

**Acknowledgements**
Special thanks go to Dr. Jiang Changyu (Department of Pain Medicine and Shenzhen Municipal Key Laboratory for Pain Medicine, The 6th Affiliated Hospital of Shenzhen University Medical School) and Dr. Tao Tao (Zhujiang Hospital, Southern Medical University), for their generous efforts in reviewing and editing the manuscript.

**Author contributions**
QDH: Designed research studies; CJB and XML: conducted experiments; CJB and QDH wrote the main manuscript text. CJB AND YJL: acquired and analyzed data: CJB. CJB prepared Figs. 1, 2, 3, 4, 5, 6. All authors reviewed and approved the final version of the manuscript.

**Funding**
This work was supported by the science foundation of Guangdong Second Provincial General Hospital (Grant No. YY2019-005).

**Availability of data and materials**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
All animals were sourced from the Animal Experimental Center, South China Agricultural University (Guangzhou, China). All procedures were approved by the Institutional Animal Ethical Care Committee of Southern Medical University Experimental Animal Centre and adhered to the guidelines of the International Association for the Study of Pain (LAEC-2021-158).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 29 December 2024 Accepted: 3 March 2025
Published online: 20 March 2025

**References**

1. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 2010;9(8):807–19. https://doi.org/10.1016/S1474-4422(10)70143-5.
2. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet. 2021;397(10289):2082–97. https://doi.org/10.1016/S0140-6736(21)00393-7.
3. Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533–48. https://doi.org/10.1038/nrd4334.
4. Krishnamoorthy S, Recchiuti A, Chiang N, et al. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proc Natl Acad Sci U S A. 2010;107(4):1660–5. https://doi.org/10.1073/pnas.0907342107.
5. Schmitz NV, Rogerio AP, Abrahao OJ. Insights into the activation mechanism of the ALX/FPR2 receptor. J Phys Chem Lett. 2020;11(21):8952–7. https://doi.org/10.1021/acs.jpclett.0c02052.
6. Rogerio AP, Haworth O, Croze R, et al. Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. J Immunol. 2012;189(4):1983–91. https://doi.org/10.4049/jimmunol.1101665.
7. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflamm. 2014;11:98. https://doi.org/10.1186/1742-2094-11-98.
8. Meesawatsom P, Hathway G, Bennett A, et al. Spinal neuronal excitability and neuroinflammation in a model of chemotherapeutic neuropathic pain: targeting the resolution pathways. J Neuroinflamm. 2020;17(1):316. https://doi.org/10.1186/s12974-020-01997-w.
9. Ji RR, Nackley A, Huh Y, et al. Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology. 2018;129(2):343–66. https://doi.org/10.1097/ALN.0000000000002130.
10. Park CK, Lu N, Xu ZZ, et al. Resolving TRPV1- and TNF-alpha-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. J Neurosci. 2011;31(42):15072–85. https://doi.org/10.1523/JNEUROSCI.2443-11.2011.
11. Choi JI, Svensson CI, Koehrn FJ, et al. Peripheral inflammation induces tumor necrosis factor dependent AMPA receptor trafficking and Akt phosphorylation in spinal cord in addition to pain behavior. Pain. 2010;149(2):243–53. https://doi.org/10.1016/j.pain.2010.02.008.
12. Constandil L, Aguilera R, Goich M, et al. Involvement of spinal cord BDNF in the generation and maintenance of chronic neuropathic pain in rats. Brain Res Bull. 2011;86(5–6):454–9. https://doi.org/10.1016/j.brainresbull.2011.08.008.
13. Chen Y, Zeng J, Cen L, et al. Multiple roles of the p75 neurotrophin receptor in the nervous system. J Int Med Res. 2009;37(2):281–8. https://doi.org/10.1177/147323000903700201.
14. Teng KK, Felice S, Kim T, et al. Understanding proneurotrophin actions: Recent advances and challenges. Dev Neurobiol. 2010;70(5):350–9. https://doi.org/10.1002/dneu.20768.
15. Sasi M, Vignoli B, Canossa M, et al. Neurobiology of local and intercellular BDNF signaling. Pflugers Arch. 2017;469(5–6):593–610. https://doi.org/10.1007/s00424-017-1964-4.
16. Hu Z, Yu X, Chen P, et al. BDNF-TrkB signaling pathway-mediated microglial activation induces neuronal KCC2 downregulation contributing to dynamic allodynia following spared nerve injury. Mol Pain. 2023;19:814386719. https://doi.org/10.1177/17448069231185439.
17. Theodosiou M, Rush AR, Zhou FX, et al. Hyperalgesia due to nerve damage: role of nerve growth factor. Pain. 1999;81(3):245–55. https://doi.org/10.1016/S0304-3959(99)00018-4.
18. Groth R, Aanonsen L. Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia. Pain. 2002;100(1–2):171–81. https://doi.org/10.1016/s0304-3959(02)00264-6.
19. Calabrese F, Rossetti AC, Racagni G, et al. Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front Cell Neurosci. 2014;8:430. https://doi.org/10.3389/fncel.2014.00430.
20. Ding H, Chen J, Su M, et al. BDNF promotes activation of astrocytes and microglia contributing to neuroinflammation and mechanical allodynia in cyclophosphamide-induced cystitis. J Neuroinflamm. 2020;17(1):19. https://doi.org/10.1186/s12974-020-1704-0.

21. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000;87(2):149–58. https://doi.org/10.1016/S0304-3959(00)00276-1.
22. Lu Y, Zhang J, Zeng F, et al. Human PMSCs-derived small extracellular vesicles alleviate neuropathic pain through miR-26a-5p/Wnt5a in SNI mice model. J Neuroinflamm. 2022;19(1):221. https://doi.org/10.1186/s12974-022-02578-9.
23. Xu ZZ, Zhang L, Liu T, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med. 2010; 16(5): 592–7, 1p-597p. https://doi.org/10.1038/nm.2123.
24. Bonin RP, Bories C, De Koninck Y. A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. Mol Pain. 2014;10:26. https://doi.org/10.1186/1744-8069-10-26.
25. Moalem G, Tracey DJ. Immune and inflammatory mechanisms in neuropathic pain. Brain Res Rev. 2006;51(2):240–64. https://doi.org/10.1016/j.brainresrev.2005.11.004.
26. Ellis A, Bennett DL. Neuroinflammation and the generation of neuropathic pain. Br J Anaesth. 2013;111(1):26–37. https://doi.org/10.1093/bja/aet128.
27. Chen G, Zhang YQ, Qadri YJ, et al. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100(6):1292–311. https://doi.org/10.1016/j.neuron.2018.11.009.
28. Fukushima S, Furube E, Itoh M, et al. Robust increase of microglia proliferation in the fornix of hippocampal axonal pathway after a single LPS stimulation. J Neuroimmunol. 2015;285:31–40. https://doi.org/10.1016/j.jneuroim.2015.05.014.
29. Xiao H, Xu J. Isaindigotone as an inhibitor of the lipopolysaccharide-induced inflammatory reaction of BV-2 cells and corresponding mechanisms. Mol Med Rep. 2019;19(4):2890–6. https://doi.org/10.3892/mmr.2019.9909.
30. Coull JA, Beggs S, Boudreau D, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005;438(7070):1017–21. https://doi.org/10.1038/nature04223.
31. Sikandar S, Minett MS, Millet Q, et al. Brain-derived neurotrophic factor derived from sensory neurons plays a critical role in chronic pain. Brain. 2018;141(4):1028–39. https://doi.org/10.1093/brain/awy009.
32. Xu Q, Ji XF, Chi TY, et al. Sigma 1 receptor activation regulates brain-derived neurotrophic factor through NR2A-CaMKIV-TORC1 pathway to rescue the impairment of learning and memory induced by brain ischaemia/reperfusion. Psychopharmacology. 2015;232(10):1779–91. https://doi.org/10.1007/s00213-014-3809-6.
33. Apfel SC, Wright DE, Wiideman AM, et al. Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system. Mol Cell Neurosci. 1996;7(2):134–42. https://doi.org/10.1006/mcne.1996.0010.
34. Ge Y, Huang M, Yao YM. Autophagy and proinflammatory cytokines: Interactions and clinical implications. Cytokine Growth Factor Rev. 2018;43:38–46. https://doi.org/10.1016/j.cytogfr.2018.07.001.
35. Bang S, Yoo S, Yang TJ, et al. Resolvin D1 attenuates activation of sensory transient receptor potential channels leading to multiple anti-nociception. Br J Pharmacol. 2010;161(3):707–20. https://doi.org/10.1111/j.1476-5381.2010.00909.x.
36. Tao X, Luo X, Zhang T, et al. Spinal cord stimulation attenuates mechanical allodynia and increases central resolvin d1 levels in rats with spared nerve injury. Front Physiol. 2021;12: 687046. https://doi.org/10.3389/fphys.2021.687046.
37. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: an update. Biochim Biophys Acta. 2010;1801(12):1260–73. https://doi.org/10.1016/j.bbalip.2010.08.002.
38. Xu ZZ, Liu XJ, Berta T, et al. Neuroprotectin/protectin D1 protects against neuropathic pain in mice after nerve trauma. Ann Neurol. 2013;74(3):490–5. https://doi.org/10.1002/ana.23928.
39. Zhang L, Terrando N, Xu ZZ, et al. Distinct analgesic actions of DHA and DHA-derived specialized pro-resolving mediators on post-operative pain after bone fracture in mice. Front Pharmacol. 2018;9:412. https://doi.org/10.3389/fphar.2018.00412.
40. Benabdoun HA, Kulbay M, Rondon EP, et al. In vitro and in vivo assessment of the proresolutive and antiresorptive actions of resolvin D1: relevance to arthritis. Arthritis Res Ther. 2019;21(1):72. https://doi.org/10.1186/s13075-019-1852-8.
41. Wang M, Zhang J, Zhao M, et al. Resolvin D1 attenuates doxorubicin-induced cardiotoxicity by inhibiting inflammation, oxidative and endoplasmic reticulum stress. Front Pharmacol. 2021;12: 749899. https://doi.org/10.3389/fphar.2021.749899.
42. Cox RJ, Phillips O, Fukumoto J, et al. Enhanced resolution of hyperoxic acute lung injury as a result of aspirin triggered resolvin D1 treatment. Am J Respir Cell Mol Biol. 2015;53(3):422–35. https://doi.org/10.1165/rcmb.2014-0339OC.
43. Wu B, Mottola G, Chatterjee A, et al. Perivascular delivery of resolvin D1 inhibits neointimal hyperplasia in a rat model of arterial injury. J Vasc Surg. 2017;65(1):207–17. https://doi.org/10.1016/j.jvs.2016.01.030.
44. Li C, Wu X, Liu S, et al. Role of Resolvins in the inflammatory resolution of neurological diseases. Front Pharmacol. 2020;11:612. https://doi.org/10.3389/fphar.2020.00612.
45. Mattoscio D, Isopi E, Lamolinara A, et al. Resolvin D1 reduces cancer growth stimulating a protective neutrophil-dependent recruitment of anti-tumor monocytes. J Exp Clin Cancer Res. 2021;40(1):129. https://doi.org/10.1186/s13046-021-01937-3.
46. Velly AM, Mohit S. Epidemiology of pain and relation to psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2018;87(Pt B):159–67. https://doi.org/10.1016/j.pnpbp.2017.05.012.
47. Magalhaes DM, Mampay M, Sebastiao AM, et al. Age-related impact of social isolation in mice: young vs middle-aged. Neurochem Int. 2024;174: 105678. https://doi.org/10.1016/j.neuint.2024.105678.
48. Wang YH, Gao X, Tang YR, et al. Resolvin D1 alleviates mechanical allodynia via ALX/FPR2 receptor targeted nod-like receptor protein 3/extracellular signal-related kinase signaling in a neuropathic pain model. Neuroscience. 2022;494:12–24. https://doi.org/10.1016/j.neuroscience.2022.04.019.
49. Ji J, Xue TF, Guo XD, et al. Antagonizing peroxisome proliferator-activated receptor gamma facilitates M1-to-M2 shift of microglia by enhancing autophagy via the LKB1-AMPK signaling pathway. Aging Cell. 2018;17(4): e12774. https://doi.org/10.1111/acel.12774.
50. Teixeira-Santos L, Albino-Teixeira A, Pinho D. Neuroinflammation, oxidative stress and their interplay in neuropathic pain: focus on specialized pro-resolving mediators and NADPH oxidase inhibitors as potential therapeutic strategies. Pharmacol Res. 2020;162: 105280. https://doi.org/10.1016/j.phrs.2020.105280.
51. Rey C, Nadjar A, Buaud B, et al. Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. Brain Behav Immun. 2016;55:249–59. https://doi.org/10.1016/j.bbi.2015.12.013.
52. Xu MX, Tan BC, Zhou W, et al. Resolvin D1, an endogenous lipid mediator for inactivation of inflammation-related signaling pathways in microglial cells, prevents lipopolysaccharide-induced inflammatory responses. Cns Neurosci Ther. 2013;19(4):235–43. https://doi.org/10.1111/cns.12069.
53. Li L, Wu Y, Wang Y, et al. Resolvin D1 promotes the interleukin-4-induced alternative activation in BV-2 microglial cells. J Neuroinflamm. 2014;11:72. https://doi.org/10.1186/1742-2094-11-72.
54. Seidel MF, Hugle T, Morlion B, et al. Neurogenic inflammation as a novel treatment target for chronic pain syndromes. Exp Neurol. 2022;356: 114108. https://doi.org/10.1016/j.expneurol.2022.114108.
55. Carroll P, Lewin GR, Koltzenburg M, et al. A role for BDNF in mechanosensation. Nat Neurosci. 1998;1(1):42–6. https://doi.org/10.1038/242.
56. Leuti A, Fava M, Pellegrini N, et al. Role of specialized pro-resolving mediators in neuropathic pain. Front Pharmacol. 2021;12: 717993. https://doi.org/10.3389/fphar.2021.717993.
57. Beggs S, Trang T, Salter MW. P2X4R+ microglia drive neuropathic pain. Nat Neurosci. 2012;15(8):1068–73. https://doi.org/10.1038/nn.3155.
58. Kaila K, Price TJ, Payne JA, et al. Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat Rev Neurosci. 2014;15(10):637–54. https://doi.org/10.1038/nrn3819.
59. Lin YT, Ro LS, Wang HL, et al. Up-regulation of dorsal root ganglia BDNF and trkB receptor in inflammatory pain: an in vivo and in vitro study. J Neuroinflamm. 2011;8:126. https://doi.org/10.1186/1742-2094-8-126.
60. Notaras M, van den Buuse M. Brain-derived neurotrophic factor (BDNF): novel insights into regulation and genetic variation. Neuroscientist. 2019;25(5):434–54. https://doi.org/10.1177/1073858418810142.
61. Wang H, Wei Y, Pu Y, et al. Brain-derived neurotrophic factor stimulation of T-type Ca(2+) channels in sensory neurons contributes to increased peripheral pain sensitivity. Sci Signal. 2019. https://doi.org/10.1126/scisignal.aaw2300.

62. Merighi A, Salio C, Ghirri A, et al. BDNF as a pain modulator. Prog Neurobiol. 2008;85(3):297–317. https://doi.org/10.1016/j.pneurobio.2008.04.004.
63. Phan TT, Jayathilake NJ, Lee KP, et al. BDNF/TrkB signaling inhibition suppresses astrogliosis and alleviates mechanical allodynia in a partial crush injury model. Exp Neurobiol. 2023;32(5):343–53. https://doi.org/10.5607/en23031.
64. Zhao X, Li X, Guo H, et al. Resolvin D1 attenuates mechanical allodynia after burn injury: Involvement of spinal glia, p38 mitogen-activated protein kinase, and brain-derived neurotrophic factor/tropomyosin-related kinase B signaling. Mol Pain. 2023;19:814361250. https://doi.org/10.1177/17448069231159970.
65. Liang YJ, Feng SY, Qi YP, et al. Contribution of microglial reaction to increased nociceptive responses in high-fat-diet (HFD)-induced obesity in male mice. Brain Behav Immun. 2019;80:777–92. https://doi.org/10.1016/j.bbi.2019.05.026.
66. Ferrini F, Trang T, Mattioli TA, et al. Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl(-) homeostasis. Nat Neurosci. 2013;16(2):183–92. https://doi.org/10.1038/nn.3295.
67. Liu ZH, Miao GS, Wang JN, et al. Resolvin D1 inhibits mechanical hypersensitivity in sciatica by modulating the expression of nuclear factor-kappaB, phospho-extracellular signal-regulated kinase, and pro- and antiinflammatory cytokines in the spinal cord and dorsal root ganglion. Anesthesiology. 2016;124(4):934–44. https://doi.org/10.1097/ALN.0000000000001010.
68. Xiaoyu L, Dandan L, Tianzhao O, et al. Resolvin D1 combined with exercise rehabilitation alleviates neurological injury in mice with intracranial hemorrhage via the BDNF/TrkB/PI3K/AKT pathway. Sci Rep. 2024;14(1):31447. https://doi.org/10.1038/s41598-024-83019-w.

## Publisher's Note