ORIGINAL ARTICLE

BDNF-VGF Pathway Aggravates Incision Induced Acute Postoperative Pain via Upregulating the Neuroinflammation in Dorsal Root Ganglia

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Abstract
Approximately one-third of postoperative patients are troubled by postoperative pain. Effective treatments are still lacking. The aim of this study is to investigate the role of brain-derived neurotrophic factor (BDNF)-VGF (non-acronymic) in dorsal root ganglia (DRG) in postoperative pain. Pain behaviors were assessed through measurements of paw withdrawal threshold (PWT) and paw withdrawal latency (PWL). Transcriptome analysis was conducted to identify potential targets associated with postoperative pain. Western blotting, immunofluorescence, and ELISA were employed to further detect macrophage activation as well as the expression of BDNF, VGF, TNF-α, IL-1β, and IL-6. Results showed that plantar incision induced both mechanical and thermal hyperalgesia. Transcriptome analysis suggested that plantar incision caused upregulation of BDNF and VGF. The expressions of BDNF and VGF were upregulated in isolectin B4-positive (IB4$^+$) and calcitonin gene-related peptide-positive (CGRP$^+$) neurons, rather than neurofilament 200-positive (NF200$^+$) neurons. The activation of BDNF-VGF pathway upregulated expression of IL-6, TNF-α, and IL-1β and promoted the activation of macrophages. In conclusion, BDNF-VGF pathway aggravates acute postoperative pain by promoting macrophage activation and pro-inflammatory cytokine production, which may provide a new target for the treatment of postoperative pain.

Keywords BDNF · VGF · Postoperative pain · Macrophage · Neuroinflammation

Introduction

According to the figures provided by the World Health Organization (WHO), over 320 million people undergo surgery each year [1]. And it was reported that 20–40% of surgical patients experience severe pain 24 h after surgery [2]. The acute postoperative pain, associated with tissue injury, gradually diminishes as the wound heals. However, severe acute postoperative pain is strongly associated with the development of postoperative complications, such as cardiopulmonary dysfunction and cognitive dysfunction, which hampers postoperative recovery [3]. In the meantime, the intensity of postoperative pain is proportional to the incidence of transition to chronic pain [4], resulting in an enormous social, psychological, and economic burden [5]. Despite the fact that pain management has always been taken very seriously, this situation has not been improved significantly compared to three decades ago [6]. Therefore, it is essential to find new therapeutic targets to relieve postoperative pain.

Brain-derived neurotrophic factor (BDNF), a neurotrophic factor widely expressed in the central and peripheral nervous systems, rapidly increased in response to neuronal stimulation [7]. Numerous studies have revealed the promoting effects of BDNF in inflammatory pain and neuropathic pain [8–10], yet there is limited research on the involvement of BDNF in dorsal root ganglia (DRG) in postoperative pain. In addition, the downstream effectors of BDNF in

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postoperative pain are still unclear. VGF (non-acronymic), a secreted granin protein and peptide precursor, is synthesized in specific neurons and neuroendocrine cells [11]. VGF is strongly regulated by BDNF, which has been shown to play an important role in anxiety, depression, and memory processes [12]. It is widely distributed throughout the central and peripheral nervous systems [12]. VGF contains multiple potential cleavage sites. And it is easily converted into small peptides by neuroendocrine-specific prohormone convertases [13]. Moreover, as secreted proteins, they can be released into the extracellular space. They act on macrophage/microglia and modulate its function, promoting the releaseof pro-inflammatory cytokines [14]. Currently, VGF has been found to be upregulated at both mRNA and protein levels in the DRG and spinal cord in neuropathic pain models [15, 16]. However, there are still no studies to show whether BDNF-VGF signaling is involved in the development of postoperative pain.

Based on the aforementioned evidence, we hypothesized that BDNF-VGF pathway was involved in the development of postoperative pain. To test these hypotheses, a plantar incision model was employed to simulate postoperative pain. Furthermore, a series of experiments including behavioral assessments, transcriptomic sequencing, and molecular biology techniques were conducted for validation. Ultimately, our findings provided crucial evidence for the participation of BDNF-VGF pathway in the development of postoperative pain.

Method

Animals

The 2-month-old male Sprague–Dawley (SD) rats (180-200 g) were bought from the Bentel Biotechnology (Hubei, China) (License number: SYXK (e) 2021-0057). Three rats were housed in a cage, drinking and eating freely, using a 12-h light-dark cycle. The ambient temperature was maintained at 23-25 °C and the relative humidity was maintained at 55-57%. Rats were acclimatized for 1 week before all experiments were performed. All the experiments were conducted with the permission of Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology.

Plantar Incision

Plantar incision was used to simulate postoperative pain as previously described [17]. First, rats were anesthetized with 2.5-3% isoflurane (Reward, Shenzhen, China). After anesthesia, a 1-cm incision was made 0.5 cm from the heel to the end of the toe on the sole of the foot. The incision was deep into the muscle layer and the flexor digitorum brevis muscle was divided and retracted. Finally, the incision was closed with 5-0 silk sutures and the skin was disinfected three times. Rats in the control group were anesthetized without incision.

Intrathecal Injection

Recombinant human/murine/rat BDNF (rBDNF) was purchased from PeproTech (NJ, USA). The drug was dissolved in saline at a concentration of 5 ng/μl. Immediately after plantar incision, 100ng rBDNF was injected intrathecally, twice in succession with a 12-h interval. The dose and injection protocol were determined based on previous studies and experimental purpose [18]. Rats were anesthetized with 2.5-3% isoflurane. Exogenous rBDNF was injected into the L5-L6 intervertebral space of the lumbar spine using a microsyringe (High Pigeon, Shanghai, China). The presence of cerebrospinal fluid in the retraction or a tail flick after the injection was regarded as a successful intrathecal injection.

Behavior Tests

Prior to testing, the animals were allowed to acclimate to the testing environment for at least 30 min. We repeatedly measured paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) 1 day before surgery, 2, 4, 8, and 24 h, and 3, 5, and 7 days after surgery to assess changes in pain thresholds. The testing protocol remained the same as described previously [19–21]. Each group consisted of eight rats.

The PWT of rats was assessed using the up-and-down method of the Von Frey test. A series of Von Frey filaments were applied perpendicularly to the hind paw in sequence. The rat's response to each filament was observed and recorded as positive or negative. The positive response included paw withdrawal, licking, or shaking. The negative response was characterized by no response, or a delayed or incomplete response. Five consecutive tests with a 30-s interval between each test was conducted and the paw withdrawal response was recorded. If the positive response was observed in three or more out of the five tests, it was recorded as the PWT for that rat.

To measure PWL in response to thermal stimulation, a thermal phonometer (Ugo Basile, Milan, Italy) was applied to the rat's hind paw at a constant temperature for a duration of no more than 20 s. The time interval between the onset of the thermal stimulus and the withdrawal of the paw was recorded. Three consecutive tests with a 5-min interval between each test were conducted. The average of three measurements represented PWL.
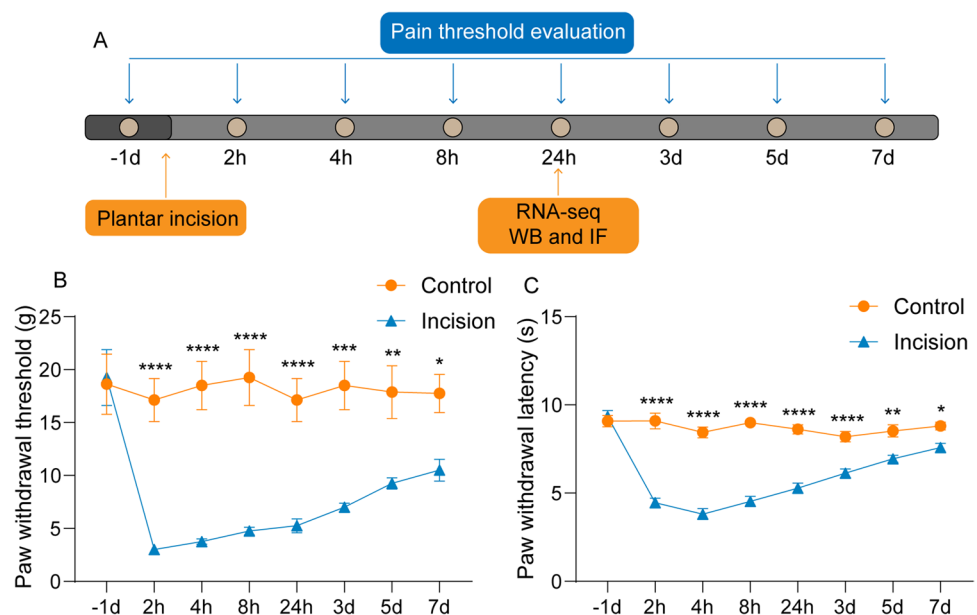
Western Blotting

Twenty-four hours after the plantar incision, rats were deeply euthanized by isoflurane. Each group consisted of three rats. The ipsilateral L4-L6 DRG were immediately dissected and stored at -80 °C. Tissues were homogenized in pre-cooling RIPA lysis buffer (Beyotime, Beijing, China) with protease and phosphatase inhibitors (Roche, Shanghai, China). After centrifugation at 12,000 rpm for 20 min at 4 °C, the supernatant was collected. Protein concentration was determined using the BCA assay (Cwbio, Beijing, China) and adjusted to equal concentrations. Loading buffer (Solarbio, Beijing, China) was added to the samples to denature proteins. Proteins were scattered by SDS-PAGE and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Massachusetts, USA). Membranes were blocked with rapid blocking buffer (Epizyme, Shanghai, China), and then were incubated overnight at 4 °C with the following primary antibodies: mouse anti-β-actin (1:1000; Proteintech, Wuhan, China), rabbit anti-BDNF (1:1000; Abcam, Cambridge, UK), rabbit anti-VGF (1:1000; Abmart, Shanghai, China), rabbit anti-IL6 (1:1000; Affinity, OH, USA), rabbit anti-TNF-α (1:1000; Proteintech, Wuhan, China), and rabbit anti-IL-1β (1:1000; Abcam, Cambridge, UK). After washing, the membranes were incubated with anti-mouse horseradish peroxidase (HRP)-conjugated IgG antibody H&L (1:5000; Antgene, Wuhan, China) or anti-rabbit HRP-conjugated IgG antibody H&L (1:5000; Antgene, Wuhan, China) at room temperature for 1 h. Finally, the images were captured by the imaging system (Media Cybernetics, Silver Spring, MD, USA). The intensity of each band was quantified using ImageJ (NIH, Washington, USA) and normalized to the loading control.

Immunofluorescence

Twenty-four hours after the plantar incision, rats were deeply euthanized by isoflurane and perfused by phosphate buffer saline (PBS) (Servicebio, Wuhan, China) followed by fixation with 4% paraformaldehyde. Each group consisted of three rats. The fixed DRG were subsequently embedded in paraffin and sectioned into 5-μm-thick slices. The baked paraffin sections were deparaffinized in xylene twice and hydrated in a series of gradient dilutions of ethanol. Antigen retrieval was performed by microwave heating in sodium citrate repair buffer (Servicebio, Wuhan, China). Then, the sections were blocked with 5% BSA and 0.3% Triton X-100 for 1 h and incubated overnight at 4 ℃ with the following primary antibodies: mouse anti-BDNF (1:500; Abcam, Cambridge, UK), rabbit anti-BDNF (1:200; Abmart, Shanghai, China), rabbit anti-VGF (1:200; Abmart, Shanghai, China), mouse anti-Iba1 (1:500; Abcam, Cambridge, UK), mouse anti-NeuN (1:500; Abcam, Cambridge, UK), mouse anti-GFAP (1:1000; Abcam, Cambridge, UK), mouse anti-NF200 (1:400; Cell Signaling Technology, Boston, USA), mouse anti-CGRP (1:100; Santa, California, USA), FITC-conjugated IB4 (1:200; Sigma, St. Louis, Missouri, USA), rabbit anti-IL-6 (1:100; Proteintech, Wuhan, China), and rabbit anti-TNF-α (1:100; Proteintech, Wuhan, China). At last, the sections were incubated with the following secondary antibodies: Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:1000; Abcam, Cambridge, UK), Alexa Fluor 488-conjugated

Fig. 1 Plantar incision induced a decrease in pain thresholds. **A** The flowchart for the first part of the experiment. **B** Paw withdrawal threshold (PWT) was decreased after plantar incision, which was measured with the Von Frey test ($n = 8$, two-way ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$). **C** Paw withdrawal latency (PWL) was decreased after plantar incision, which was assessed using a thermal phonometer ($n = 8$, two-way ANOVA, $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$). All data were reported as the mean ± standard error of the mean (SEM)
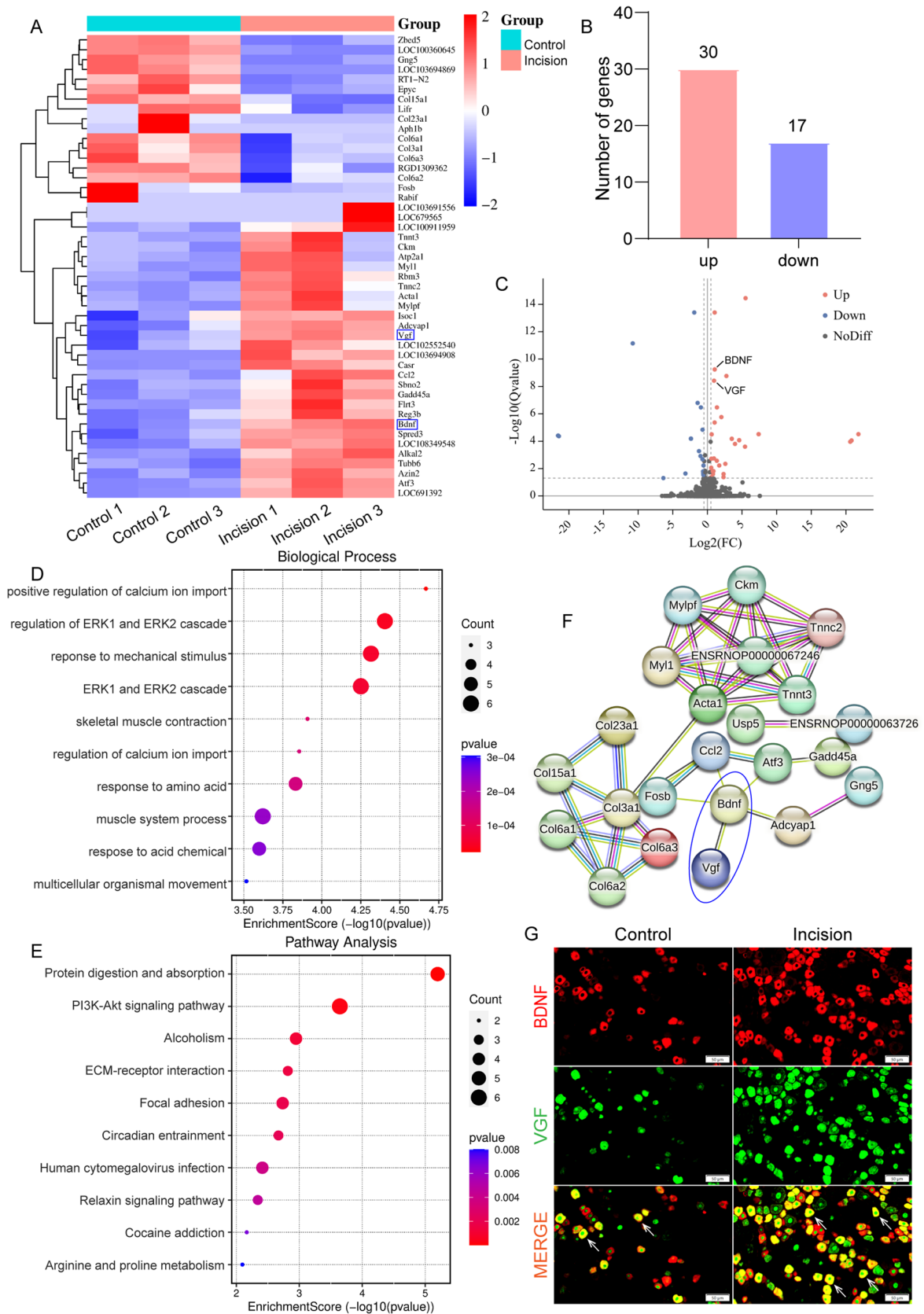
goat anti-mouse IgG (1:1000; Abcam, Cambridge, UK), Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000; Abcam, Cambridge, UK), and Alexa Fluor 594-conjugated goat anti-mouse IgG (1:1000; Abcam, Cambridge, UK). Last, the sections were mounted with antifade mounting medium containing diamidino-phenyl-indole (DAPI) (SouthernBiotech, Alabama, USA). The images were captured using a slide scanner (Olympus, Tokyo, Japan) and analyzed using ImageJ (NIH, Washington, USA).

ELISA

Twenty-four hours after the plantar incision, rats were deeply euthanized by isoflurane. Each group consisted of four rats. The ipsilateral L4-L6 DRG were immediately dissected. Experiments were performed according to the instruction of the ELISA kit (Bioswamp, Wuhan, China). In brief, tissues were homogenized in pre-cooling PBS with protease inhibitor. After centrifugation at 10,000g for 10 min at 4 °C, the supernatant was collected to be used for ELISA. Six tubes were prepared for gradient standard dilution. Then, samples, standard, biotinylated detection antibody, and HRP-conjugated reagent were added into the wells and incubate it for 30 min. After washing, chromogen solution was added. Ten minutes later, stop solution was used to stop the reaction and the solution changed from blue to yellow. Finally, the OD value was measured and the concentration of IL-6, TNF-α, and IL-1β was analyzed.

RNA Sequencing

The ipsilateral L4-L6 DRG were used for RNA sequencing at BGI Genomics to investigate gene expression profiles. The control and incision groups consisted of three samples each. Firstly, TRIzol lysis buffer was used to extract total RNA from DRG. And then, the extracted RNA was fragmented and reverse transcribed into cDNA. After end repair, adding a single "A" nucleotide to the 3′ ends and adaptor ligation, the product was enriched by PCR. The PCR product was denatured to single-stranded

and then cyclized to obtain a single-stranded cyclic product. After rolling cycle amplification, the DNA nanoball was generated. The DNA nanoballs were loaded into patterned nanoarrays and then sequenced through combinatorial Probe-Anchor Synthesis. Fragments per kilobase per million reads (FPKM) was used to evaluate the transcript levels. Differentially expressed genes (DEGs) were analyzed by DESeq2 (v1.4.5). DEGs were identified based on the criteria of |log$_2$(fold change, FC)| ≥ 0.5 and adjusted P-value < 0.05, with the additional requirement that at least one sample among all the samples satisfied the FPKM value ≥ 1. Volcano plot was plotted by https://www.chiplot.online/index.html (last accessed on 30 Apr 2023), an online tool for data visualization. Heatmap, GO enrichment analysis, and KEGG pathway analysis were plotted by https://www.bioinformatics.com.cn (last accessed on 30 Apr 2023), another online platform for data analysis and visualization. Protein-protein interaction (PPI) network was constructed based on the STRING database.

Statistical Analysis

Statistical results analysis was performed using GraphPad Prism 9.0 Software (GraphPad Software, La Jolla, CA). All data were expressed as mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test was conducted to analyze the statistical difference over time between the control and incision groups. Multiple t-test with false discovery rate (FDR) correction was chosen to explore significant differences between the vehicle and rBDNF groups. Unpaired t-test was conducted to analyze the statistical difference between two groups at the same time point. Statistically significant difference was defined as a $P < 0.05$.

Results

The Plantar Incision Pain Was Accompanied with Upregulation of BDNF, VGF, and Neuroinflammation in the DRG

We established the rat model of postoperative pain by making plantar incision as previously described [17]. Consistent with previous reports, plantar incision resulted in a significant decrease in PWT and PWL (Fig. 1B, C). Both PWT and PWL were at their lowest within 24 h postoperatively, and then gradually recovered over a period of more than 7 days.
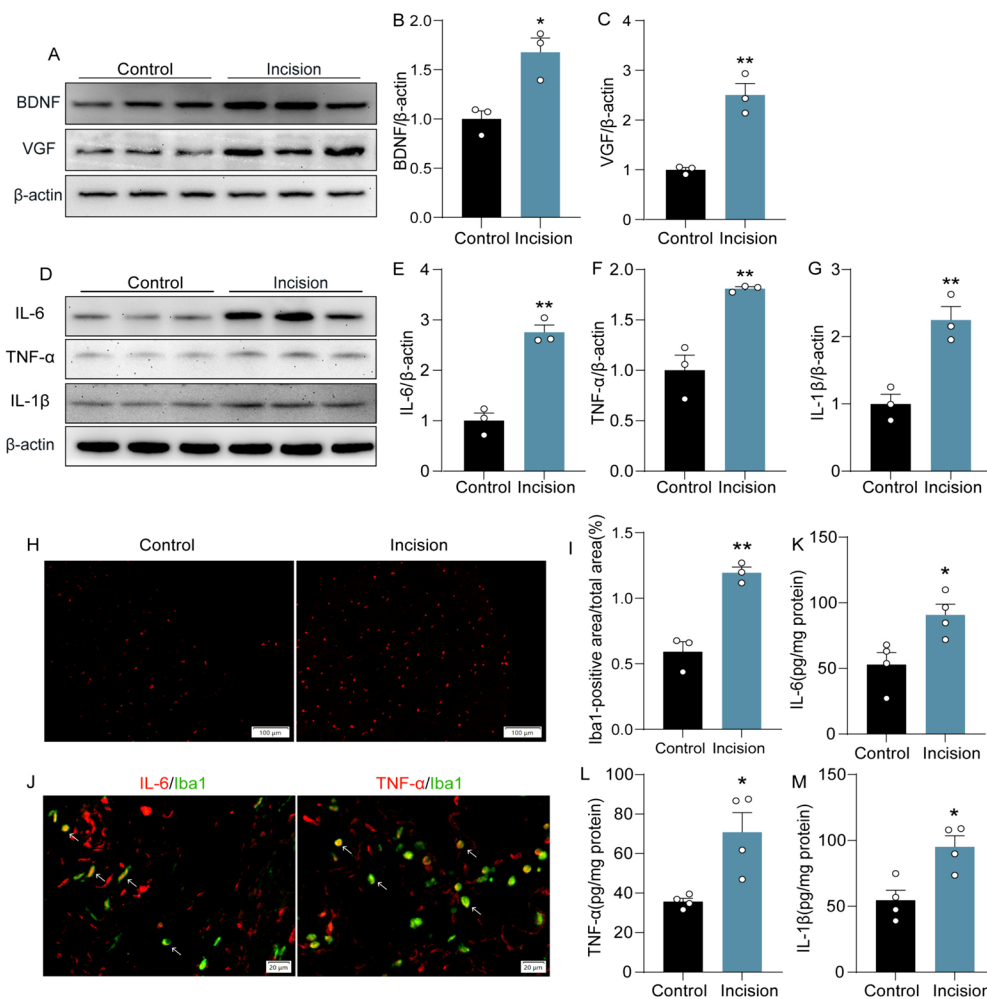
Fig. 3 BDNF and VGF in the DRG were upregulated after plantar incision, accompanied by exaggerated neuroinflammation. **A** The representative images of western blotting of BDNF and VGF in the DRG after plantar incision. **B** Statistical results of western blotting of BDNF ($n=3$, unpaired t-test, *$P<0.05$). **C** Statistical results of western blotting of VGF ($n=3$, unpaired t-test, **$P<0.01$). **D** The representative images of western blotting of IL-6, TNF-α, and IL-1β in the DRG after plantar incision. **E** Statistical results of western blotting of IL-6 ($n=3$, unpaired t-test, **$P<0.01$). **F** Statistical results of western blotting of TNF-α ($n=3$, unpaired t-test, **$P<0.01$). **G** Sta-tistical results of western blotting of IL-1β ($n=3$, unpaired t-test, **$P<0.01$). **H** Immunofluorescence images of Iba1 in the DRG after plantar incision. Scale bar, 100 μm. **I** Percentage of Iba1-posi-tive expression in DRG after plantar incision ($n=3$, unpaired t-test, **$P<0.01$). **J** Immunofluorescence image for co-localization of Iba1 and IL-6/TNF-α. Scale bar, 20 μm. **K** ELISA results of IL-6 after surgery ($n=4$, unpaired t-test, *$P<0.05$). **L** ELISA results of TNF-α after surgery ($n=4$, unpaired t-test, *$P<0.05$). **M** ELISA results of IL-1β after surgery ($n=4$, unpaired t-test, *$P<0.05$). All data are presented as mean ± SEM

To explore the underlying mechanisms of postoperative pain, we examined the gene expression profiles of the injured ipsilateral DRG by RNA sequencing 24 h after incision pain model. A total of 47 DEGs (adjusted $P<0.05$ and $\log_2 FC \geq 0.5$) were identified by RNA sequencing after plantar incision (Fig. 2A). Among them, 30 genes were upregulated and 17 genes were downregulated (Fig. 2B). The upregulated and downregulated DEGs were illustrated in volcano plot (Fig. 2C). To study the function of DEGs, we further conducted GO enrichment analysis and KEGG pathway analysis on DEGs (Fig. 2D, E). GO Biological Processes functional annotations showed that DEGs were primarily involved in positive regulation of calcium ion import, regulation of ERK1 and ERK2 cas-cade, and response to mechanical stimulus (Fig. 2D). KEGG enrichment analysis displayed that DEGs were principally related to protein digestion and absorption, PI3K-Akt signaling pathway and alcoholism (Fig. 2E). Both ERK1/2 and PI3K-Akt signaling pathways were strongly associated with inflammation, which is one of the most common mechanisms in pain development.

Among these DEGs, BDNF and VGF were found sig-nificantly upregulated (Fig. 2A, C). PPI network analysis implicated a close relationship between BDNF and VGF
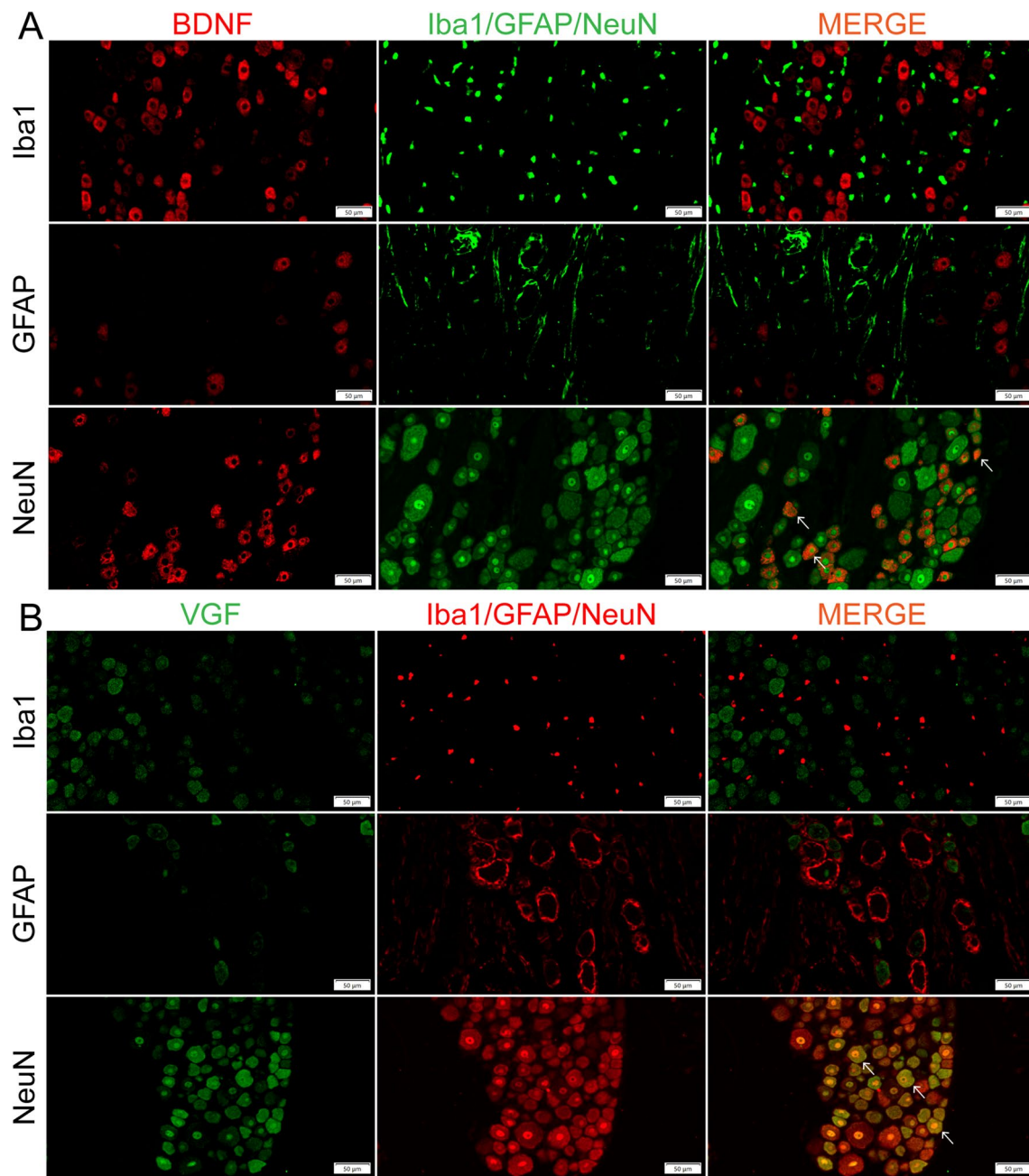
Fig. 4 BDNF and VGF were expressed exclusively on neurons in the DRG. **A** Schematic depiction of BDNF co-localization with Iba1, GFAP, and NeuN, respectively. Scale bar, 50 μm. **B** Schematic depiction of VGF co-localization with Iba1, GFAP, and NeuN, respectively. Scale bar, 50 μm

(Fig. 2F). We further demonstrated the co-localization of the two proteins by fluorescent staining (Fig. 2G). In addition, many previous studies have shown that VGF was regulated by BDNF, which was closely associated with the development of depression [22]. These results suggested that BDNF-VGF pathway might be involved in regulating the development of incision pain.

We further demonstrated by western blotting that the expression of BDNF and VGF at 24 h postoperatively significantly increased after plantar incision (Fig. 3A–C). In addition, the expressions of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β, in ipsilateral DRGs were markedly upregulated after plantar incision (Fig. 3D–G and K–M). And the expression of Iba1 significantly increased (Fig. 3H–I), which means that a large number of macrophages have been activated in the DRG. We further demonstrated the co-localization of Iba1 and IL-6/TNF-α (Fig. 3J), which indicated that macrophages
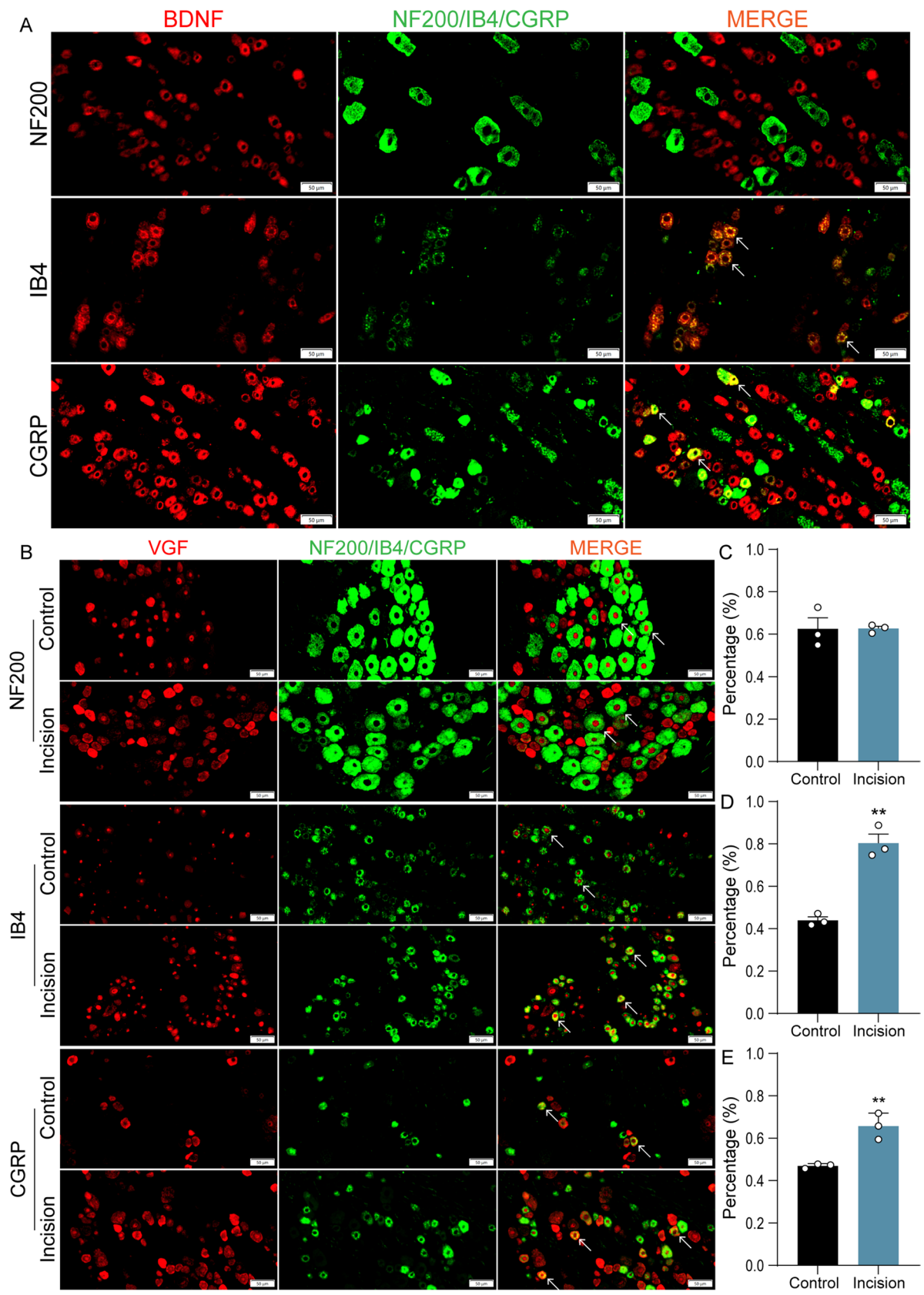
can produce inflammatory factors. These results represented the increased neuroinflammation.

Plantar Incision Causes Increased Expression of BDNF and VGF Mainly in IB4$^+$ and CGRP$^+$ Neurons

To explore the localization of BDNF and VGF, we co-labeled BDNF and VGF with macrophages (Iba1), satellite glial cells (GFAP), and neurons (NeuN) respectively using immunofluorescence. The results showed that both BDNF and VGF expressed in the neurons, rather than macrophages or satellite glial cells (Fig. 4A, B).

We further investigated the localization of the two molecules in different types of neurons. The results showed that BDNF was mainly expressed in isolectin B4-positive (IB4$^+$) neurons, with a small proportion distributed in calcitonin gene-related peptide-positive (CGRP$^+$) neurons and almost no expression in neurofilament 200-positive (NF200$^+$) neurons (Fig. 5A). VGF was expressed in all three types of neurons. After plantar incision, the proportion of VGF expression in IB4$^+$ neurons and CGRP$^+$ neurons increased, while the proportion of expression in NF200$^+$ neurons was unchanged (Fig. 5B–E).

Intrathecal Injection of rBDNF Aggravates Nociceptive Hyperalgesia and Neuroinflammation

To test whether increased expression of BDNF could promote the development of postoperative pain, rBDNF was injected intrathecally into the L5-L6 lumbar spinal space. As expected, compared to intrathecal injection of vehicle, intrathecal injection of rBDNF significantly increased the expression of BDNF in DRG (Fig. 6B, C). The results of immunofluorescence showed that intrathecal injection of rBDNF did not result in a shift from the absence to the presence of BDNF in NF200$^+$ neurons, and the increased BDNF remains only in IB4$^+$ and CGRP$^+$ neurons (Fig. 6D).

Meanwhile, as expected, intrathecal injection of rBDNF further reduced PWT and PWL during the first day following the surgery (Fig. 7A, B). However, this aggravating

effect disappeared on the seventh postoperative day (Fig. 7A, B). Unexpectedly, the expression of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β, in ipsilateral DRG were markedly upregulated in rBDNF group 24 h after incision (Fig. 7C–F and I–K). The expression of Iba1 further increased as well (Fig. 7G, H).

## Intrathecal Injection of rBDNF Increased the Expression of VGF in IB4$^+$ and CGRP$^+$ Neurons

We analyzed the effect of intrathecal injection of rBDNF on VGF expression by western blotting and immunofluorescence. Compared to intrathecal injection of vehicle, intrathecal injection of rBDNF significantly increased the expression of VGF in DRG (Fig. 8A, B). The results of immunofluorescence showed that intrathecal injection of rBDNF caused an increase in VGF expression mainly in IB4$^+$ and CGRP$^+$ neurons, while VGF remained unchanged in NF200$^+$ neurons (Fig. 8C–F). These results showed a consistent change in BDNF and VGF and demonstrated that BDNF was involved in plantar incision-induced pain through regulating VGF in IB4$^+$ and CGRP$^+$ neurons.

Discussion

Nociceptive hyperalgesia induced by the plantar incision is able to simulate postoperative pain to some extent [23]. Studying the mechanisms of incision pain may provide new therapeutic targets for the treatment of acute postoperative pain. In this study, we found that postoperative pain induced by plantar incision significantly upregulated the expression of BDNF and VGF, accompanied by increased neuroinflammation. Intrathecal injection of rBDNF can aggravate postoperative pain, mainly by upregulating VGF and neuroinflammation. Our study is the first to report that BDNF-VGF pathway contributes to the development of postoperative pain.

We found an upregulation of the expression of BDNF and VGF in the incision pain model. Intrathecal administration of rBDNF can further aggravate incision pain. BDNF in DRG has been reported significantly upregulated in diabetic neuropathic pain models and complete Freund's adjuvant (CFA)-induced inflammatory pain models [24, 25]. A previous study found that overexpression of BDNF in DRG aggravates chronic constriction injury (CCI)-induced neuropathic pain, while inhibition of its expression relieves the pain [26]. Our finding demonstrated that BDNF in DRG was upregulated after plantar incision, which is consistent with previous studies. However, in contrast, hippocampal BDNF expression exhibits an opposing trend, showing a

Fig. 6 Expression and distribution of BDNF in the DRG following intrathecal rBDNF injection. **A** The flowchart for the second part of the experiment. **B** The representative images of western blotting of BDNF in the DRG after intrathecal injection of rBDNF. **C** Statistical results of western blotting of BDNF ($n = 3$, unpaired t-test, ** $P < 0.01$). Data were presented as mean ± SEM. **D** Immunofluorescence images of co-localization of BDNF with NF200, IB4, and CGRP following intrathecal injection of rBDNF. Scale bar, 50 μm
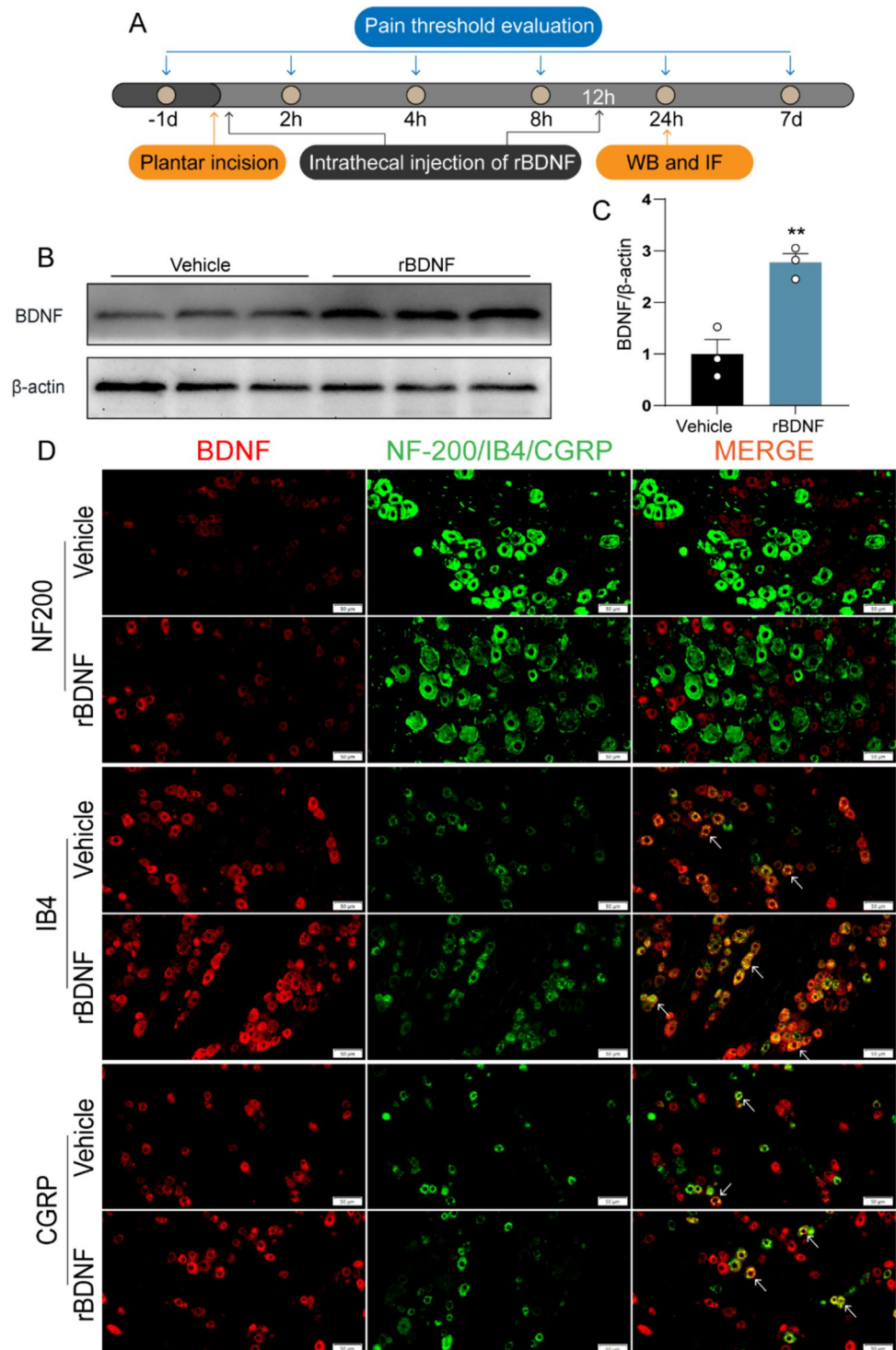
decrease in various pain models including diabetic neuropathic pain and post-stroke pain [24, 27]. BDNF in different sites may play different roles in regulating pain. And BDNF in the hippocampus is involved in both chronic pain and the associated cognitive and emotional disturbance, suggesting a complex role [28]. Many studies also have reported the upregulation of VGF in neuropathic and inflammatory pain and its pronociceptive effect [29–31]. VGF in the DRG was significantly upregulated in the spared nerve injury (SNI) model and remained at a high level for a long time [32].
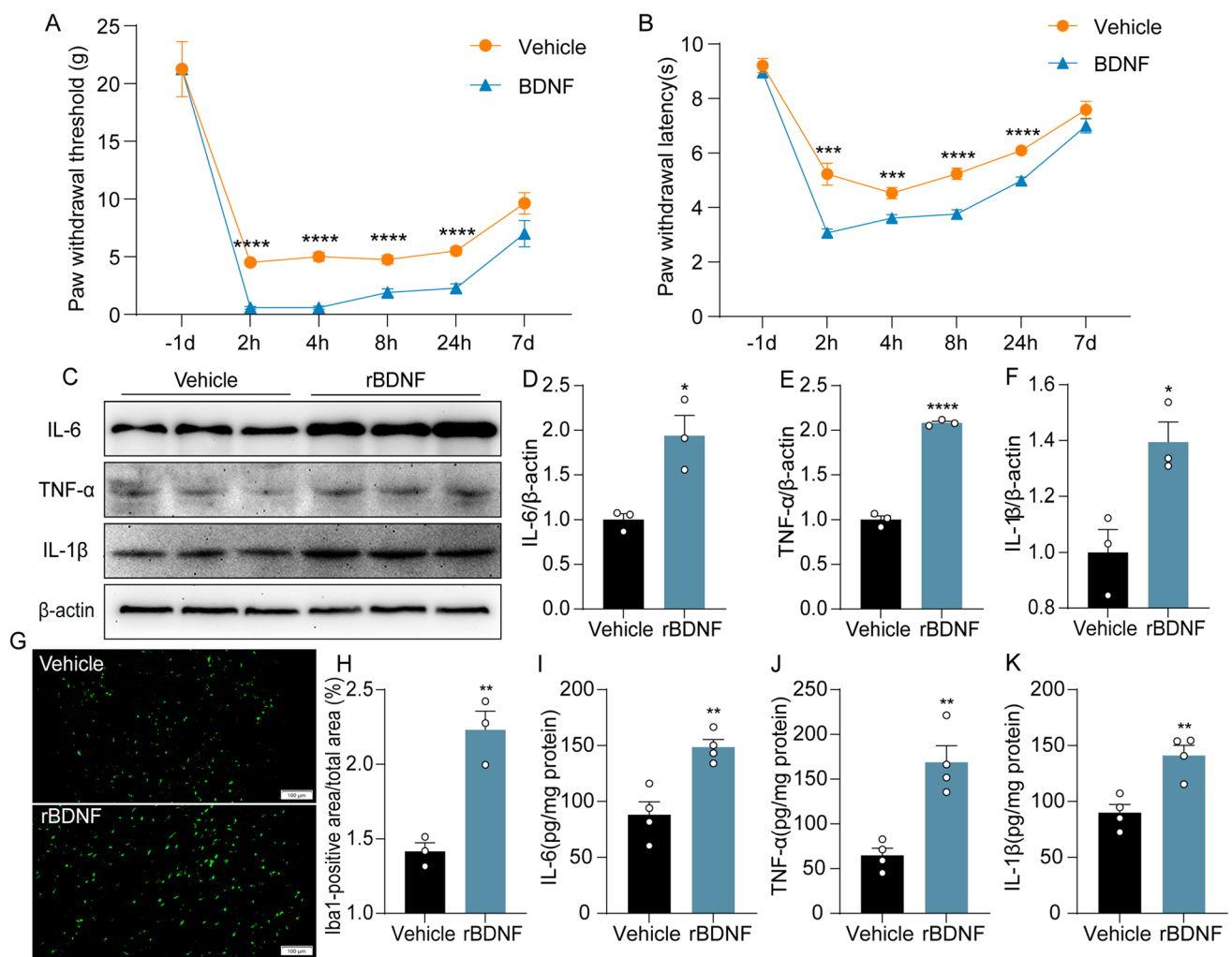
Fig. 7 Exacerbation of nociceptive hyperalgesia and neuroinflammation after intrathecal injection of rBDNF. **A** Intrathecal rBDNF injection resulted in significant decrease of PWT within 24 h, followed by recovery within 7 days ($n=8$, multiple t-test, ****$P_{FDR}<0.0001$). **B** Intrathecal rBDNF injection resulted in significant decrease of PWL within 24 h, followed by recovery within 7 days ($n=8$, multiple t-test, ***$P_{FDR}<0.001$, ****$P_{FDR}<0.0001$). **C** The representative images of western blotting of IL-6, TNF-α, and IL-1β in the DRG after intrathecal injection of rBDNF. **D** Statistical results of western blotting of IL-6 after intrathecal injection of rBDNF ($n=3$, unpaired t-test, *$P<0.05$). **E** Statistical results of western blotting of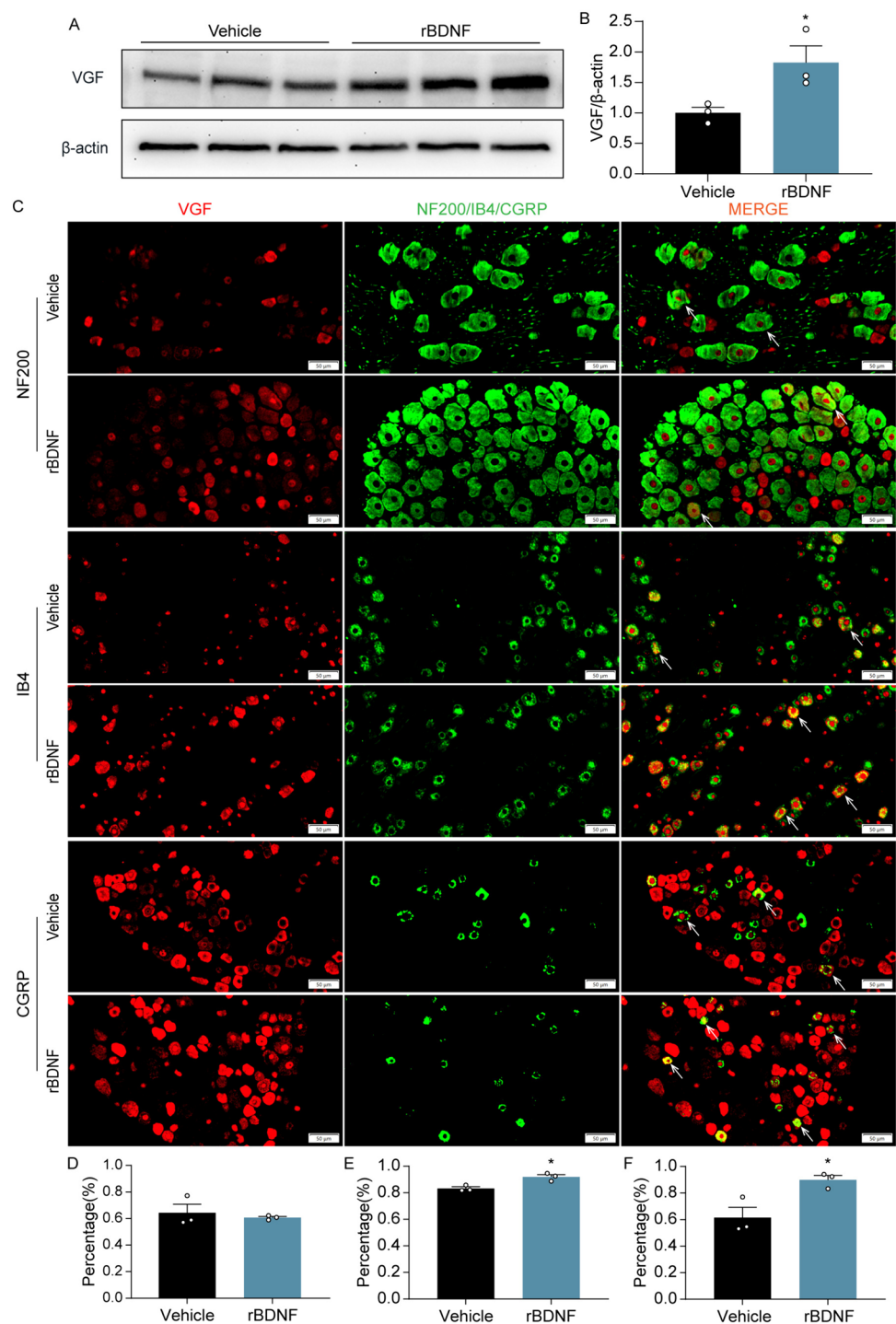 TNF-α after intrathecal injection of rBDNF ($n=3$, unpaired t-test, ****$P<0.0001$). **F** Statistical results of western blotting of IL-1β after intrathecal injection of rBDNF ($n=3$, unpaired t-test, *$P<0.05$). **G** Immunofluorescence images of Iba1 in the DRG after intrathecal rBDNF injection. Scale bar, 100 μm. **H** Percentage of Iba1-positive expression in DRG after intrathecal rBDNF injection ($n=3$, unpaired t-test, **$P<0.01$). **I** ELISA results of IL-6 after intrathecal injection of rBDNF ($n=4$, unpaired t-test, **$P<0.01$). **J** ELISA results of TNF-α after intrathecal injection of rBDNF ($n=4$, unpaired t-test, **$P<0.01$). **K** ELISA results of IL-1β after intrathecal injection of rBDNF ($n=4$, unpaired t-test, **$P<0.01$). All data are presented as mean±SEM

Intrathecal injection of anti-TLQP-21 neutralizing antibody, an antibody of the VGF-derived peptide, significantly ameliorated SNI-induced neuropathic pain and CFA-induced inflammatory pain [33]. Thus, our findings of VGF in postoperative pain complement the role of VGF in different pain models.

At the same time, BDNF exerted a notable regulatory influence on VGF, which was consistent with other studies [34–36]. This regulatory effect was manifested in two distinct aspects. Firstly, BDNF and VGF exhibited the same alterations, as an increase in BDNF levels was accompanied by a concurrent increase in VGF expression. Secondly, the distribution patterns of BDNF and VGF in distinct neuronal populations exhibit a close correlation. In the control group, BDNF exhibited predominant distribution in IB4^{+} neurons, with a minor presence in CGRP^{+} neurons, while its expression in NF200^{+} neurons was virtually absent. In contrast, VGF exhibited robust expression not only in IB4^{+} and CGRP^{+} neurons but also in NF200^{+} neurons. However, in the incision group, only the expression of VGF in

Fig. 8 Expression and distribution of VGF in DRG following intrathecal rBDNF injection. **A** The representative images of western blotting of VGF in the DRG after intrathecal injection of rBDNF. **B** Statistical results of western blotting of VGF ($n = 3$, unpaired t-test, $*P < 0.05$). **C** Schematic depiction of co-localization of VGF with NF200, IB4, and CGRP following intrathecal injection of rBDNF. Scale bar, 50 μm. **D** Statistical analysis of VGF changes in NF200$^+$ neurons after intrathecal injection of rBDNF ($n = 3$, unpaired t-test, NS). **E** Statistical analysis of VGF changes in IB4$^+$ neurons after intrathecal injection of rBDNF ($n = 3$, unpaired t-test, $*P < 0.05$). **F** Statistical analysis of VGF changes in CGRP$^+$ neurons after intrathecal injection of rBDNF ($n = 3$, unpaired t-test, $*P < 0.05$). All data are presented as mean ± SEM

IB4$^+$ and CGRP$^+$ neurons increased, while no significant changes were observed in VGF expression in NF200$^+$ neurons. This phenomenon was consistent with the distribution pattern of BDNF. Furthermore, intrathecal injection of rBDNF failed to induce substantial expression of BDNF in NF200$^+$ neurons, while VGF expression remained upregulated exclusively in IB4$^+$ and CGRP$^+$ neurons. In conclusion, our study showed that BDNF contributed to the incision pain model by regulating VGF in IB4$^+$ and CGRP$^+$ neurons.

Different-sized neurons in the DRG exhibit distinct responses to different types of stimulation. Previous research showed that IB4$^+$ neurons were responsive primarily to noxious mechanical stimulation [37]. Selective deletion of IB4$^+$

neurons specifically relieved mechanical pain induced by tissue damage and inflammatory invasion [38], while CGRP+ neurons were predominantly involved in the transmission of thermal pain [39]. The reduction in the number of CGRP+ neurons was closely correlated with the desensitization of the body to noxious thermal stimulation [40]. Recently, CGRP receptor antagonists were proved to be effective for migraine headache and achieved the successful translation from the lab to the clinic [41]. NF200 is a kind of non-nociceptive mechanoreceptor marker [42]. Many studies have recognized the importance of BDNF in pain [8–10]. Our results showed that BDNF was expressed in IB4+ and CGRP+ neurons, which is consistent with its role in pain. Besides IB4+ and CGRP+ neurons, VGF was also expressed in NF200+ neurons. The divergent expression of BDNF and VGF suggests that VGF plays an important role not only in the transmission of nociceptive impulses but also in the transmission of non-nociceptive impulses. Further investigation is needed to confirm this.

We have made an intriguing discovery that BDNF-VGF pathway can promote activation of macrophages and upregulate pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β. And macrophages can produce inflammatory factors. Macrophage/microglia-induced neuroinflammation is widely recognized as a key mechanism underlying many forms of pain [43]. The activated macrophage/microglia released pro-inflammatory or pro-nociceptive mediators, such as IL-6, TNF-α, and IL-1β, to increase the neuronal excitability, thus resulting in pain hypersensitivity [44]. And the inhibition of macrophage/microglia could relieve incision-induced pain [45, 46]. Currently, increasing evidence showed that VGF, undergoing proteolytic cleavage, was released to regulate the function of macrophage/microglia [14, 16, 29]. Lee et al. found that VGF can effectively activated macrophages through ERK signaling pathway in vitro [47]. Besides, VGF was found to activate spinal microglia through P38 signaling pathway to promote the development of both inflammatory pain and neuropathic pain [16]. Similarly, this study also found that BDNF-VGF pathway activated macrophages and upregulated the pro-inflammatory cytokines, resulting in pain hyperalgesia induced by plantar incision. Therefore, we concluded that BDNF-VGF pathway promote the development of postoperative pain by increasing the macrophages induced neuroinflammation.

Limitations still exist in our study. We only focused on the effect of BDNF-VGF pathway on pro-inflammatory cytokines, ignoring its role in anti-inflammatory cytokines. It may also have an effect on anti-inflammatory factors.

Conclusion

Our finding revealed the role of BDNF-VGF pathway in IB4+ and CGRP+ neurons in postoperative pain, which was attributed to upregulated neuroinflammation in the DRG.

Declarations

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