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Original Article

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BDNF/TrkB Signaling Inhibition Suppresses Astrogliosis and Alleviates Mechanical Allodynia in a Partial Crush Injury Model

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Neuropathic pain presents a formidable clinical challenge due to its persistent nature and limited responsiveness to conventional analgesic treatments. While significant progress has been made in understanding the role of spinal astrocytes in neuropathic pain, their contribution and functional changes following a partial crush injury (PCI) remain unexplored. In this study, we investigated structural and functional changes in spinal astrocytes during chronic neuropathic pain, employing a partial crush injury model. This model allowes us to replicate the transition from initial nociceptive responses to persistent pain, highlighting the relevance of astrocytes in pain maintenance and sensitization. Through the examination of mechanical allodynia, a painful sensation in response to innocuous stimuli, and the correlation with increased levels of brain-derived neurotrophic factor (BDNF) along with reactive astrocytes, we identified a potential mechanistic link between astrocytic activity and BDNF signaling. Ultimately, our research provides evidence that inhibiting astrocyte activation through a BDNF/TrkB inhibitor alleviates mechanical allodynia, underscoring the therapeutic potential of targeting glial BDNF-related pathways for pain management. These findings offer critical insights into the cellular and molecular dynamics of neuropathic pain, paving the way for innovative and targeted treatment strategies for this challenging condition.

Key words: Chronic pain, Sciatic nerve lesion, Astrogliosis, BDNF, Mechanical allodynia

INTRODUCTION

Neuropathic pain is a complex and debilitating form of chronic pain presenting a significant clinical challenge due to its persistent nature and limited response to conventional analgesic treatments, compelling researchers to seek deeper insights into its underlying mechanisms. Spinal astrocytes, a type of glial cell in the central nervous system, play a pivotal role in shaping pain perception [1-3]. When activated by injury or inflammation, these astrocytes release signaling molecules that amplify pain signals, contribute to neuroinflammation, hinder neural recovery, and alter neurotransmitter balance. Despite significant progress in unravelling the role of spinal astrocytes in neuropathic pain, a comprehensive understanding of the contribution and functional changes of these astrocytes undergo a partial crush injury, remains an unexplored area of research.

The primary objectives of this study are to investigate specific changes in spinal astrocytes during chronic neuropathic pain using a partial crush injury (PCI) and to elucidate their functional implications contributing to a deeper understanding of the cellular mechanisms underlying this chronic pain condition. For this study, we utilized the partial crush injury model as its ability to replicate both acute and chronic pain [4], making it an ideal platform to study spinal astrocytes in neuropathic pain. This model captures the transition from initial nociceptive responses to per-

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sistent pain, enabling the exploration of astrocytic involvement in pain maintenance and sensitization. Additionally, it offers a valuable opportunity to investigate molecular and cellular changes in spinal astrocytes and their role in central sensitization.

Various studies initially reported that peripheral nerve injury in mice and rat resulted in pain hypersensitivity and astrocyte hypertrophy in the spinal dorsal horn [1]. Furthermore, robust astrogliosis is induced under several pain conditions such as spinal cord injury [5, 6], chronic opioid exposure [7], CFA-induced pain [8, 9]. In mouse models of neuropathic pain, persistent and early astrogliosis appeared both in early and persistent indicates its involvement in the shift from acute to chronic pain and maintaining pain. For example, in a mouse model of spinal cord injury, GFAP upregulation remains robust nine months while reactions of other cell types like microglia have diminished [10]. Mice lacking GFAP or intrathecal knockdown of GFAP in nerve-injured animals were observed to have pain reduction [11]. Importantly, direct manipulation of astrocyte activity using pharmacological and optogenetic approaches revealed the probable role for astrocytes in pain [12-15]. One of the important factors related to the spinal mechanisms of chronic pain and central sensitization is BDNF (Brain-derived neurotrophic factor) [16, 17]. Studies have shown that inhibition of BDNF-TrkB signaling restrained activation of astrocytes and microglia and alleviated neuroinflammation in the spinal dorsal horn of cyclophosphamide-induced cystitis [18]. Importantly, astrocytes activated by BDNF was suggested to contribute to mechanical allodynia development in neuropathic pain in rats [19]. However, whether and how BDNF and reactive astrocytes [20] regulates neuropathic pain in partial crush injury model remains unknown.

In this study, we observed the crucial role of spinal astrogliosis in the development of mechanical allodynia, which is characterized by a painful sensation in response to innocuous stimuli, using a partial crush injury model. The correlation between mechanical allodynia and enhanced BDNF levels along with reactive astrocytes highlights a potential mechanistic relation, suggesting that astrocytic activity and BDNF signaling might play an important role in pain sensitization. The notable observation that inhibiting BDNF/TrkB signaling reduces astrocyte activation and mechanical allodynia underscores the therapeutic potential of targeting glial BDNF-related pathways for pain management. Overall, these findings will provide valuable insights into the cellular and molecular mechanisms of neuropathic pain, offering the way for innovative strategies in developing more effective and targeted treatments for this challenging condition.

MATERIALS AND METHODS

Animal and partial crush injury (PCI) model

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Institute of Basic Science (Approval number: IBS-2022-029). Mice were housed in groups of five, a 12:12 hour light/dark cycle (7 am~7 pm) was maintained. Behavioral testing was conducted between 9 am and 6 pm. Colonies of C57BL/6J (RRID: IMSR_JAX:000664).

The sciatic nerve injury model was conducted as previously explained [4]. Adult male mice (8~10 weeks old) received a single unilateral crush injury to the sciatic nerve under isoflurane anesthesia. The right thigh was prepared by shaving and applying iodine treatment, followed by an incision. The sciatic nerve was exposed using blunt forceps, after which an ultra-fine hemostat (Cat. No 13020-12, Fine Science Tools, Germany) was set up with an aluminum foil spacer (15 μm thick) to create a 30 μm gap. The sciatic nerve was then gently lifted using a fire-polished glass rod (Cat. No. 10061-12, Fine Science Tools, Germany) and placed within the gap on the hemostat (2~3 mm from the tip). After closing the hemostat's first locking position for 15 seconds, the nerve was carefully released. Sutures closed the wound, and the mice recovered in the standard animal housing at the Institute of Basic Science. All tools were autoclaved before surgery, and strict aseptic techniques were maintained throughout.

Von Frey hair test

All sensory testing was performed between 9 am~6 pm in an isolated room maintained at 22±2°C and 50±10% humidity. For the mechanical threshold (Von-Frey filament) testing, mice were transferred from the standard keeping animal room to a transparent plastic cylinder (7 cm diameter and 20 cm height) placed on a mental mesh floor with 5×5 mm holes (mesh floor, Jeung Do Bio & plant co.). The mice were then habituated for at least one hour before the test. To access mechanical sensitivity, the withdrawal threshold of the injured hind paw was measured using a series of Von-Frey filaments (touch test sensory evaluation kit of 20, Cat. No 58011, Stoelting co.) (2.44, 2.83, 3.22, 3.61, 3.84, 4.08, 4.17, 4.31 nM, Exacta, North Coast Medical, USA; equivalent in grams to 0.04, 0.07, 0.16, 0.4, 0.6, 1, 1.4, 2). The 50% withdrawal threshold was determined using the 'up-down' method as previously described [21]. The withdrawal response to the filaments regards as hind paw lift or flinch. The 0.4 g filament was the first stimulus to be used, when there was no response then a higher diameter were applied, if there were response then the next lower diameter filament were applied. This process was repeated until no response was obtained.

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Immunohistochemistry

Mice were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) through a peristaltic pump. Spinal cord tissue was immediately collected and incubated in 4% PFA overnight. The tissue was then sectioned at 30 μM into cold PBS using vibratome Leica VT1000S and store at 4°C. For spinal cord immunohistochemistry, spinal sections were immunolabeled for GFAP (1:1000, Millipore AB5541), BDNF (1:1000, abcam ab108319), Iba1 (1:1000, ab225260). Briefly, tissue was washed with PBS/0.3% Triton X-100 (PBST) for 4 times, blocked with PBST containing 5% normal goat serum for 2 hours at RT, and then incubated overnight with primary antibodies at 4°C. The next day, tissue was washed with PBST for 4 times followed by 2 hour of secondaries (Goat anti-Rabbit Alexa Fluor® 488, Goat Anti-Chicken Alexa Fluor® 647, 1:800; abcam) at RT. Tissue was then washed with PBST for 4 times before mounted onto glass slides and cover with DAPI-containing mounting medium VECTASHIELD® (Vector laboratories). Imaging for quantification of GFAP, Iba1 and BDNF expression were conduct at 40× on Nikon Ti-e Eclipse with PFS microscopy and whole spinal cord section at 20× on Axio Scan Z1.

Immunoblotting

Proteins were isolated and pooled from spinal cord, homogenized with pestle after freeze and thaw in Tissue Lysis/Extraction Reagent (Sigma-Aldrich). Next, SDS–PAGE and western blotting were performed Thirty micrograms of proteins were loaded for each lane on a 4~12% Tris-glycine gel (Invitrogen), resolved and transferred onto polyvinylidene difluoride membrane. On blocking 1 hour at RT with 5% milk powder (AppliChem, Darmstadt, Germany) diluted in double distillate water, the blotted proteins were incubated overnight at 4°C with anti-BDNF antibody (1:1000, abcam ab108319), anti-TrkB antibody (1:1000, abcam ab18987) and anti-GAPDH (1:2000, abcam ab9485). Next, the membrane was incubated for 2 hours at RT with the secondary antibodies ECL peroxidase-labelled anti-rabbit antibody (1:2000; Amersham Biosciences, Freiburg, Germany). Labelled proteins were detected by chemiluminescence using the ECL Prime Western Blotting Detection Reagents (Amersham Biosciences) on Image lab software. Band intensities were analyzed using the open-source software ImageJ and calculated as expression relative to GAPDH. Every lane was analyzed with the plot analysis tool of ImageJ and the area of the peak corresponding to the band was used as intensity value.

Pharmacological test

ANA12 (N-[2-[[(Hexahydro-2-oxo-1H-azepin-3-yl) amino] carbonyl] phenyl] benzo [b] thiophene-2-carboxamide) at doses of 0.5 mg/kg (Tocris; Cat. No. 4781) was injected intraperitoneally (IP) following partial crush injury. Briefly, ANA12 was injected at day 6 and 7 after injury to examine the analgesic effect in acute pain model, while injected at day 27 and 28 after injury in chronic pain model. Perfusions were made 4 hours after the final injection. ANA12 was dissolved in 5% dimethyl sulfoxide (DMSO) and stored at -20°C in aliquots.

RESULTS

Morphological analysis of spinal astrocytes in partial crush injury model

To investigate the characteristics of astrocytes during the transition of chronic neuropathic pain, we employed a partial crush injury on the sciatic nerve [4] and evaluated the withdrawal threshold using the von-Frey test (Fig. 1A). Following the injury for 7 days, mice exhibited a significantly elevated withdrawal threshold on the ipsilateral side in comparison to the contralateral side: 430.2%±139 and 105%±15, respectively. After 2 weeks of the injury, mice exhibited very low level of withdrawal threshold, and this level remained consistently for at least another 4 weeks (Fig. 1B, C). This indicates the development and transition to persistent chronic pain. We conducted immunohistochemistry in the lumbar sacral spinal region to further investigate the changes in astrocyte morphology during the development of neuropathic pain. At day 7 post-injury, a significant increase in glial fibrillary acidic protein (GFAP), a marker for astrogliosis in astrocytes, was observed on the ipsilateral side of the dorsal spinal horn, in comparison to the contralateral side and the control group (Fig. 1D) indicating the occurrence of astrogliosis from the early stages of pain initiation. Intriguingly, at day 28 post-injury, GFAP signals exhibited a significantly larger increase, and this distribution was observed equally on both the ipsilateral and contralateral sides, unlike the PCI day 7 group. This implies the continuous involvement of astrocytes in both the development and maintenance of neuropathic pain in the partial crush injury model. For a more comprehensive characterization of spinal astrogliosis throughout the pain progression, we performed structural analysis of astrocytes using IMARIS software. At day 7 post-injury, the number of astrocyte filaments was substantially higher on the ipsilateral side compared to the contralateral side: 879±59.7 and 632±25.6, respectively. Moreover, filament area and volume were observed to increase exclusively on the ipsilateral side (Fig. 1E, F). We further confirmed a significant expression of astrogliosis on both the ipsilateral and contralateral dorsal sides on day 28 post-injury, corresponding to the chronic pain phase, evidenced by an increase in astrocytic volume and the

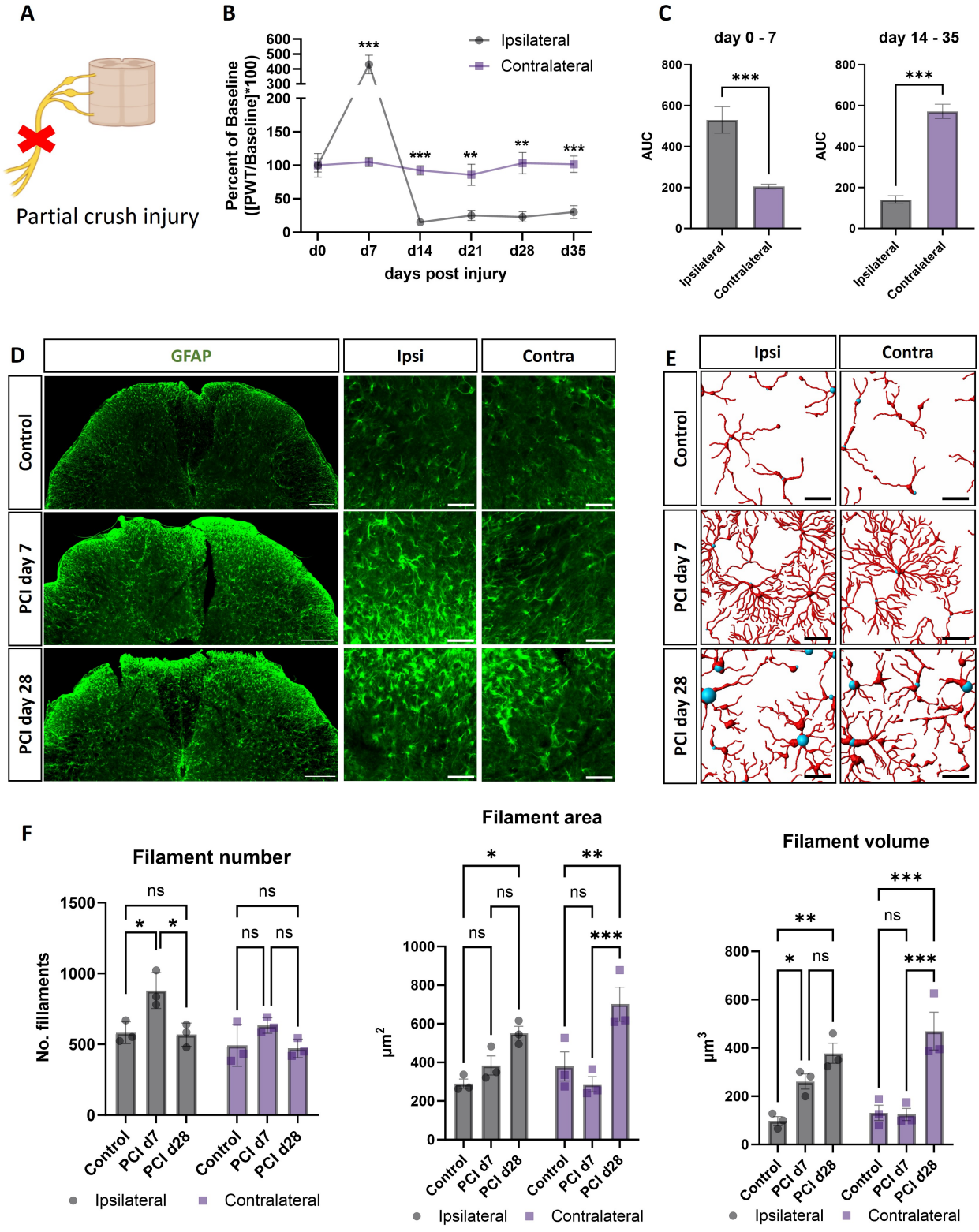

Fig. 1. Characterization of spinal astrocytes in partial crush injury model. (A) Partial crush injury model. (B) Percentage of withdrawal threshold in PCI model. (C) Area under curve between ipsilateral and contralateral from day 0 to day 7 and from day 14 to day 35. Unpaired two-tailed-t-test: **p=0.001, ***p<0.001. (D) Representative images for the GFAP expression (Green: GFAP). Scale bar: 200 µm (left), 50 µm (right). (E) Representative astrocyte structure using IMARIS. Scale bar: 20 µm. (F) Analysis of astrocytic dendrite structure, represented in filament number, area and volume. Two-way ANOVA with Tukey's multiple comparison test (*p<0.01, **p<0.005, ***p<0.001, ns: non-significant, p>0.05).

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number of astrocyte filaments. These findings demonstrate the progression of spinal reactive astrocytes during chronic pain development in the partial crush injury model and reveal the pivotal role of astrocytes in the initiation and perpetuation of the pain process.

Spinal microglia dynamics in the progression of the partial crush injury model

Since spinal reactive astrocytes and microglia are known to play significant roles in the initiation and maintenance of chronic neuropathic pain [22, 23], we investigated the role of spinal microglia in the progression of chronic pain in the PCI model. Through immunohistochemistry analysis, we observed that Iba1, a pain-microglial marker, signals displayed a pronounced increase and accumulation on the ipsilateral side at PCI day 7, followed by a reduction at PCI day 28 (Fig. 2A). To gain a deeper understanding of spinal microglia structural changes, we employed IMARIS analysis (Fig. 2B, C). Notably, at PCI day 7, the ipsilateral side exhibited a significant increase in microglia filament numbers compared to the contralateral side (ipsilateral: 619±37.3; contra-

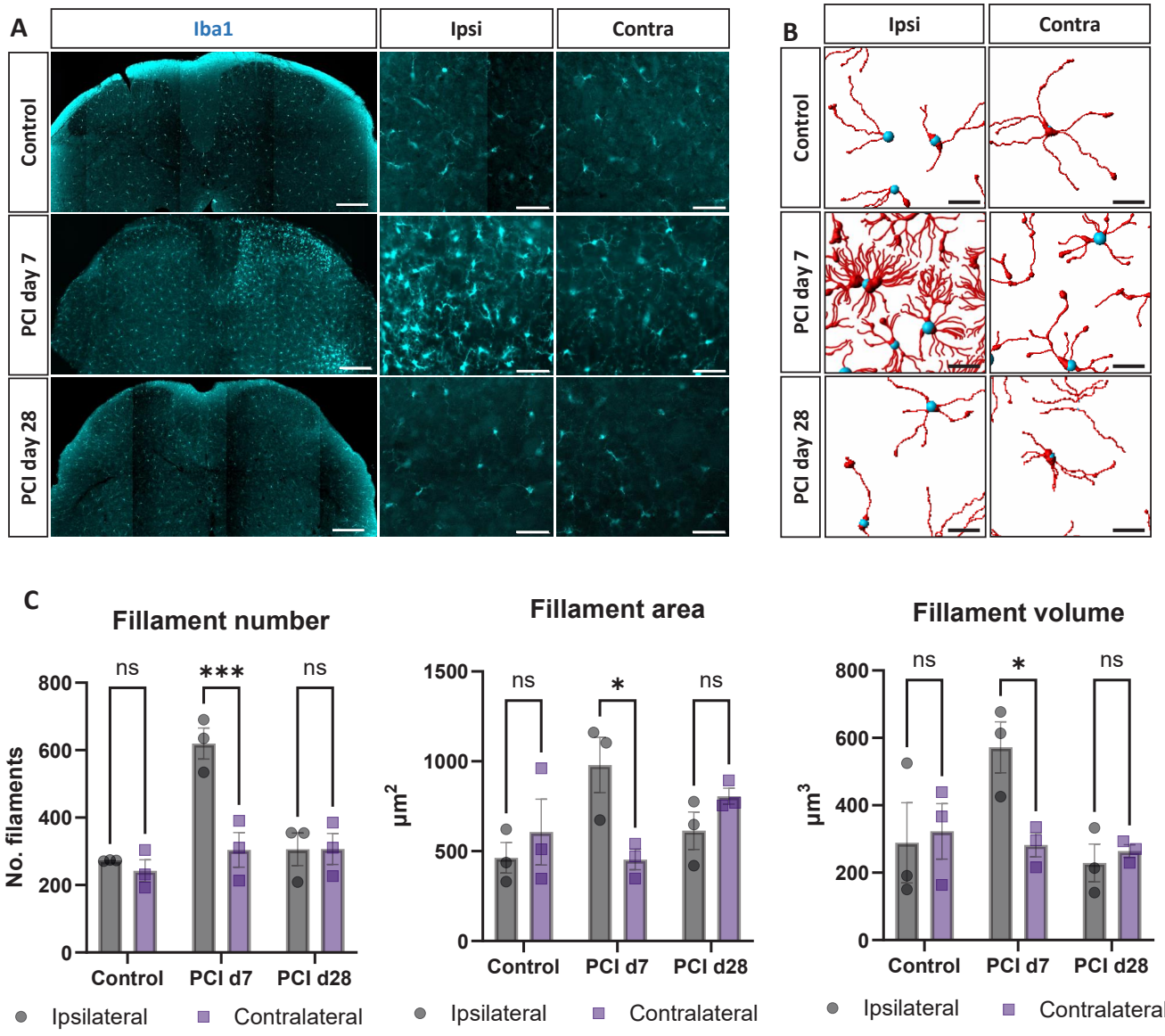

Fig. 2. Characterization of microglia in the pain initiation and maintenance duration. (A) Representative images for the Iba1 expression (Cyanin: Iba1). Scale bar: 200 μm (left), 50 μm (right). (B) Representative microglia structure using IMARIS. Scale bar: 20 μm. (C) Analysis of microglia dendrite structure, represented in filament number, area and volume. Two-way ANOVA with Tukey's multiple comparison test (*p<0.05, ***p<0.001, ns: non-significant, p>0.05).

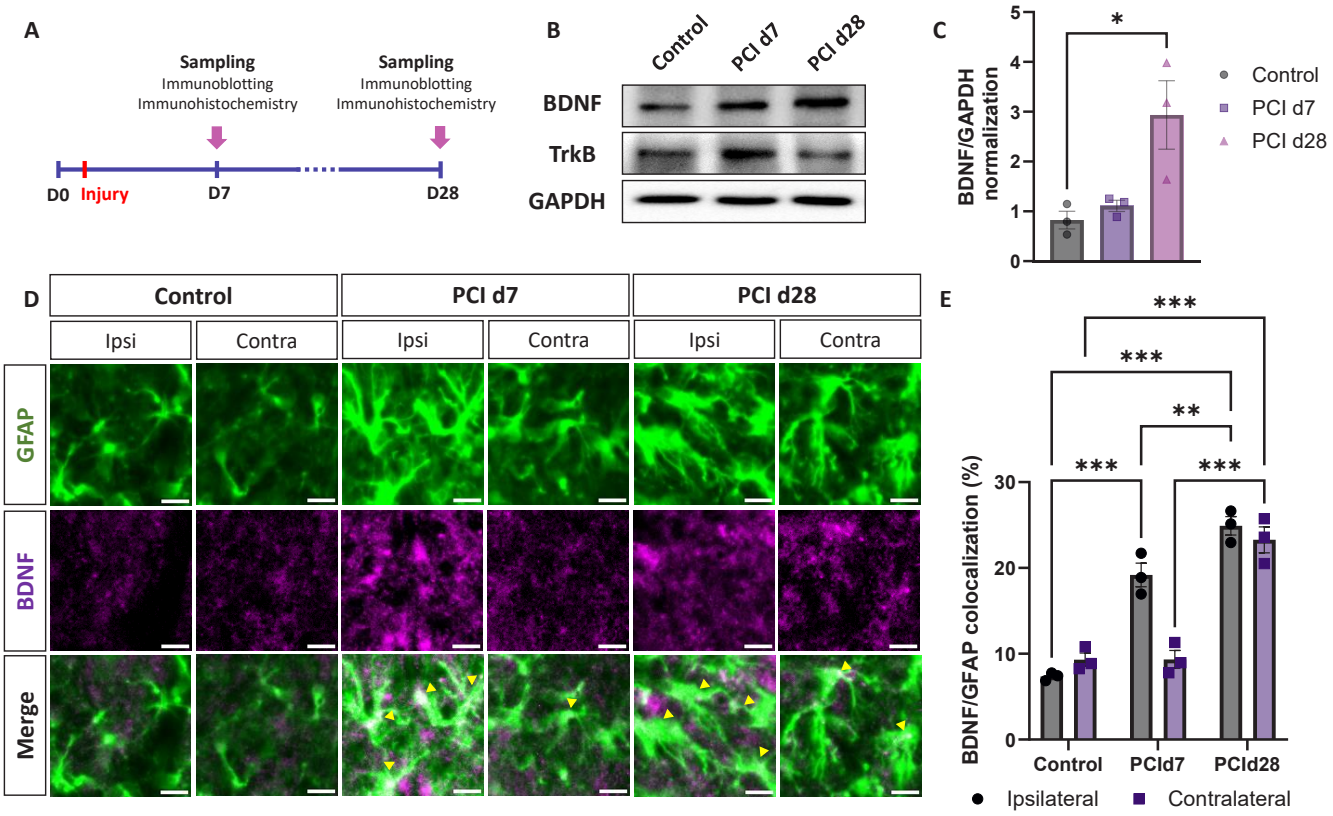

Fig. 3. Spinal astrocytic BDNF increases during the pain maintenance. (A) Experimental timeline for examining BDNF level in acute and chronic pain period. (B) Protein expression level of BDNF and TrkB in control, PCId7 and PCId28. (C) Normalization of BDNF expression in three groups. Unpaired-two-tailed-t-test, *p<0.05. (D) Immunohistochemistry representative images of GFAP and BNDF expression between ipsilateral and contralateral in control, PCId7 and PCId28. Scale bar: 10 μm. (E) Colocalization of BDNF in astrocytes positive among control, PCId7 and PCId28. Two-way ANOVA with Tukey's multiple comparison test (**p=0.009, ***p<0.001, p>0.99).

lateral: 404±93.7), alongside heightened filament area (ipsilateral: 978±125.6; contralateral: 653±201.5) and filament volume (ipsilateral: 572±61.6; contralateral: 415±131.8). However, in the PCI day 28 group, no substantial differences were observed between the ipsilateral and contralateral sides regarding microglia filament numbers (ipsilateral: 306±39.6; contralateral: 307±37.4), filament area (ipsilateral: 614±85.2; contralateral: 831±36.2), and filament volume (ipsilateral: 229±45.8; contralateral: 250±12.2). These results emphasize the intriguing possibility of a dynamic interaction and mutual support between microglia and astrocytes in the complex process of neuropathic pain induced by partial crush injury. Consequently, these findings align with earlier research suggesting that microglia primarily play a specific role during the initiation of pain, while astrocytes significantly contribute to the sustenance of the pain process.

Spinal BDNF and astrocytic co-localization during chronic pain development in the PCI model

Several research studies have consistently demonstrated a significant increase in brain-derived neurotrophic factor (BDNF) across various pain models [16, 17, 24], with its secretion attributed to glial cells [1, 23]. We therefore examined the total spinal BDNF protein levels in the PCI model (Fig. 3A). Immunoblotting results revealed a notable elevation in total spinal BDNF protein expression as the chronic pain progressed, particularly noticeable at PCI day 7 and PCI day 28 compared to the control group (Fig. 3B). Notably, spinal BDNF level reached their peak during the chronic pain phase on PCI day 28, measuring 2.93±0.56, in comparison to the control group's level 0.82±0.14 (Fig. 3C). Combined with our earlier results that demonstrated time-dependent morphological changes in astrocytes during pain development (Fig. 1D~F), it is suggested that this overexpression of BDNF on PCI day 28 might be associated with spinal astrocytes. To further investigate this possibility, we conducted immunohistochemistry to examine the

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co-localization of GFAP and BDNF staining (Fig. 3D). Our results revealed an increase in BDNF-positive astrocytes on the ipsilateral side at PCI day 7 (19.2%±1.12), contrasting with the contralateral side (9.3%±0.84). Particularly, the co-localization of GFAP and BDNF on PCI day 28 exhibited a substantial increase on both the ipsilateral (24.9%±0.87) and contralateral sides (23.6%+1.23) when compared to the control group: 7.3%±0.21 and 9.3%±0.63, respectively (Fig. 3E). The increase in astrocytic BDNF aligns with the astrogliosis observed during the transition from acute to chronic pain (Fig. 1D, E, 3D). These results suggest the relevance of reactive astrocytes and elevated astrocytic BDNF in mechanical allodynia induced by partial crush injury.

Increase in mechanical withdrawal threshold and reduction of astrogliosis by BDNF/TrkB inhibitor

It has been reported that BDNF inhibitors, such as ANA12, effectively mitigate cystitis-related pain by suppressing the activation of astrocytes and microglia [18]. Furthermore, the administration of fluorocitrate, an astrocytic metabolism inhibitor, could successfully inhibited BDNF-induced allodynia and GFAP upregulation [19]. We therefore investigated causal relationship between BDNF and reactive astrocytes concerning PCI-induced mechanical allodynia. To examine the role of BDNF and reactive astrocytes during the acute pain phase, we administered the BDNF inhibitor ANA-12 at a dose of 0.5 mg/kg via intraperitoneal injection to PCI mice on days 6 and 7 (Fig. 4A). We observed significant increase in the withdrawal threshold level on PCI days 4 (D4) and 5 (D5) on the ipsilateral side, contrasting with the control group (Fig. 4B). This increase indicates a temporary loss of motor and sensory functions related to mechanical sensation, a condition referred to as neurapraxia, caused by partial compression of peripheral nerves [25, 26]. In addition, the 6th and 7th days post-injury, D6 and D7, respectively, also represented temporary loss of mechanical sensation occurs, leading to an abnormally increased mechanical withdrawal threshold, as documented in previous studies [27, 28]. In other words, in the PCI model, mechanical thresholds for responding to mechanical stimuli are significantly elevated compared to the control group during the initial 7 days following nerve injury. What is noteworthy here is that despite not showing a significant difference on D6, the consecutive administration of ANA-12 for two days in the PCI group managed to restore the previously elevated mechanical response to the level observed in the saline-injected control group by D7, even reaching the mechanical threshold level of the control group when compared to the saline-injected PCI group. This suggests the potential involvement of spinal BDNF and TrkB signaling in the process of sensory or motor abnormalities observed shortly after nerve injury (Fig. 4B). Next, we extended our investigation to the relationship between spinal BDNF and reactive astrocytes during the chronic pain phase, where ANA-12 was injected on PCI day 27 (D27) and PCI day 28 (D28) (Fig. 4C). ANA-12 administration markedly restored the withdrawal threshold level on the ipsilateral side in comparison to the saline-treated PCI group: 47.4%±6.9 in PCI d27+ANA-12 and 13.2%±2.96 in PCI d27+saline; 62.2%±10.3 in PCI d28+ANA-12 and 12.3%±5.35 in PCI d28+saline. In the PCI model, the increase in the mechanical withdrawal threshold was not observed until the application of ANA-12, strongly suggesting the pivotal role of the BDNF inhibitor in diminishing chronic neuropathic pain. We further evaluated the spinal BDNF protein levels through immunoblotting, and the excessive BDNF expression observed on PCI days 7 and 28 was effectively suppressed by ANA-12 administration (Fig. 4E, F). Although additional research is required to fully understand the reduction in spinal BDNF levels due to BDNF/TrkB inhibition, these results suggest that BDNF/TrkB signaling inhibition may be effective not only shortly after nerve injury but also in treating neuropathic pain that develops chronically. Finally, we explored the impact of the BDNF inhibitor on spinal reactive astrocyte expression. Immunohistochemistry results revealed a reduction in reactive astrocytes within both the acute and chronic phases upon ANA-12 treatment, as demonstrated in the PCI day 7+ANA-12 and PCI day 28+ANA-12 groups (Fig. 4G). Additionally, BDNF inhibitor injection successfully reduced the spinal astrocytic BDNF observed in PCI d7 and PCI d28 group. These results all together demonstrated the potential of BDNF/TrkB signaling inhibition to effectively suppress reactive astrocytes, ultimately leading to a reduction in allodynia within the PCI model.

DISCUSSION

We demonstrate that BDNF/TrkB signaling inhibition has the potential to effectively suppress reactive astrocytes, resulting in a reduction in allodynia within the PCI model. These key findings underscore the important role of spinal astrocytes in the progression of chronic pain in the context of a partial crush injury model. Astrogliosis, marked by increased cell volume and numbers, was prominent during chronic pain development, with microgliosis primarily implicated in pain initiation rather than maintenance. This was consistent with other studies showed the microglia activation generally precedes and subsides prior to astrocytes activation [22, 29, 30]. Indicating that microglia and astrocytes have separate roles in the induction, maintenance and resolution of chronic pain, with the initiation involves microgliosis while later phase involves astrogliosis. Specifically, astrocyte activation has been associated with various signaling molecules and neuromod-

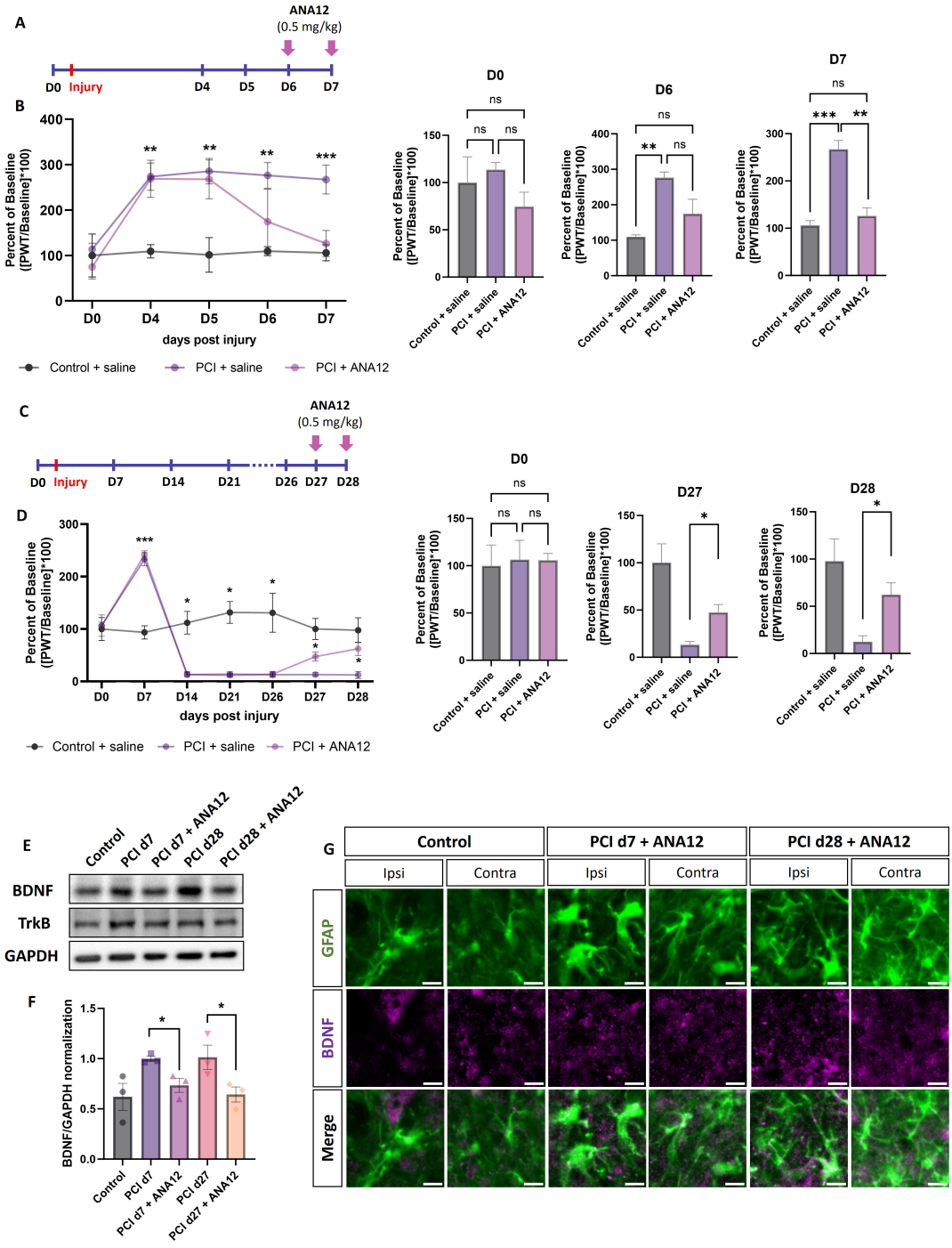

Fig. 4. BDNF inhibitor effectively reduced pain behavior and spinal astrocytic BDNF. (A) Experimental timeline for BDNF inhibitor injection during acute pain period, ANA12 was injected at 0.5 mg/kg on day 6 and day 7 post injury. (B) (Left) Percentage of withdrawal threshold within 7 days post injury with and without ANA12 injection, (Right) bar graphs represent significant differences in percentage of withdrawal threshold among three groups on day 0, day 6 and day 7 post injury. Ordinary one-way ANOVA with Tukey's multiple comparison test (**p=0.009, ***p<0.001, ns: non-significant, p>0.05). (C) Experimental timeline for BDNF inhibitor injection during chronic pain period, ANA12 was injected at 0.5 mg/kg on day 27 and day 28 post injury. (D) (Left) Percentage of withdrawal threshold within 28 days post injury with and without ANA12 injection, (Right) bar graphs represent significant differences in percentage of withdrawal threshold among three groups on day 0, day 7, day 21, day 27 and day 28 post injury. Ordinary one-way ANOVA with Tukey's multiple comparison test (*p<0.01, ***p<0.001, ns: non-significant, p>0.99). (E) Protein expression level of BDNF and TrkB in control, PCId7, PCId7+ANA12, PCId28 and PCId28+ANA12. (F) Normalization of BDNF expression in five groups. Unpaired-two-tailed-t-test, *p<0.05. (G) Immunohistochemistry representative images of GFAP and BNDF expression between ipsilateral and contralateral in control, PCId7+ANA12 and PCId28+ANA12. Scale bar: 10 μm.

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ulators contributing to neuropathic pain [1]. TNF, NFκB, STAT3 leads to astrocytes activation and upregulation of chemokine expression in nerve injury model [31-33]. Secreted trophic factor as bFGF (FGF2) in sciatic nerve ligation rat models maintains late phase of neuropathic pain and activates JNK pathway in astrocytes [14]. Among several factors, research findings have reported that BDNF plays a crucial role in maintaining neuropathic pain [23, 24, 34], but the BDNF-astrocytes causal relationship in neuropathic pain remained less understood. This study highlights a significant connection between elevated BDNF levels and reactive astrocytes, suggesting their collaborative involvement in pain sensitization. Notably, inhibiting astrocyte activation through a BDNF inhibitor effectively increased the mechanical withdrawal threshold. This aligns with previous research findings indicating the significant role of spinal glia activation induced by exogenous BDNF in various pain conditions [18, 35]. In addition to a few research on the significance of astrocytic BDNF in neuropathic pain [19], our study offers novel contributions by focusing on a partial crush injury model to examine the transition from acute to chronic pain. Furthermore, our study highlights the temporal changes in astrocytes and microglia throughout chronic pain development and explores the potential therapeutic role of inhibiting glial BDNF/TrkB signaling, providing valuable insights into glial pathways for pain management.

Previous research has established that injury-triggered activation of P2X receptors in microglia can lead to the BDNF secretion, contributing to neuropathic pain in the spinal cord [36, 37]. Additionally, BDNF-induced secretion of BDNF has been observed in neurons during axon development via several complex signaling pathways [38] and an exogenous BDNF treatment to astrocyte could produce morphological complexities in resting astrocytes via TrkB.T1 [39]. Our study shows the involvement of microglia prominently during the initial phase of pain (approximately 7 days post-injury), but subsequently returned to the normal state, and the treatment of ANA12 at day 6 and 7 effectively restored pain threshold in PCI. This might be attribute to the microglial BDNF secretion in the initial phase of pain, leading to reactive astrocyte phenotypes via astrocytic TrkB.T1 receptors, subsequently initiating inflammatory signaling in the spinal cord. The observed restoration of the withdrawal threshold of PCI-induced pain at 28 days may also be attributed to the prolonged effects of microglial BDNF-triggered signaling in astrocytes or neurons. To selectively indicate the causal relationship and sequential event of microglial and astrocytic BDNF in neuropathic pain, additional experiments including the selective downregulation of BDNF/TrkB signaling in astrocytes, are warranted in the future.

A comparative analysis with alternative neuropathic pain models reveals the distinct advantages of the partial crush injury model. While the partial crush injury model faithfully replicates the transition from acute to chronic pain, with a strong emphasis on the role of spinal astrocytes, some studies utilizing nerve ligation models may provide more specific insights into neuropathic pain initiation mechanisms [40, 41]. Additionally, while the partial crush injury model captures multifaceted aspects of neuropathic pain, other models involving chemical induction might offer complementary perspectives on astrocytic contributions, necessitating a comprehensive approach for a holistic understanding of neuropathic pain pathways.

In conclusion, our study delves into the intricate characteristics of spinal astrocytes following a partial crush injury, revealing their dynamic role in the development and maintenance of neuropathic pain. By elucidating the molecular underpinnings of astrocytic involvement and their interaction with pain pathways, our research contributes to a deeper comprehension of neuropathic pain mechanisms, offering new avenues for therapeutic interventions targeting astrocyte-mediated processes.

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