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BDNF contributes to the neonatal incision-induced facilitation of spinal long-term potentiation and the exacerbation of incisional pain in adult rats

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ABSTRACT

Neonatal surgical injury exacerbates spinal microglial reactivity, modifies spinal synaptic function, leading to exaggerated pain hypersensitivity after adult repeated incision. Whether and how the alteration in microglial reactivity and synaptic plasticity are functionally related remain unclear. Previously, we and others have documented that spinal brain-derived neurotrophic factor (BDNF), secreted from microglia, contributes to long-term potentiation (LTP) in adult rodents with neuropathic pain. Here, we demonstrated that the mRNA and protein expression of spinal BDNF are significantly upregulated in adult rats subjected to neonatal incision and adult repeated incision (nIN-IN). Neonatal incision facilitates spinal LTP induced by BDNF or high frequency electrical stimulation after adult incision, including a decreased induction threshold and an increased magnitude of LTP. Coincidently, inhibition of spinal BDNF abrogates the LTP facilitation, alleviates the mechanical allodynia and thermal hyperalgesia in nIN-IN rats. By contrast, spinal application of exogenous BDNF in the adult rats with a single neonatal incision mimics the LTP facilitation and pain hypersensitivity, which have been found in nIN-IN rats. Exogenous BDNF-induced exacerbation of pain hypersensitivity could be blocked by BDNF inhibitor. In addition, blockade of microglial reactivity by intrathecal application of minocycline attenuates the elevation of BDNF and the LTP facilitation, and also, alleviates pain hypersensitivity in nIN-IN rats. In conclusion, spinal BDNF, at least partly derived from microglia, contributes to the neonatal incision-induced facilitation of spinal LTP and to the exacerbation of incisional pain in adult rats. Thus, spinal BDNF may combine the changes of microglial reactivity and synaptic plasticity in nIN-IN rats.

Abbreviations: ACC, the anterior cingulate cortex; AMPAR, α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA) receptor; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; F, female; HFS, high frequency electrical stimulation; Iba1, ionized calcium binding adaptor molecule 1; IN rats, rats with only adult incision; IOD, integrated optical density; LTP, long-term potentiation; M, male; nIN rats, rats with only neonatal incision; nSham rats, rats with only neonatal sham operation; nIN-IN rats, rats with neonatal incision and adult incision; nSham-IN rats, rats with neonatal sham operation and adult incision; NGF, nerve growth factor; NMDA receptors, N-methyl-D-aspartate receptors; P3, postnatal day 3; PWL, paw withdrawal latency; PWT, paw withdrawal threshold; RVM, rostroventral medulla; TrkB, tropomyosin related kinase B; TrkB-Fc, tropomyosin related kinase B-immunoglobulin G fusion protein.

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1. Introduction

Both clinical and preclinical studies have demonstrated that tissue injury during a critical period in early life leads to an exacerbated pain hypersensitivity after repeated noxious stimulation or injury (Gong et al., 2016; Schwaller et al., 2015; Soens et al., 2015; Valeri et al., 2016), suggesting that the neonatal tissue damage may induce a long-term "priming" of pain circuits, which is proposed as a contributor of exaggerated pain response to the repeated trauma. Spinal dorsal horn may play a vital role in the neonatal incision-induced exacerbation of adult incisional pain, for the reason that the spinal dorsal horn-mediated priming of adult pain sensitivity is revealed by electrical stimulation of the tibial nerve without adult repeated incision (Beggs et al., 2012). However, the underlying mechanisms remain unclear.

Spinal microglial reactivity is elevated in adult incision with previous neonatal incision (Beggs et al., 2012; Schwaller et al., 2015). Spinal microglia can synthesize and secrete brain-derived neurotrophic factor (BDNF), which can modify neuronal axonal growth, dendritic maturation and synaptic plasticity, thus participates in many types of pain (Benarroch, 2015). Especially, we have previously found that spinal BDNF contributes to neuropathic pain in adult rats with spinal nerve ligation (Ding et al., 2015; Geng et al., 2010), and spinal nerve ligation-evoked neuropathic pain and incisional pain both arise, at least partly, from injuries to peripheral nerves (Kehlet et al., 2006). Moreover, only microglia-derived BDNF leads to neuropathic pain in adult rodents by shifting the neuron-glia interactions (Coull et al., 2005; Zhao et al., 2006). Additionally, spinal microglia-secreted BDNF is involved in immune profile switches in adolescent mice with neonatal peripheral nerve injury (McKelvey et al., 2015). However, the potential role of spinal BDNF in neonatal incision-induced exacerbation of adult incisional pain is unknown.

A growing body of evidence shows that neonatal injury alters synaptic function within adult spinal nociceptive circuits. Adult spinal projection neurons from neonatal incised mice exhibit a significant increase in the efficacy of primary afferent synapses (Li et al., 2013, 2015). Neonatal incision facilitates the long-term potentiation (LTP) in adult projection neurons (Li and Baccei, 2016). Moreover, it has been proved that BDNF is essential in several protocols-induced LTP in adult spinal cord and some brain regions (Garraway and Huie, 2016; Meis et al., 2012; Sakata et al., 2013). Also, we have previously documented that BDNF induces spinal LTP in mature spinal cord (Ding et al., 2015). However, whether microglia-derived BDNF regulates spinal LTP in neonatal incision-induced exacerbation of adult incisional pain is still unknown.

In this study, we investigated whether the upregulation of spinal BDNF contributes to the facilitation of spinal LTP and plays a role in neonatal incision-facilitated adult incisional pain. Our results show that the repeated incisions in neonatal period and adulthood produce a remarkable upregulation of spinal BDNF, at least partly derived from microglia, which facilitates both BDNF- and electrical-induced spinal LTP and exacerbates pain hypersensitivity. Thus, spinal BDNF may combine the alternations of microglial reactivity and synaptic plasticity in adult rats subjected to repeated incisions.

2. Materials and methods

2.1. Chemicals, antibodies, and animals

BDNF (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% sterile saline as a 0.5 μg/μl stock solution. Tropomyosin related kinase B-immunoglobulin G fusion protein (TrkB-Fc) (R&D systems, Minneapolis, USA) and IgG (Sigma-Aldrich) were dissolved in 0.01 M phosphate buffer saline (PBS) containing 0.1% bovine serum albumin (BSA) as a concentrated stock solution (1 μg/μl). The stock solution was stored at −20 °C, and freshly diluted to working concentration. Minocycline (Sigma-Aldrich) was dissolved to 5.25 μg/μl in saline immediately before administration. Lidocaine hydrochloride (Sigma-Aldrich) was freshly prepared at a concentration of 1% by dissolving it in saline (pH of the final solution was 6.6–6.7). Goat polyclonal anti-rat ionized calcium binding adaptor molecule 1 (Iba1) antibody was purchased from Abcam (Cambridge, MA, USA).

Pregnant Sprague-Dawley rats were provided by and were housed in the Department of Experimental Animal Sciences, Peking University Health Science Center. After birth, rat pups were randomly divided into different experimental groups. Approximately equal numbers of males and females were contained in each group. And each group contained more than one litter to rule out potential litter variability. Litters were weaned at postnatal day 21 and then housed with the same sex. All the animals were raised with free access to food and water under natural light–dark cycle and constant ambient room temperature. All animal experiments followed the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by the Animal Care and Use Committee of Peking University Health Science Center. There were 7 different experimental groups in our present study as presented in Fig. 1A. Experimental groups contain: (1) Naive: non-operated adult controls; (2) nSham: rats with equivalent anaesthesia, maternal separation and handling on postnatal day 3 (P3) and follow up for 10 weeks (w); (3) nIN: rats having only neonatal incision and follow up for 10 w; (4) Sham: control animals with equivalent anaesthesia and handling in adulthood; (5) IN: age-matched animals have only hindpaw incision in adulthood; (6) nSham-IN: rats with equivalent anaesthesia, maternal separation and handling on P3 and incision in adulthood; (7) nIN-IN: neonatal incision on P3 and repeated incision in adulthood after 10 w.

2.2. Planter hindpaw incision

Rat pups aged 3 postnatal days were anaesthetized with 2–3% isoflurane (Sigma-Aldrich). A small incision through the skin and fascia was made and the underlying plantaris muscle was elevated and incised longitudinally (Brennan et al., 1996). The same relative length of incision was carried out in neonatal and adult rats, extending from the mid-point of the heel to the first footpad, which can result in a longer incision in adults compared with neonatal rats. The skin was closed as soon as possible with 5-0 silk in rat pups and 4-0 silk in adults (Schwaller et al., 2015; Sun et al., 2004; Tobe et al., 2010; Walker et al., 2015). Animals were kept warm during recovery from anaesthesia, immediately returned to their cage. The remaining sutures were removed 5 days later according to previous report (Beggs et al., 2012). Incisions were carried out in rat pups from nIN-IN, nIN groups, and adult rats from nIN-IN, nSham-IN and IN groups (Fig. 1A).

2.3. Intrathecal injection

Intrathecal cannula implantation was carried out (Ding et al., 2015). A PE-10 catheter was implanted to L4 and L5 of the spinal cord by a guide cannula. Operations were carried out under sterile conditions. Twenty μl of drugs or vehicles were intrathecally given by the PE-10 catheter, and then 10 μl of vehicles were given to flush. Each injection continued for more than 5 min (min), then the needle of Hamilton syringe kept in situ for another 2 min. Intrathecal cannula implantation was carried out on day 7 pre-intrathecal injection in order to give enough time for rats to recover.

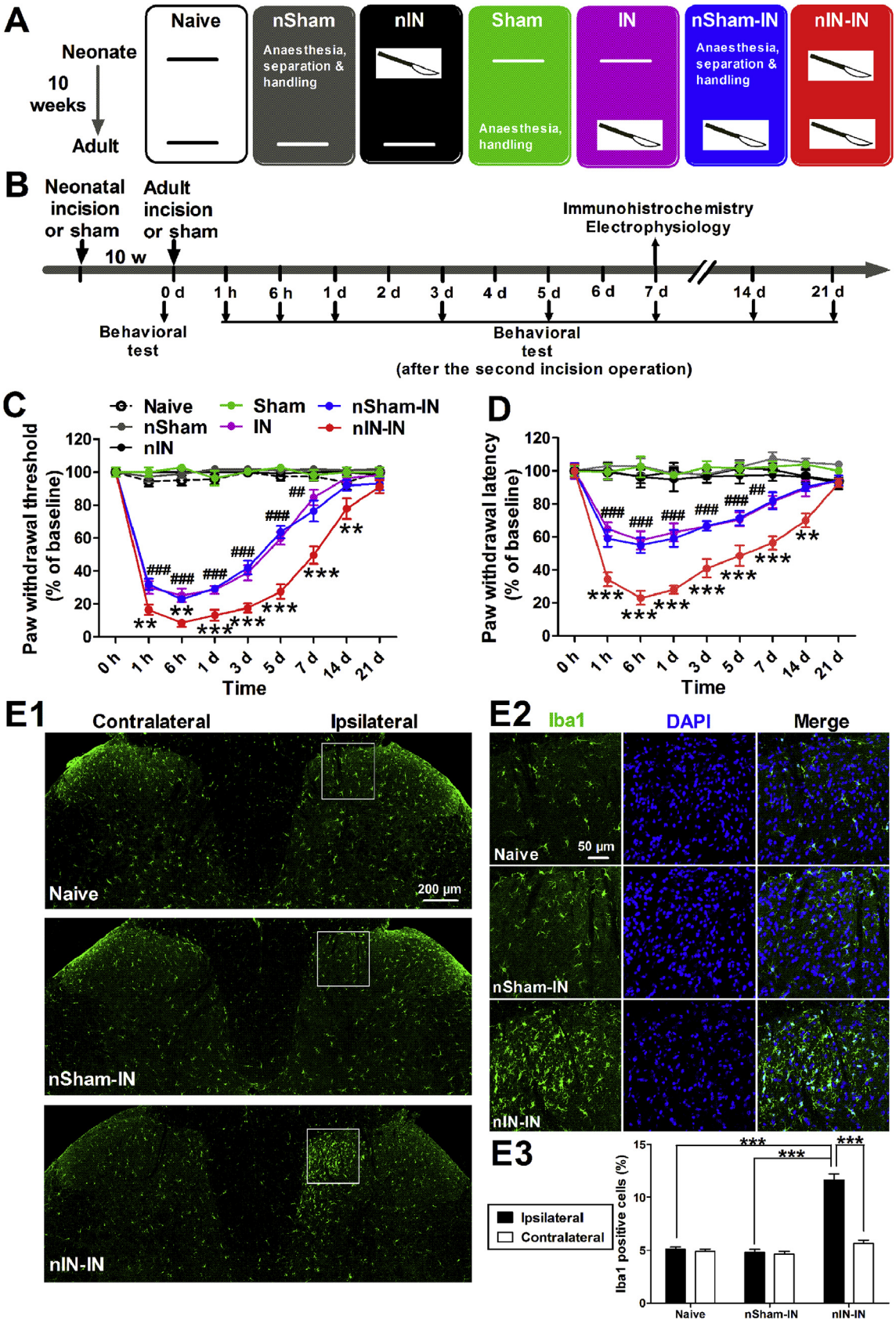

Fig. 1. Neonatal incision exacerbates adult incision-induced mechanical allodynia and thermal hyperalgesia, enhances the immunoreactivity of ionized calcium binding adaptor molecule 1 (Iba1) as a microglial marker in the superficial spinal dorsal horn. (A) Schematic diagram of experimental groups. Experimental groups contain: (1) Naive: non-operated adult controls; (2) nSham: rats with equivalent anaesthesia, maternal separation and handling on postnatal day 3 (P3) and follow up for 10 weeks (w); (3) nIN: rats having only neonatal incision and follow up for 10 w; (4) Sham: control animals with equivalent anaesthesia and handling in adulthood; (5) IN: age-matched animals have only adulthood

2.4. Behavioral testing

2.4.1. Assessment of mechanical allodynia

Paw withdrawal threshold (PWT) was measured to assess mechanical allodynia. The 50% PWT was tested by the Up-and-Down method (Chaplan et al., 1994). The adult rat was allowed to habituate for 20 min on a metal mesh floor covered with an inverted clear plastic cage. Eight von Frey hairs (0.41 g, 0.70 g, 1.20 g, 2.00 g, 3.63 g, 5.50 g, 8.50 g, 15.10 g) (Stoelting Wood Dale, IL) were applied. Each test began with the fourth von Frey filaments (2.00 g) applied to the mid-plantar surface of the rats hindpaw for approximately 2–3 s. Positive response was an sudden withdrawal of the foot during stimulation or just after the removal of the filament. The next weaker or stronger von Frey hair was used when a positive or negative response appeared. Until 6 stimuli were completed, this procedure was performed. The formula used to measure the 50% PWT was the following: 50% PWT $= 10^{[Xf+ k\delta]}$, where X_f is the value of the last von Frey hair applied (in log units), k is a value from the pattern of responses, and $\delta = 0.224$, which is the average interval (in log units) between the von Frey hairs (Dixon, 1980). If a rat had positive response to the lowest von Frey hair, the 50% PWT was 0.25 g. If a rat had negative response to the highest von Frey filament, the 50% PWT was 15.0 g. Mechanical allodynia is defined as that the 50% PWT is < 4.0 g (i.e. withdrawal of the foot induced by non-noxious tactile stimulus) in animals (Zimmermann, 2001).

2.4.2. Assessment of thermal hyperalgesia

Paw withdrawal latency (PWL) was performed to determine heat hyperalgesia. Adult rats were allowed to acclimate for a minimum of 30 min. The mid-plantar surface of the hindpaw was focused by a radiant heat source. Measurements of PWL were taken by a timer that was started by the activation of the heat source and stopped when withdrawal of the paw was detected with a photo detector. Thirty seconds were used as a maximal cutoff time to avoid tissue damage. After testing 3 times, and the average of PWL values was calculated for each rat. Intervals between consecutive tests in the hindpaw were more than 5 min.

2.4.3. Assessment of locomotor function

In order to determine the locomotor function, inclined-plane test was performed for all behavioral experiments in our present study. Adult animals were put on an inclined plate. Fifty degree was the initial angle of the inclined plate. The angle increased 5° each time. The maximum angle that the animal did not falling for 5 s was recorded (Rivlin and Tator, 1977). Three measurements were taken and were averaged as the result of each rat. In this study, inclined plate test was performed for all behavioral experiments in which the animals received intrathecal drugs.

2.4.4. Measurement of drug effects

The first behavioral experiment was carried out to test the effects of neonatal incision on adult incision-induced mechanical allodynia and thermal hyperalgesia, the ipsilateral PWT to von Frey filaments and PWL to heat radiation were detected on day 0 (before adult incision) and on 1 h, 6 h, 1, 3, 5, 7, 14 and 21 days after adult incision in all the seven different groups (Figs. 1–3).

The second behavioral experiment determined the effect of pretreatment or posttreatment with TrkB-Fc, a tropomyosin related kinase B (TrkB) immunoglobulin G fusion protein that is often used to scavenge endogenous BDNF, on mechanical allodynia and thermal hyperalgesia in adult rats with neonatal and adult incisions. In the pretreatment experiments, TrkB-Fc (350 ng/20 μl), or IgG at an equal dose, was intrathecally injected to animals 30 min before adult incision, repeated on day 0, and twice per day from day 1 to day 7 after adult hindpaw incision. The PWT and PWL were measured on day 0 (before adult incision) and on days 1, 3, 5, 7, 14 and 21 after adult incision (Fig. 4A). In order to investigate the role of BDNF in nSham-IN rats, high dose of TrkB-Fc (10 μg/20 μl) or IgG was intrathecally injected to animals 30 min before adult incision, repeated on day 0. The PWT and PWL were measured on day 0 (before adult incision) and on 1 h, 6 h, 1, 2 and 3 days after adult incision (Supplementary data: Fig. S1E). In the posttreatment experiments, TrkB-Fc (350 ng/20 μl) or IgG was intrathecally delivered twice per day from day 1 to day 3 after adult incision. Nociceptive behaviors were detected on day 0 (before adult operation) and on days 1, 3, 5 and 7 after adult injury (Supplementary data: Fig. S2A).

In the third behavioral test, we investigated whether intrathecally delivery of BDNF in adult rats with only neonatal incision mimics the alternations in rats with neonatal and adult incisions. As reported before, acute and gradual enhancements in BDNF activate distinct downstream signaling, and only slow BDNF delivery facilitates hippocampal LTP. Additionally, endogenous BDNF is upregulated slowly when it is constitutively secreted or from a distant source (Balkowiec and Katz, 2002; Canossa et al., 1997; Griesbeck et al., 1999; Hartmann et al., 2001; Ji et al., 2010; Lessmann et al., 2003). Thus, as we previously used before (Ding et al., 2015), BDNF (20 ng/20 μl) or vehicle saline was intrathecally delivered to the nIN or nSham group, repeated twice at a 30 min-interval, and continued for 3 days for a long-lasting effect. In order to confirm that impacts of pain behaviors were directly caused by BDNF injection, TrkB-Fc (350 ng/20 μl) or IgG was intrathecally applicated to animals (at 8:00–9:00 a.m. and 8:00–9:00 p.m., twice per day at a 12 h-interval) along with BDNF injection (at 2:00–3:00 p.m., 20ng/20 μl, twice per day at a 30 min-interval) for 3 days in the nIN group (see Fig. 5A). The PWT and PWL were detected on day 0 (before adult incision) and on days 1, 3, 5, 7, 14 and 21 after adult operation. To test the effective time, BDNF (20 ng/20 μl) or vehicle saline was intrathecally delivered to the nIN or nSham group, repeated twice at a 30 min-interval, the PWT and PWL were detected on day 0 (before adult operation) and on 6 h, 1 day, 2 days and 3 days after adult operation. (Supplementary data: Fig. S3B).

In the fourth behavioral test, minocycline, a microglial inhibitor, was used to determine whether BDNF, which contributes to neonatal incision-exaggerated pain hypersensitivity, is secreted from microglia. In the pretreatment experiments, minocycline at

hindpaw incision; (6) nSham-IN: rats with equivalent anaesthesia, maternal separation and handling on P3 and incision in adulthood; (7) nIN-IN: neonatal incision on P3 and repeated incision in adulthood after 10 w. (B) Scheme of the experimental procedure. Hindpaw incisions were performed in neonatal or/and adult rats. Pain behaviors were measured before adult operation, and were also detected on 1 h, 6 h, 1, 3, 5, 7, 14 and 21 days after adult operation in each group. (C, D) The hindlimb mechanical withdrawal threshold and thermal withdrawal latency are expressed as percentage change from baseline for 21 days after adult incision. Note that the paw withdrawal threshold and paw withdrawal latency are decreased in the nIN-IN group compared with the nSham-IN group from 1 h to 14 days following adult incision. $^{**}P < 0.01$, $^{***}P < 0.001$, nIN-IN *vs.* nSham-IN, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, IN *vs.* Sham, 2-way ANOVA, n = 12/group, 6 each gender. (E) Effects of neonatal incision on Iba1 immunoreactivity in the superficial spinal dorsal horn (lamina I and II) in naïve, nSham-IN and nIN-IN rats. (E1, E2) Representative staining of Iba1 is shown from naïve, nSham-IN and nIN-IN rats. Spinal cord sections stained with Iba1 (green) and DAPI (blue) on day 7 after adult operation. (E2) High power images of the white squares in Figure E1. (E3) The numbers of Iba1 positive cells in the superficial laminas were quantified. Note that Iba1 immunoreactivity in the ipsilateral superficial dorsal horn is increased in the nIN-IN group compared with naïve or nSham-IN group, and the positive staining of Iba1 elevates in the ipsilateral dorsal horn compared with the contralateral side in the nIN-IN group. $^{***}P < 0.001$, 1-way ANOVA, n = 6 animals/group, 3 each gender, 4–6 tissues per animal. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

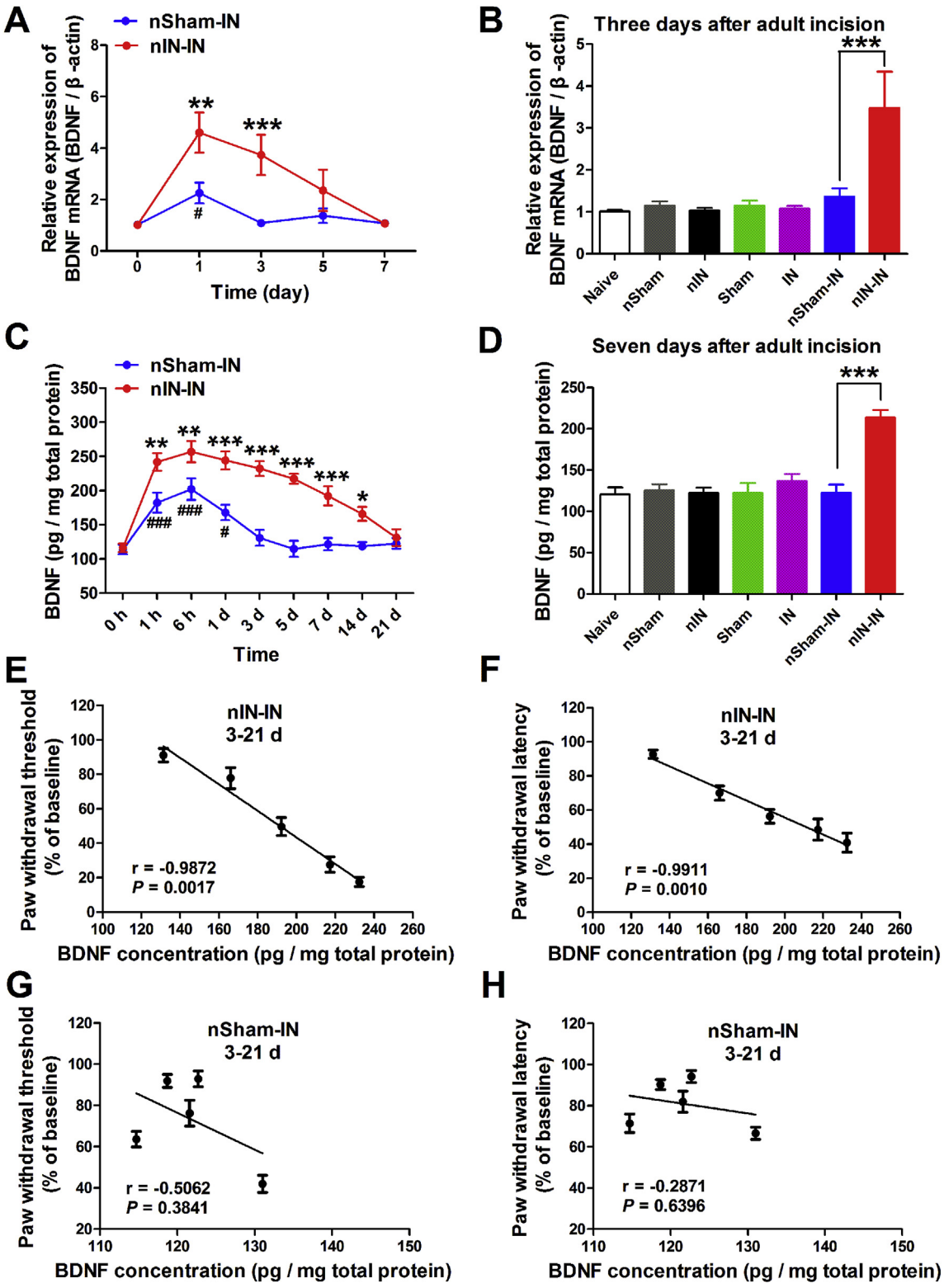

Fig. 2. Neonatal and adult repeated hindpaw incisions increase the expression of BDNF in the spinal dorsal horn. (A, B) Quantitative real-time polymerase chain reaction assay of BDNF mRNA expression in the spinal dorsal horn. (A) The expression of BDNF mRNA is prominently increased from day 1 to day 3 after adult incision in rats with neonatal and adult incision (nIN-IN) compared with rats with neonatal sham operation and adult incision (nSham-IN). $^{**}P<0.01$, $^{***}P<0.001$, 2-way ANOVA, $^{\#}P<0.05$, *vs.* nSham-IN day 0, 1-way ANOVA, n = 6/group in each time point, 3 each gender. (B) The expression of BDNF mRNA is upregulated in the nIN-IN group compared with the nSham-IN group 3 days after adult incision, while other controls show no statistically significant change. $^{***}P<0.001$, 1-way ANOVA, n = 9–11/group, Naïve: male (M) 6 female (F) 5, nSham: M 4 F 5, nIN: M 5 F 4, Sham: M 5 F 5, IN: M 5 F 6, nSham-IN: M 5 F 5, nIN-IN: M 5 F 4. (C, D) Effects of repeated hindpaw incisions on the concentration of BDNF protein in the spinal dorsal horn. (C) Dynamic changes of BDNF protein expression in the spinal dorsal horn in different time points in the nIN-IN and nSham-IN groups. Note that the peak of the increased BDNF concentration occurs on 6 h after adult incision, and continues to day 14 after adult incision, according to the duration of behavioral hypersensitivity. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, 2-way ANOVA, $^{\#}P<0.05$, $^{\#\#\#}P<0.05$, *vs.* nSham-IN 0 h, 1-way ANOVA, n = 14/group in each time point, 7 each gender. (D) The concentration of BDNF protein is upregulated in the nIN-IN group compared with the nSham-IN group, while other controls show no statistically significant change. $^{***}P<0.001$, 1-way ANOVA, n = 12–16/group, Naïve: 6 each gender; nSham, Sham, nSham-IN:

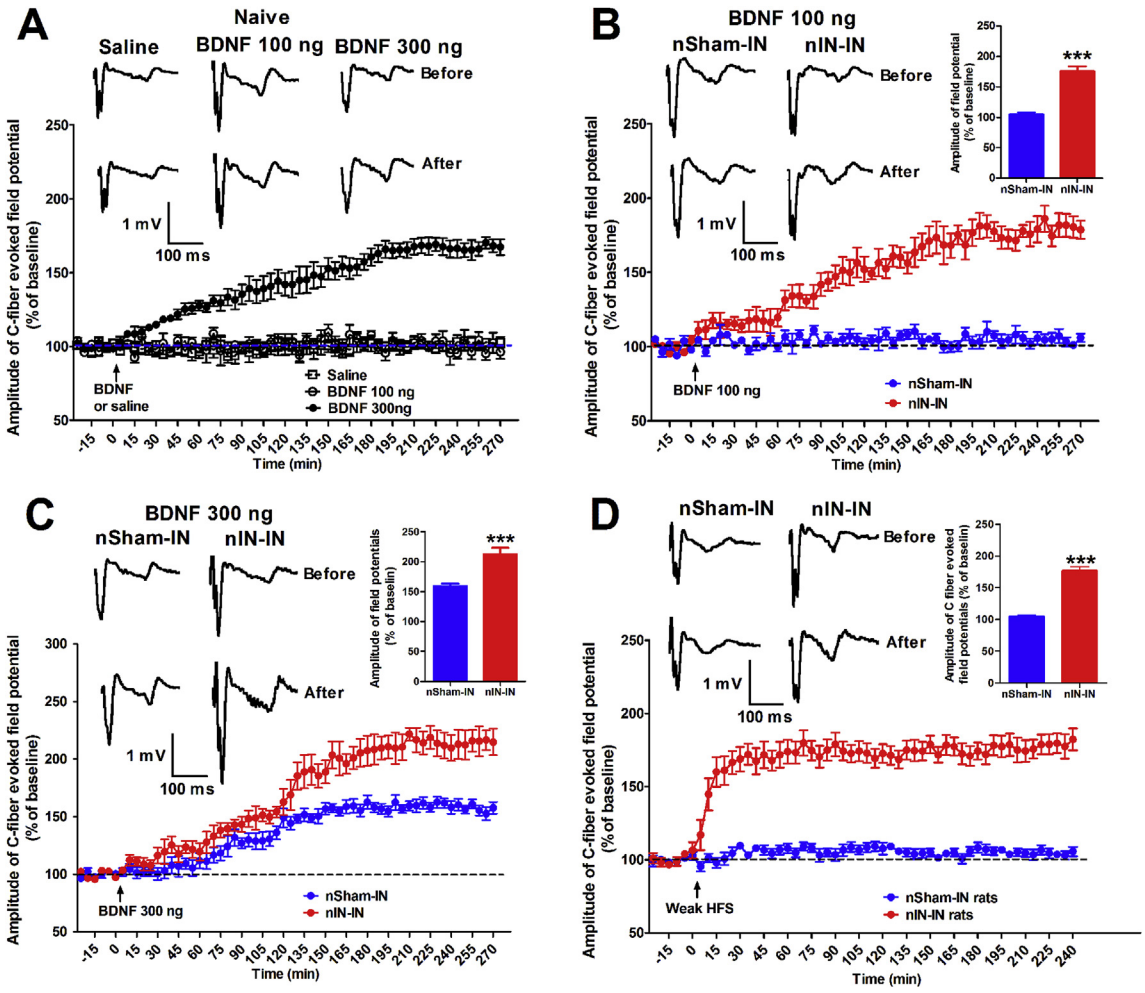

Fig. 3. Neonatal and adult repeated hindpaw incisions facilitate BDNF-induced spinal LTP and HFS-induced spinal LTP. (A) Effects of BDNF 100 ng or 300 ng on C-fiber-evoked field potentials in the mature spinal cord of intact rats. Note that spinal application BDNF at 300 ng induces LTP in intact rats compared with saline, while BDNF at 100 ng cannot induces LTP. Histogram displays the time course of C-fiber-evoked field potentials before and after spinal application of BDNF (100 ng or 300 ng) or saline in intact rats. Traces at top are recorded before and after BDNF or saline application, respectively. n = 5–6/group, Saline: male (M) 2 female (F) 3, BDNF 100 ng: M 2 F 3, BDNF 300 ng: 3 per sex. (B, C) Effects of repeated incisions on the dose of BDNF needed to induce spinal LTP (B) and the magnitude of LTP induced by full-dose BDNF (C). Note that spinal application of BDNF 100 ng induces LTP in rats with repeated incisions (nIN-IN) but not in neonatal sham operated and adult incision (nSham-IN) rats (B), and nIN-IN rats have significantly higher magnitude of 300 ng BDNF-induced LTP compare with nSham-IN rats (C). Histograms display the time course of C-fiber-evoked field potentials before and after spinal application of BDNF in intact rats, nSham-IN or nIN-IN rats. Traces at top are recorded before and after BDNF application, respectively. The bar graph shows the mean C-fiber-evoked field potentials during the 180–240 min following BDNF given in nIN-IN and nSham-IN rats. $^{***}P<0.001$, 1-way ANOVA or two-tailed unpaired *t*-test, (B): n = 7–8/group, nSham-IN: M 4 F 3, nIN-IN: 4 each gender; (C): n = 6–7/group, nSham-IN: M 3 F 4, nIN-IN: 3 each gender. (D) Effects of repeated incisions on the threshold of HFS-induced spinal LTP. Note that weak HFS at a low intensity (5–10V) induces LTP in nIN-IN rats but not in nSham-IN rats. Histograms display the time course of C-fiber-evoked field potentials before and after weak HFS in nSham-IN or nIN-IN rats. Traces at top are recorded before and after weak HFS, respectively. The bar graph shows the mean C-fiber-evoked field potentials during the 180–240 min following weak HFS in nIN-IN and nSham-IN rats. $^{***}P<0.001$, two-tailed unpaired *t*-test, n = 10/group, 5 each gender.

5.25 μg/μl (105 μg in 20-μl volume) or saline was intrathecal administrated 30 min before adult incision surgery, repeated on day 0, and twice per day from day 1 to day 7 after adult hindpaw incision. The PWT and PWL were detected on day 0 (before adult incision) and on days 1, 3, 5, 7, 14 and 21 after adult incision (Fig. 6A). In the posttreatment experiments, minocycline (105 μg/20 μl) or saline was intrathecally delivered to rats twice per day from day 1 to day 3 after adult incision. Nociceptive behaviors were detected on day 0 (before adult operation) and on days 1, 3 and 5 after adult injury (Supplementary data: Fig. S4A).

Finally, we investigated whether LTP-inducible HFS exacerbates nociceptive behaviors, and whether sciatic nerve block with lidocaine attenuates HFS-induced exacerbation of nociceptive behaviors in nIN-IN rats pre-treated with TrkB-Fc. TrkB-Fc (350 ng/20 μl) or IgG was intrathecally injected as above (i.e. 30 min before adult incision, repeated on the same day, and twice per day for 7 days after adult hindpaw incision). Lidocaine (1 mg in 0.1-ml volume) or saline was injected at the sciatic notch, 20 min before HFS of the sciatic nerve. The PWT and PWL were tested on day 0 and on days 1, 3, 5, 7 after adult incision (Supplementary data: Fig. S5C).

2.5. Immunohistochemistry

Anaesthetized animals were perfused with 37 °C saline followed

7 each gender; nIN, IN, nIN-IN: 8 each gender. (E–H) The correlation analysis between the BDNF protein expression and the paw withdrawal threshold as well as paw withdrawal latency in the timeline after adult incision from day 3 to day 21 after adult incision. Note that increases in the protein expression of BDNF in the spinal dorsal horn are highly negative correlated with mechanical allodynia and thermal hyperalgesia in nIN-IN rats (E, F), but not in nSham-IN rats (G, H) from day 3 to day 21 after adult incision.

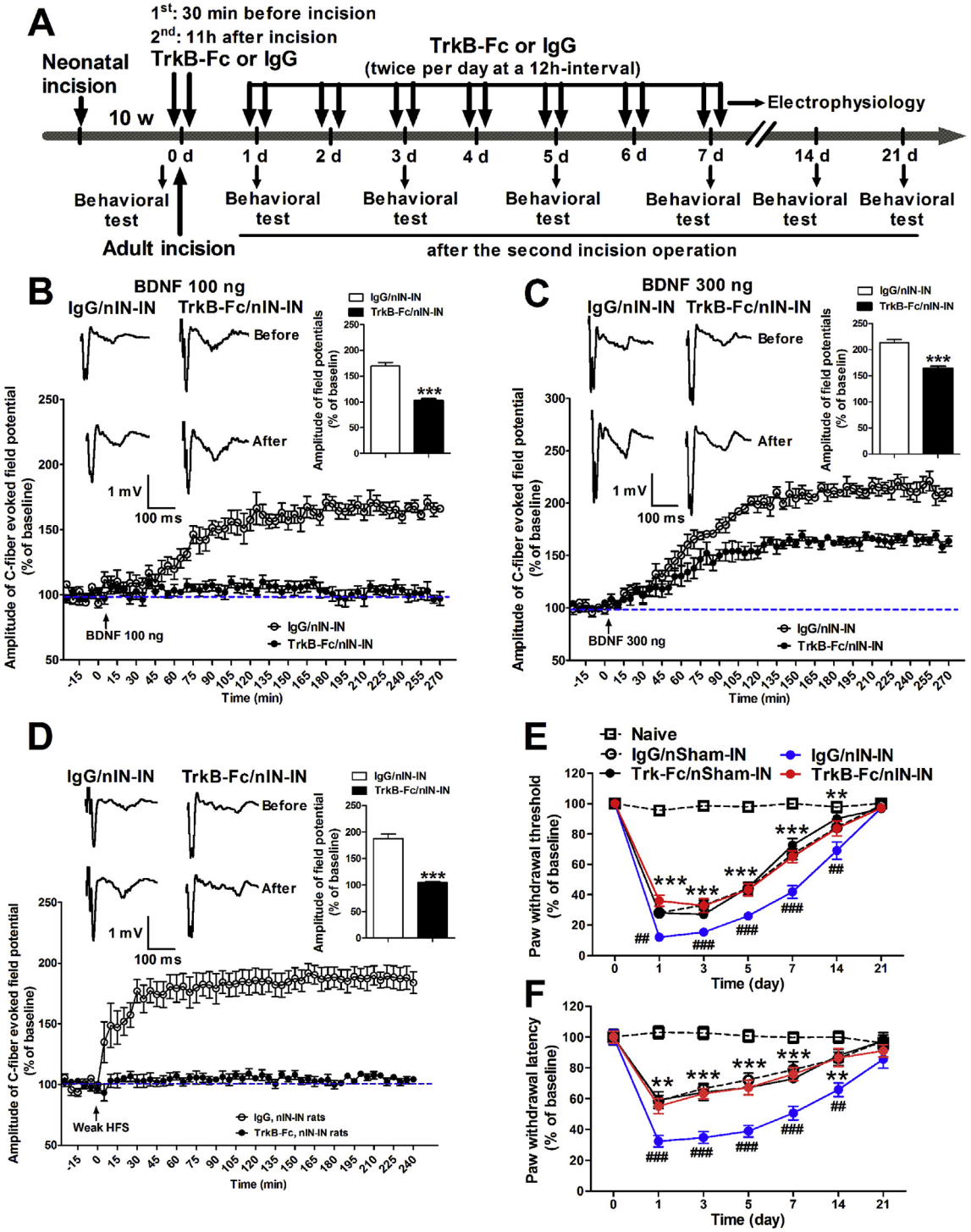

Fig. 4. Pretreatment with TrkB-Fc attenuates spinal LTP facilitation and alleviates pain hypersensitivity in rats with neonatal and adult incisions. (A) Schematic diagram of the pretreatment experiment. TrkB-Fc, TrkB-immunoglobulin G fusion protein, is applied to scavenge endogenous BDNF. TrkB-Fc (350 ng in 20-μl volume) or IgG was intrathecally delivered to rats 30 min prior to adult plantar incision, repeated on day 0, twice per day from day 1 to day 7 after adult incision. Pain behaviors were detected on day 0 (before adult operation) and on days 1, 3, 5, 7, 14 and 21 after adult injury. Electrophysiological recording was performed 8 days after adult incision, and day 7 was not used to avoid the acute effect of drugs administrated in the same day. (B, C) Effects of pretreatment with TrkB-Fc on the dose of BDNF needed to induce spinal LTP and the magnitude of BDNF-induced LTP in rats with repeated incisions (nIN-IN). Histogram displays the mean time course of C-fiber-evoked field potentials before and after spinal application of BDNF at 100 ng (B) or 300 ng (C) in nIN-IN rats injected with TrkB-Fc or IgG. Traces at top are recorded before and after BDNF application, respectively. The bar graph indicates the mean amplitude of C-fiber-evoked field potentials during the 180–240 min following BDNF given in nIN-IN rats injected with TrkB-Fc or IgG. Note that spinal injection of TrkB-Fc before adult incision completely blocks the 100 ng BDNF-induced LTP of C-fiber-evoked field potentials in nIN-IN rats (B), abolishes the double incisions-induced increase in magnitude of 300 ng BDNF-induced LTP (C), suggesting pre-treatment with TrkB-Fc abolishes the facilitation of BDNF-induced LTP in nIN-IN rats. $^{***}P$<0.01, two-tailed unpaired *t*-test, (B): n = 7–8/group, IgG/nIN-IN: 4 each gender, TrkB-Fc/nIN-IN: male (M) 3 female (F) 4; (C): n = 5–7/group, IgG/nIN-IN: M 3 F 2, TrkB-Fc/nIN-IN: M 3 F 4. (D) Effects of pretreatment with TrkB-Fc on the threshold of HFS-induced spinal LTP in nIN-IN rats. Note that weak HFS at a low intensity only induces LTP in nIN-IN rats. $^{***}P$<0.01, two-tailed unpaired *t*-test, n = 6–7, IgG/nIN-IN: M 3 F 4, TrkB-Fc/nIN-IN: 3 each gender. (E–F) Effects of pretreatment with TrkB-Fc on nociceptive behaviors The paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) are expressed as percentage change from baseline for 21 days in nIN-IN rats and neonatal sham operation and adult incision (nSham-IN) rats received TrkB-Fc or IgG respectively. Note that preventively intrathecal administration of TrkB-Fc alleviates the decrease in both PWT and PWL in nIN-IN rats. $^{**}P$<0.01, $^{***}P$<0.001, TrkB-Fc/nIN-IN *vs.* IgG/nIN-IN, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, IgG/nIN-IN *vs.* IgG/nSham-IN, 2-way ANOVA, n = 14/group, 7 each gender.

by 4% paraformaldehyde in 0.1 M phosphate buffer. L4 and L5 spinal segments were removed and post-fixed overnight at 4 °C. Following cryoprotection in 20% and 30% sucrose, spinal cords were cut into 30-μm free-floating sections and washed in PBS contained 0.3% Triton. Sections were incubated with 4% normal rabbit serum and 0.3% Triton in PBS for 1 h at room temperature, incubated in goat anti-Iba1 antibody (1:400) in PBS with 4% normal rabbit serum contained 0.3% Triton overnight at 4 °C. Tissues were washed 3 times for 5 min in PBS contained 0.3% Triton. Sections were incubated with FITC-labeled rabbit anti-goat secondary antibody (1:500, Jackson ImmunoResearch Laboratories) for 1 h at room temperature. After staining with the nuclear marker 4′, 6-diamidino-2-phenylindole (DAPI) for 10 min, tissue was washed 3 times as before. At last, sections were allowed to dry and cover slip.

The images were captured using a Leica confocal microscope controlled by Leica Application Suite V4.2 software. Relative quantification of immunoreactivity was used for Image Pro Plus (version 6.0, Media Cybernetics, USA). The ratio of Iba1-positive staining cells to the total cells stained by DAPI, the ratio of Iba1-positive cells to areas of the superficial dorsal horn (laminae I and II) were measured. In addition, the integrated optical density (IOD) of the immunoreactivity intensity in the superficial laminae of the spinal dorsal horn also was recorded, and the ratio of IOD in the ipsilateral side and contralateral side was calculated. All quantitative analyses were blindedly carried out. Each group involved three to four rats, and at least three distinct sections were measured for each rat.

2.6. Reverse transcription polymerase chain reaction and quantitative real-time polymerase chain reaction assay

Total RNA of the spinal dorsal horn was homogenized with TRIzol reagent (Invitrogen, USA). Reverse transcription was carried out with 1 μg of total RNA by using PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara, Japan). Quantitative real-time PCR was carried out using an Applied Biosystems Model ABI 7500 Fast Real-Time PCR System. A 20-μl PCR reaction was used which included 10 μl 2 × qPCR Master Mix (Promege). β-actin in parallel for each run was used as an internal control. The amplification conditions were as follows: 95 °C for 10 min (initial denaturation), 40 cycles of 25 s at 95 °C (denaturation), 45 s at 60 °C (annealing), 30 s at 72 °C (elongation), and 8 s at fluorescence measurement temperature (60 °C). Relative quantification of the expression of BDNF was measured using the $2^{-(\Delta\Delta Ct)}$ method and the Ct value represents the cycle number at which the fluorescence signal crosses the threshold. The ΔCt represented the difference between the PCR product and β-actin. The ΔΔCt was the difference between the naive group and the other group. Data are presented as $2^{-(\Delta\Delta Ct)}$, that is, the fold change in BDNF mRNA. The following primers were used: BDNF, 5′-CTGACACTTTTGAGCACGTGATC-3’ (forward) and 5′-AGGCTCCAAAGGCACT TGACT-3’ (reverse); β-actin, 5′-AGCCATG-TACGTAGCCAT CC-3’ (forward) and 5′-GCCATCTCTTGCTCG AAGTC-3’ (reverse).

2.7. Enzyme-linked immunosorbent assay (ELISA)

Under deep anaesthesia, the animal lumbar enlargement of the spinal cord was removed, and the dorsal horn was separated from the ventral horn and stored at −80 °C. Samples from different time points were processed at the same time. The dorsal horns were homogenized in ice-chilled lysis buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% NP40 (Sigma-Aldrich), 0.5% sodium deoxycholate (Sigma-Aldrich), 0.1% sodium dodecyl sulfate (SDS). The extract was centrifuged at 10 000 × g for 15 min. The concentration of total protein in the supernatants was determined with a BCA assay kit (Pierce, Rockford, USA). A commercially available ELISA kit (Promege, USA) was used to detect BDNF in the supernatant following the instructions. The plates were incubated overnight at 4 °C with the anti-BDNF monoclonal primary antibody. After blocking, 100 μl BDNF standards (7.8–500 pg/ml) and supernatant sample were added and incubated 2 h at room temperature with shaking. This was followed by 2 h incubation with 100 μl BDNF polyclonal antibody and a species-specific anti-IgG antibody conjugated to horseradish peroxidase, respectively. Finally, the substrate reaction was stopped with 100 μl 1N hydrochloric acid, and plates were recorded at 450 nm. BDNF levels in the spinal dorsal horns were normalized to the amount of total protein.

2.8. Electrophysiological studies

2.8.1. Surgery

Rats were anaesthetized with urethane (1.2–1.5 g/kg, i.p.). The trachea was inserted by a cannula for artificial respiration. Another catheter was placed in the jugular vein for continuous infusion of Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.4 mM $CaCl_2$, 1.0 mM $MgCl_2$, 6.0 mM $NaHCO_3$, 2.1 mM NaH_2PO_4, 6.5 mM D-(+)-glucose, pH 7.4), at a rate of 1.5–2 ml/h. A pair of bipolar silver hook electrode was placed under the sciatic nerve immediately proximal to the trifurcation for electrical stimulation. The vertebral column was tightly fixed in the frame with two clamps. The lumbar enlargement of the spinal cord was exposed by laminectomy at the vertebrae T13 and L1 and the dura covering lumbosacral spinal segments was cautiously removed. A small well was built with 3% agar on the lumbar enlargement of the spinal cord to permit application of drugs or vehicles (Ding et al., 2015; Xing et al., 2007). The exposed spinal tissue was covered with 37 °C saline. After surgery, the rat was artificially ventilated with a small animal ventilator and paralyzed with curare (2.0 mg/kg, i.v.), and continuous anaesthesia and paralysis were maintained with urethane (0.10–0.15 g/kg/h) and curare (0.20 mg/kg/h) during the whole experiment. The physiological condition of the animal was monitored by recording the electrocardiogram (330–460 beats/min), endexpiratory CO_2 (3.5–4.5%), and rectal temperature (36.5–37.5 °C), and was maintained within the range mentioned above.

2.8.2. C-fiber-evoked field potential recording

The C-fiber-evoked field potentials were recorded at a depth of 100–500 μm from the dorsal surface of L4-L5 spinal dorsal horn with parylene-coated tungsten micro-electrodes (1–3 MΩ, FHC, Bowdoinham, ME), driven by a micro-stepping motor. A band width of 0.1–300 Hz was used to remove artifacts without altering the C-fiber-evoked field potentials. The signals were amplified, filtered and displayed on an oscilloscope, and fed to a Pentium computer via a CED1401 interface for off-line analysis using the Spike 2.0 software (Cambridge Electronic Design, Cambridge, UK).

The test stimulation of a single square pulse (0.5 ms duration, applied every 2 s) was applied to the sciatic nerve for assessing the threshold of the evoked field potentials. The intensity of the stimulation was increased gradually from 0 V to the voltage intensity just evoking the C-fiber-evoked field potentials. The intensity of stimulation that would just elicit the C-fiber-evoked field potentials was defined as the threshold of the C-fiber-evoked field potentials (Ding et al., 2015; Xing et al., 2007). The intensity of test stimulations was 2 times of the threshold of C-fiber-evoked field potentials. Test stimulations of a single square pulse (0.5 ms, 10–20 V, delivered every 5 min) were applied to the sciatic nerve to evoke spinal C-fiber field potentials for at least 30 min as baseline control.

2.8.3. Induction of spinal LTP

BDNF-induced LTP: After 6 stable control field potentials were

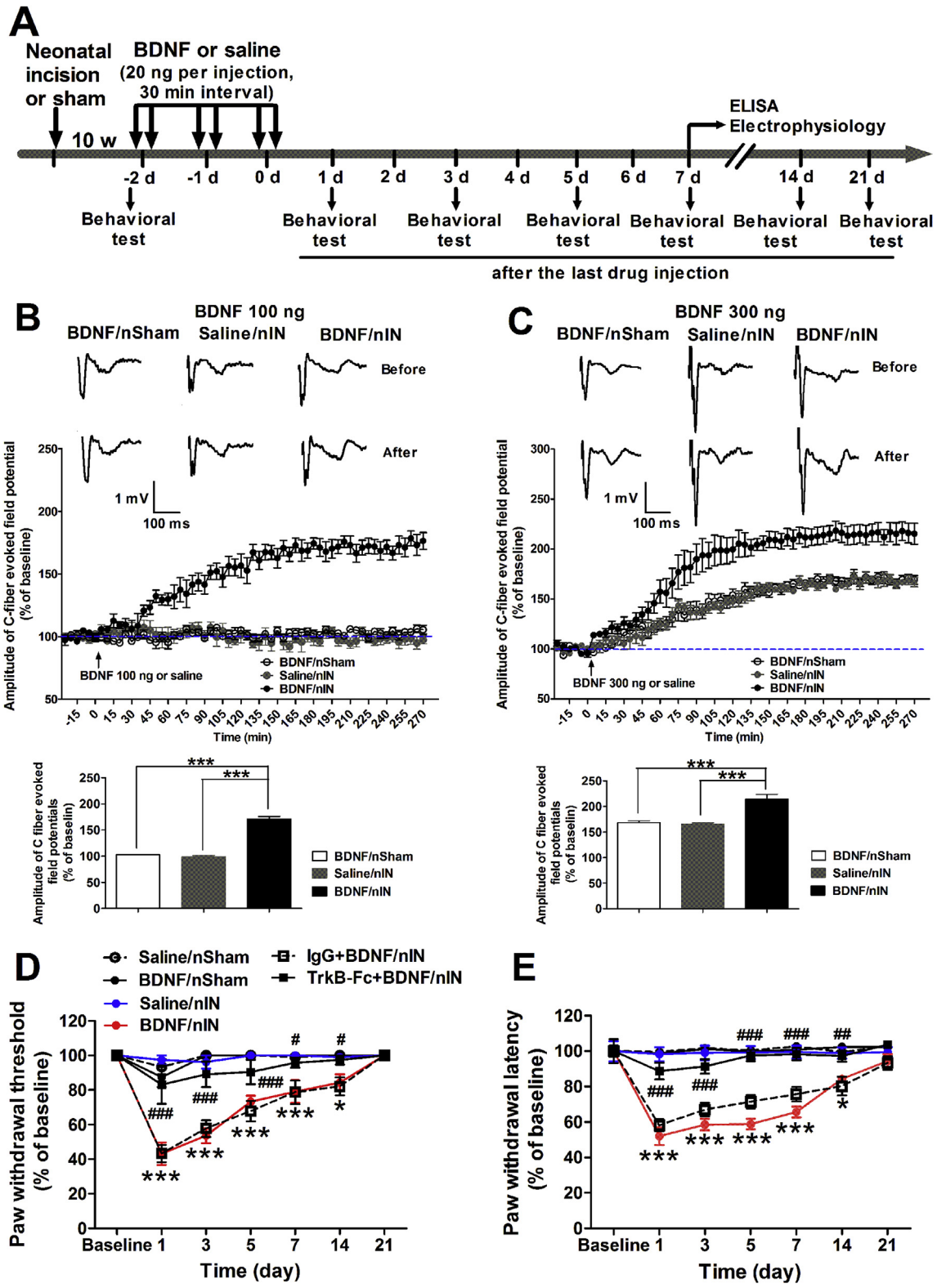

Fig. 5. Spinal administration of exogenous BDNF to adult rats with only neonatal incision mimics the LTP facilitation and pain hypersensitivity induced by repeated incisions in neonatal and adult periods. (A) Scheme of the experimental procedure. BDNF or saline was intrathecally administrated to adult animals with only neonatal incision, repeated twice at a 30min-interval per day, and continued for 3 days. The PWT and PWL were measured before drug injection, and on days 1, 3, 5, 7, 14 and 21 after the drug injection. And in order to confirm that impacts of pain behaviors in nIN rats were directly caused by injected BDNF, TrkB-Fc (350 ng/20 μl) or IgG was intrathecally delivered to rats (at 8:00–9:00 a.m. and 8:00–9:00 p.m., twice per day at a 12 h-interval) along with BDNF injection (20 ng/20 μl, at 2:00–3:00 p.m., twice per day at a 30 min-interval) for 3 days. (B, C) Effects of spinal administration of exogenous BDNF on facilitation of the BDNF-induced LTP in adult rats with only incision in the neonatal period. Intrathecal injected BDNF (20ng/injection, twice per day, 3 days) before electrophysiological recording is used to mimic the adult incision, while spinal surface given 100 ng or 300 ng BDNF during electrophysiological studies is in order to induce LTP. Histogram displays the mean time course of C-fiber-evoked field potentials before and after spinal application of BDNF at 100 ng or 300 ng in adult rats with neonatal incision. Traces at top are recorded before and after BDNF or saline application, respectively. The bar graph shows the mean C-fiber-evoked field potentials during the

recorded, BDNF (100 ng/20 μl, 300 ng/20 μl) or saline was then applied topically to the spinal dorsal horn, and the post-drug field potentials evoked by the same test stimulus (0.5 ms, 10–20 V, delivered at 5-min intervals) as described above were measured at 5-min intervals for up to 270 min. The mean amplitude of the control field potentials (the baseline responses) was obtained from an average of 6 individual test potentials (100%), and the amplitude of the field potential evoked by test stimulation after administration of BDNF or saline was normalized and expressed as the percentage of the baseline responses.

Spinal dorsal horn topically applied BDNF at 100 ng was used to investigate whether the dose of BDNF needed to induce spinal LTP is decreased in rats with neonatal and adult incisions, while BDNF at 300 ng was given to research the magnitude of BDNF-induced spinal LTP 7 days after adult operation (Fig. 1A). In addition, to determine effects of pretreatment with TrkB-Fc (Fig. 4A) or minocycline (Fig. 6A) on the facilitation of LTP, TrkB-Fc (350 ng/20 μl) or minocycline (105 μg/20 μl) was intrathecally injected to animals 30 min before adult incision, repeated on day 0 and twice per day from day 1 to day 7 after adult hindpaw incision, as mentioned in behavior experimental schedule. The rats were used for C-fiber-evoked field recording 8 days after adult incision, and day 7 was not recorded to avoid acute effects of TrkB-Fc or minocycline administration in the same day. In the posttreatment experiments, TrkB-Fc (350 ng/20 μl, Supplementary data: Fig. S2A) was intrathecally injected to animals twice per day from day 1 to day 3 after adult incision, as mentioned in behavior experimental schedule. The rats were used for C-fiber-evoked field recording 4 days after adult incision, and day 3 was not used to avoid acute effects of TrkB-Fc administration in the same day. Finally, to determine whether intrathecally delivery of BDNF mimics the effects of adult incision, BDNF (20 ng/20 μl) was intrathecally injected to adult rats with only neonatal incision for 3 days, as mentioned in behavior experimental schedule, the rats were used for recording 7 days after adult operation (Fig. 5A).

Weak HFS induced LTP: After 6 stable control field potentials were recorded, HFS at a low intensity (5–10 V, 0.5-ms duration, 100 Hz, 400 pulses given in 4 trains of 1-s duration at 10-s intervals) was then delivered to the sciatic nerve, and the post-HFS field potentials evoked by the same test stimulus (0.5-ms duration, 10–20 V, delivered at 5-min intervals) as described above were measured at 5-min intervals for up to 240 min (Fig. 3D). The mean amplitude of the control field potentials (the baseline responses) was obtained from an average of 6 individual test potentials (100%), and the amplitude of the field potential evoked by each test-stimulation after weak HFS was normalized and expressed as the percentage of the baseline responses.

In order to investigate the effect of pretreatment with TrkB-Fc on the facilitation of HFS-induced LTP in rats with neonatal and adult incisions (Fig. 4A), TrkB-Fc (350 ng/20 μl) was intrathecally applicated to animals 30 min prior to adult incision, repeated on day 0 and twice per day from day 1 to day 7 after adult hindpaw incision. The rats were used for C-fiber-evoked field recording 8 days after adult incision, and day 7 was not used to avoid acute effects of TrkB-Fc or minocycline administration in the same day. Five to eight animals were used in each group for these experiments.

2.8.4. HFS of the sciatic nerve and local nerve blockade before behavioral test

Under sodium pentobarbital (80 mg/kg, i.p.) anaesthesia, the left sciatic nerve was carefully exposed at the mid-thigh level and dissociated from the adhering tissue. The sciatic nerve was moderately hung on a pair of silver electrode hooks. HFS (40 V, 0.5-ms duration, 100 Hz, 400 pulses given in 4 trains of 1-s duration at 10-s intervals) was then delivered to the sciatic nerve, which is able to induce spinal LTP in our previous electrophysiological study (Ding et al., 2015; Xing et al., 2007). The sciatic nerves of the control group were identically exposed and manipulated but were not stimulated. Then, the muscle and skin were sutured in layers (Bian et al., 2014, 2015; Chu et al., 2012; Liang et al., 2010; Svendsen et al., 1999; Ying et al., 2006; Zhang et al., 2005).

For blockade of the sciatic nerve, 1% lidocaine solution (1 mg in 0.1-ml volume) was injected ipsilaterally at the sciatic notch with a 27-gauge needle connected to a tuberculin syringe. The control group was injected with saline (Thalhammer et al., 1995). The limited duration, low dose and postincisional injection of lidocaine tended to avoid direct effects on pain behaviors (Sun et al., 2004; Tobe et al., 2010; Wang et al., 2011). Using the same methods as above, HFS of the sciatic nerve was carried out 20 min after injection of lidocaine. The injection site was at the proximal part of the sciatic nerve compared with the electrical stimulation site. Then, the muscle and skin were sutured (Supplementary data: Fig. S5).

2.9. Statistics

GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA) was used for data analyses. Data were expressed as mean ± SEM. A two-tailed unpaired *t*-test was used for the comparison of the mean values between two groups. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test or 2-way ANOVA followed by the Bonferroni post-hoc test was used for multiple comparison. Statistically significant difference was considered at $P<0.05$.

3. Results

3.1. Neonatal incision enhances the adult incision-induced pain hypersensitivity and microglial activity in the spinal dorsal horn

To confirm the impact of neonatal incision to adult incision-induced pain hypersensitivity, the paw withdrawal threshold (PWT) and thermal withdrawal latency (PWL) were observed before and after adult hindpaw incision. For the reason that neonatal incision increased baseline pain thresholds of adult rats (Walker et al., 2015), the mechanical and heat pain thresholds were shown as percentage change from baseline in our study. We found that the PWT and PWL were both decreased by 15–63% in adult rats with repeated incisions in neonatal and adulthood periods (nIN-IN rats) compare with adult rats with neonatal sham operation and adult incision (nSham-IN rats) from 1 h (PWT: 13.8 ± 5.05% nIN-IN *vs.* 25.7 ± 2.55% nSham-IN, $P<0.05$, PWL: 37.17 ± 2.76% nIN-IN *vs.* 59.0 ± 3.25% nSham-IN, $P<0.001$) to 14 days (PWT: 82.7 ± 7.01% nIN-IN *vs.* 94.7 ± 3.63% nSham-IN, $P<0.05$, PWL: 64.2 ± 3.24% nIN-IN *vs.* 84.6 ± 1.48% nSham-IN, $P<0.01$) after adult incision (2-way

180–240 min following BDNF given in intact rats. Note that BDNF at 100 ng successfully induces spinal LTP (B), and BDNF at 300 ng induces higher magnitude of LTP (C) in adult rats with single neonatal incision, indicating that BDNF injection mimics the effect of adult incision on the facilitation of BDNF-induced LTP. $^{***}P<0.001$, two-tailed unpaired *t*-test, (B): n = 5–6/group, BDNF/nSham: 3 each gender, Saline/nIN: male (M) 3 female (F) 2, BDNF/nIN: M 3, F 2; (C): n = 5–6/group, BDNF/nSham: M 3, F 2, Saline/nIN: 3 each gender, BDNF/nIN: M 2 F 3. (D, E) Effects of exogenous BDNF on nociceptive behaviors in adult rats with only neonatal incision. Note that perfusion of BDNF (20 ng) statistically reduces the paw withdrawal threshold and paw withdrawal latency of adult rats with only neonatal incision, while injection of TrkB-Fc 30min before given exogenous BDNF significantly attenuates the reduction in rats with neonatal incision treated with BDNF. $^{*}P<0.05$, $^{***}P<0.001$, BDNF/nIN *vs.* Saline/nIN, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, TrkB-Fc + BDNF/nIN *vs.* IgG + BDNF/nIN, 2-way ANOVA, n = 8/group, 4 each gender.

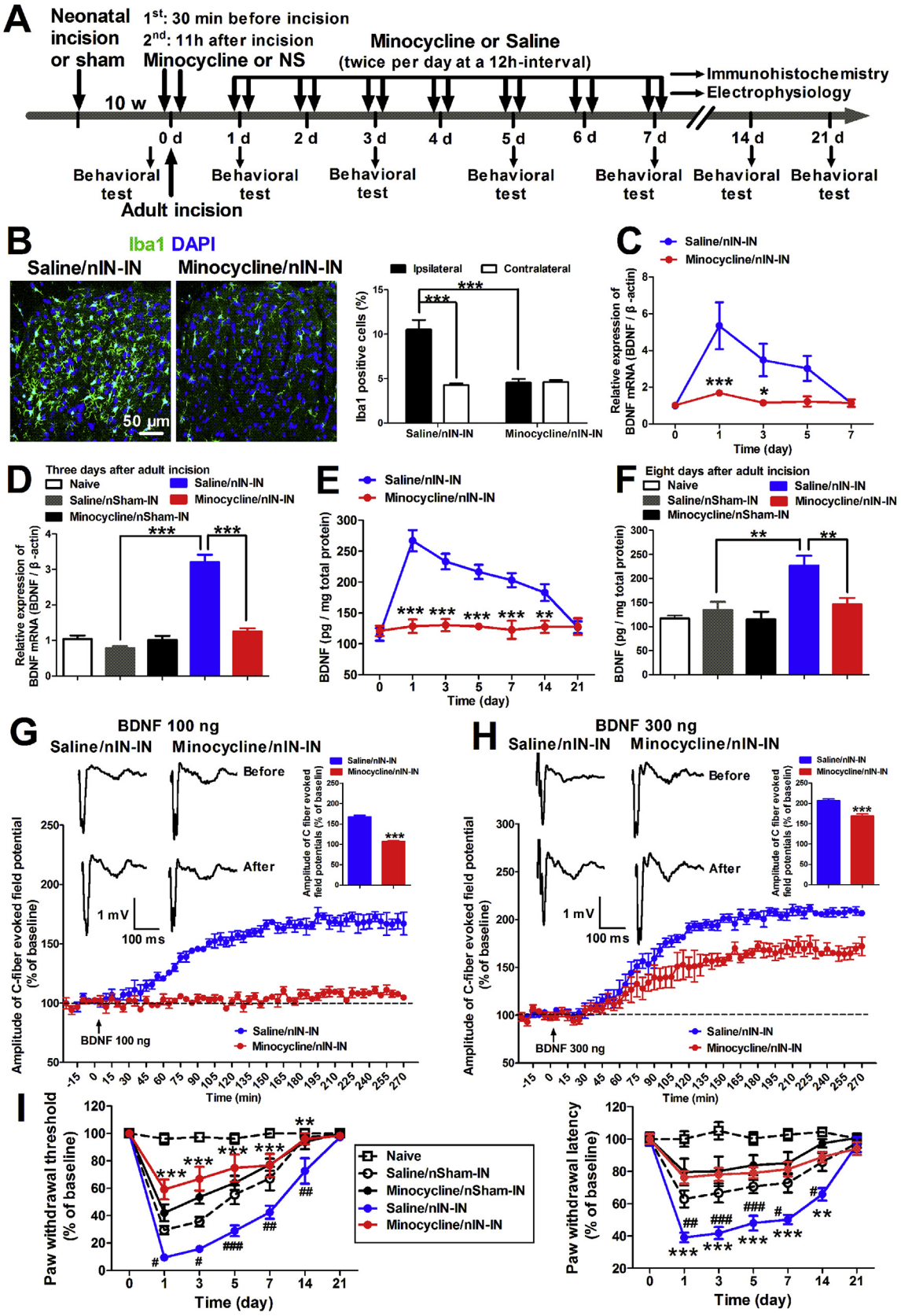

Fig. 6. Pretreatment with minocycline, a microglial inhibitor, attenuates repeated incisions-evoked ionized calcium binding adaptor molecule 1 (Iba1) upregulation as well as BDNF upregulation in the spinal dorsal horn, abolishes BDNF-induced spinal LTP facilitation, and alleviates pain hypersensitivity in adult rats. (A) Scheme of the experimental procedure. Minocycline (105 μg/20 μl) or saline was intrathecally delivered to rats 30 min before adult surgery, repeated on day 0, twice per day from day 1 to day 7 after adult incision. Pain behaviors were measured on day 0 (before adult operation), and also detected on days 1, 3, 5, 7, 14 and 21 after adult operation in each group. Iba1 staining, BDNF protein expression and electrophysiological tests were performed on day 8 after adult incision, and day 7 was not used to avoid the acute effect of minocycline administrated in the same day. (B) Iba1, a

ANOVA, n = 12, Fig. 1C and D). However, rats with single adult incision had mechanical allodynia and heat hyperalgesia lasted for only 7 days after adult incision compared with sham operated rats (2-way ANOVA, n = 12, Fig. 1C and D). Moreover, the sensory thresholds were not different between the nSham-IN and IN group at all the time points, suggesting that maternal separation, handling and neonatal anaesthesia on P3 have no statistically effect on the response to repeated incision (P>0.05, 2-way ANOVA, n = 12, Fig. 1C and D). Furthermore, although long-term effects of some neonatal trauma are affected by gender (LaPrairie and Murphy, 2010; Li and Baccei, 2011), there was no significant effect of gender on the PWT and PWL in the impact of neonatal incision on adult incisional pain (Table 1), according to other reports (Beggs et al., 2012; Gong et al., 2016; Schwaller et al., 2015; Soens et al., 2015; Walker et al., 2009). Collectively, neonatal hindpaw incision increases the degree and duration of adult incision-induced mechanical allodynia and heat hyperalgesia without gender specificity.

Ionized calcium binding adaptor molecule 1 (Iba1) was tested as a microglial morphology marker in tissues obtained from the spinal cord on day 7 after adult operation in the nIN-IN, nSham-IN or naïve group. The immunohistochemical staining revealed that the number of positive staining cells of Iba1 in the ipsilateral superficial dorsal horn was increased by 142% and 128% in nIN-IN rats in contrast to nSham-IN rats and naïve rats, respectively. Moreover, only nIN-IN rats had a 207% elevation in Iba1-positive staining cells in the ipsilateral superficial dorsal horn compared with contralateral side (P<0.001, 1-way ANOVA, n = 6 rats/group, 4–6 tissues per rat, Fig. 1E). In contrast, the ratio of the integrated optical density (IOD) of the ipsilateral side and the contralateral side was not statistically different in these 3 groups (Naïve: 1.03 ± 0.03, nSham-IN: 1.04 ± 0.03, nIN-IN: 1.13 ± 0.03, P>0.05, 1-way ANOVA, n = 6 rats/group, 4–6 tissues per rat, Supplementary data: Fig. S6). Taken together, these results demonstrate that neonatal priming increases the pain hypersensitivity and microglia activity induced by adult incision.

3.2. Upregulation of spinal BDNF in the adult rats with neonatal and adult incisions

We and others have reported that spinal BDNF takes an important part in the development of several types of chronic pain during mature and development (Coull et al., 2005; Ding et al., 2015; Geng et al., 2010; Groth and Aanonsen, 2002; Liang et al., 2016; Liu et al., 2016; Zhao et al., 2006). In addition, microglia, which is involved in the priming of adult pain responses by neonatal pain experience, can synthesize and secret BDNF (Beggs et al., 2012; Benarroch, 2015; Ding et al., 2015), thus we hypothesized that spinal BDNF would be involved in neonatal incision-exacerbated adult incisional pain. As shown in Fig. 2A, the expression of BDNF mRNA in the spinal dorsal horn was increased to 343% from day 1 (4.605 ± 0.78 nIN-IN *vs.* 2.255 ± 0.40 nSham-IN, P<0.01) to day 3 (3.735 ± 0.78 nIN-IN *vs.* 1.089 ± 0.12 nSham-IN, P<0.001) after adult incision in nIN-IN rats compared with nSham-IN rats (2-way ANOVA, n = 6), whereas the expression of BDNF mRNA was upregulated only on day 1 in nSham-IN rats (1-way ANOVA, n = 6). Also, the gene expression of BDNF is not changed in adult rats with single incision in neonatal period or adulthood 3 days after adult operation (P>0.05, 1-way ANOVA, n = 9–11, Fig. 2B). Furthermore, we found the protein expression of BDNF was increased to 189% from 1 h (241.8 ± 12.82 pg/mg total protein nIN-IN *vs.* 182.3 ± 14.77 pg/mg total protein nSham-IN, P<0.01) to 14 days (166.0 ± 10.12 pg/mg total protein nIN-IN *vs.* 118.7 ± 5.85 pg/mg total protein nSham-IN, P<0.05), and peaked on 6 h after adult incision in nIN-IN rats (2-way ANOVA, n = 14, Fig. 2C), which was according to the dynamic change of pain behaviors in the time line after adult incision (Fig. 1C and D). However, the concentration of BDNF was increased in nSham-IN rats from 1 h (P<0.001) to only 1 day (P<0.05) (1-way ANOVA, n = 14, Fig. 2C). Also, single incision in the neonatal period or adulthood respectively did not have statistically significant effects on the concentration of BDNF protein in the dorsal horn compare with sham operated rats on day 7 after adult incision (P > 0.05, 1-way ANOVA, n = 12–16, Fig. 2D). Indeed, in late stage after adult incision (3–21 days), there are significant negative correlations between the protein expression of BDNF and PWT (r = − 0.9872, P=0.0017) as well as PWL (r = − 0.9911, P = 0.0010) in the nIN-IN group (Fig. 2E and F), while there is no statistically significant correlation between the BDNF concentration and PWT (r = − 0.5062, P=0.3841) as well as PWL (r = − 0.2871, P=0.6396) in the nSham-IN group (Fig. 2 G, H), although in early stage after adult incision (0–1 day), there are significant negative correlations between BDNF expression and nociceptive behaviors in both nIN-IN and nSham-IN rats (Supplementary data: Figs. S1A–D). Collectively, neonatal hindpaw incision increases the degree and duration of adult incision-induced upregulation of BDNF. Additionally, although nerve growth factor (NGF), a member of the same family with BDNF, showed gender specificity in the long-term effects of neonatal injury (Li and Baccei, 2011), gender is not involved in the effects of repeated incisions on the protein expression of spinal BDNF at all the times points in nIN-IN and nSham-IN rats and at all the 7 groups on day 7 after adult operation tested in the present study (Table 1). These results imply

microglia marker, was stained in spinal cord sections from adult rats with neonatal and adult incisions (nIN-IN) pretreated with minocycline or saline. Left: Representative high-power images of the superficial spinal dorsal horn are shown from nIN-IN rats administrated with minocycline or saline. Right: The positive staining of Iba1 in the superficial spinal dorsal horn was quantified. Note that the upregulation of Iba1 in the superficial dorsal horn induced by repeated incisions is completely abolished by pretreatment with minocycline in nIN-IN rats. $^{***}P$<0.001, 1-way ANOVA, n = 3 animals/group, Saline/nIN-IN: male (M) 2 female (F) 1, Minocycline/nIN-IN: M 1 F 2, 3–5 tissues per animal. (C–F) Effects of minocycline on the upregulation of BDNF mRNA and protein induced by repeated incisions. (C) The upregulation of BDNF mRNA induced by repeated incisions is attenuated from day 1 to day 3 by intrathecal administration of minocycline. $^{*}P$<0.05, $^{***}P$<0.001, 2-way ANOVA, n = 6/group in each time point, 3 each gender. (D) The upregulation of BDNF mRNA induced by repeated incisions is attenuated by pretreatment with minocycline, while minocycline or saline injection has no effect on adult rats with neonatal sham operation and adult incision (nSham-IN). $^{***}P$<0.001, compared to nSham-IN, 1-way ANOVA, n = 9–10/group, Naïve: M 5 F 4; Saline/nSham-IN: M 4 F 5; Minocycline/nSham-IN: M 5 F 4; Saline/nIN-IN, Minocycline/nIN-IN: 5 each gender. (E) Dynamic changes of the BDNF protein expression in the spinal dorsal horn in different time points in nIN-IN rats administrated with minocycline or saline. Note that the upregulation of BDNF protein in the spinal dorsal horn is blocked by pretreatment with minocycline from day 1 to day 14. $^{**}P$<0.01, $^{***}P$<0.001, 2-way ANOVA, n = 8/group in each time points, 4 each gender. (F) The upregulation of BDNF concentration in the spinal dorsal horn is blocked by pretreatment with minocycline in nIN-IN rats compared with saline, while minocycline or saline injection has no effect in nSham-IN rats. $^{**}P$ < 0.01, 1-way ANOVA, n = 8/group, 4 each gender. (G, H) Effects of pretreatment with minocycline on the facilitation of LTP in rats with repeated incisions. Traces at top are recorded before and after BDNF application, respectively. Histogram displays the mean time course of C-fiber-evoked field potentials before and after spinal application of BDNF at 100 ng (G) or 300 ng (H) in nIN-IN rats injected with minocycline or saline. The bar graph indicates the mean amplitude of C-fiber-evoked field potentials during the 180–240 min following BDNF given in nIN-IN rats injected with TrkB-Fc or IgG. Note that spinal injection of minocycline before adult incision completely blocks the 100 ng BDNF-induced LTP (G), and decreases the 300 ng BDNF-induced LTP in nIN-IN rats (H), suggesting pretreatment with minocycline abolishes the facilitation of BDNF-induced LTP in nIN-IN rats. $^{***}P$<0.001, two-tailed unpaired t-test, n = 5/group, (G): Saline/nIN-IN: M 2 F 3, Minocycline/nIN-IN: M 3 F 2; (H): Saline/nIN-IN: M 3 F 2, Minocycline/nIN-IN: M 3 F 2. (I) Effects of minocycline on the repeated incisions-induced mechanical allodynia and thermal hyperalgesia. Note that the repeated incisions-induced mechanical hyperalgesia and heat allodynia are alleviated by the injection of minocycline for 14 days after adult operation. $^{**}P$<0.01, $^{***}P$<0.001, Minocycline/nIN-IN *vs.* Saline/nIN-IN group, $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001, Saline/nIN-IN *vs.* saline/nSham-IN group, 2-way ANOVA, n = 8/group, 4 each gender.

Table 1
Effects of gender on pain behaviors, microglial reactivity, protein expression of BDNF and weak HFS-induced spinal LTP in adult rats with neonatal and adult incisions, as well as pain behaviors in TrkB-Fc treated rats.

Experiment in nIN-IN rats	Figure	Group	df	F	*P*	n (/group/sex)
Pain behavior (PWT)	Fig. 1C	nSham-IN	(1, 90)	0.91	0.3417	6
		nIN-IN	(1, 90)	0.20	0.6575	
Pain behavior (PWL)	Fig. 1D	nSham-IN	(1, 90)	0.03	0.8692	6
		nIN-IN	(1, 90)	0.90	0.3465	
Microglial reactivity (positive cells %)	Fig. 1E	Naïve nSham-IN, NIN-IN	(1, 162)	0.09	0.7672	3 rats, 4–6 tissues/rat
Microglial reactivity (IOD)	Fig. S6	Naive nSham-IN, NIN-IN	(1,81)	0.42	0.5210	3 rats, 4–6 tissues/rat
Protein expression of BDNF	Fig. 2C	nSham-IN	(1, 108)	0.08	0.7813	7
		nIN-IN	(1, 108)	1.52	0.2200	
Protein expression of BDNF	Fig. 2D	Naive nSham-IN, NIN-IN	(1, 36)	0.23	0.6325	6–8
Weak HFS-induced LTP (180–240 min)	Fig. 3D (line graph)	nSham-IN	(1, 104)	0.04	0.8487	5
		nIN-IN	(1, 104)	2.15	0.1452	
Weak HFS-induced LTP (mean field potentials)	Fig. 3D (bar graph)	nSham-IN, NIN-IN	(1, 16)	0.20	0.6607	5
Pain behavior (PWT) in TrkB-Fc treated rats	Fig. 4E	IgG/nIN-IN	(1, 84)	0.05	0.8159	7
		TrkB-Fc/nIN-IN	(1, 84)	0.83	0.3640	
Pain behavior (PWL) in TrkB-Fc treated rats	Fig. 4F	IgG/nIN-IN	(1, 84)	1.04	0.3100	7
		TrkB-Fc/nIN-IN	(1, 84)	2.86	0.0946	

Two-way ANOVA was used to analyze the effects of treatment and gender in Figs. 1E, S6, 2D and 3D (bar graph), the effects of time and gender in others.
HFS: high frequency electrical stimulation, IOD: integrated optical density, nIN-IN rats: adult rats with neonatal and adult incisions, nSham-IN rats: adult rats with neonatal sham operation and adult incision, PWT: paw withdrawal threshold, PWL: paw withdrawal latency.

that spinal BDNF contributes to the neonatal incision-elevated adult incisional pain regardless of gender.

3.3. Repeated incisions facilitates the spinal LTP

According to our previous report (Ding et al., 2015), spinal surface application of BDNF at 300 ng induces spinal LTP of C-fiber-evoked field potentials in intact rats, while BDNF at 100 ng cannot (Fig. 3A, Supplementary data: Fig. S7A). To further elucidate the potential mechanisms by which spinal BDNF contributes to the pathogenesis of adult incision-induced pain hypersensitivity primed by neonatal incision, we examined the effect of 100 ng BDNF on C-fiber-evoked field potentials in adult rats with single or repeated hindpaw incision. BDNF at 100 ng successfully induced significant spinal LTP with a long latency in nIN-IN rats (Fig. 3B), but not in rats with only neonatal or adult incision (Supplementary data: Figs. S7B and C). The amplitude of C-fiber responses was elevated by 28% above baseline approximately at 65 min (127.7 ± 6.57%, $P<0.001$ *vs.* baseline), climbed gradually to a stable plateau (168.2 ± 8.12%, $P<0.001$ *vs.* baseline) at 180–240 min after BDNF application, and persisted without reduction until our experiment termination in the nIN-IN group (n = 7–8, Fig. 3B), the extent of increase was similar to 300 ng BDNF-induced LTP in intact rats (Supplementary data: Fig. S7A). The mean C-fiber-evoked field potentials during 180–240 min after 100 ng BDNF application was increased in nIN-IN rats (175.6 ± 8.12%), compare with nSham-IN rats (104.8 ± 3.08%, $P<0.001$, 1-way ANOVA, n = 7–8, Fig. 3B).

Facilitation of LTP contains both the downregulation of LTP threshold and the enhancement of LTP magnitude. And when subthreshold stimulation induces LTP successfully, the magnitude of LTP induced by suprathreshold stimulation can be increased (Lalo et al., 2016; Suo et al., 2017; Zhang et al., 2012), decreased (Ding et al., 2015; Lalo et al., 2016) or unchanged (Lu et al., 1999; Pyapali et al., 1998; Song et al., 2009).Therefore, we further detected whether the magnitude of 300 ng BDNF-induced spinal LTP was changed in nIN-IN rats. We observed that the nIN-IN rats had higher magnitude of BDNF-induced LTP compared with the nSham-IN group (Fig. 3C). When compared to the baseline responses (averaged at 30-0 min prior to drug), the mean C-fiber-evoked field potentials during 180–240 min after BDNF application was increased in the nIN-IN group (212.6 ± 10.19%), compared with the nSham-IN group (159.1 ± 4.01%, $P<0.001$, two-tailed unpaired *t*-test, n = 6–7, Fig. 3C). Taken together, we found that repeated incisions decreases the dose of BDNF needed to induce LTP, increasing the magnitude of LTP elicited by full-dose BDNF.

HFS at a low intensity (5–10 V, 0.5-ms duration, 100 Hz, 400 pulses given in 4 trains of 1-s duration at 10-s intervals) induced spinal LTP only in the nIN-IN group, but not the nSham-IN group (Fig. 3D). When compared to the baseline responses, the mean C fiber-evoked field potentials 180–240 min after BDNF application were 177.1 ± 6.28% in nIN-IN rats and 104.8 ± 2.31% in nSham-IN rats ($P < 0.001$, two-tailed unpaired *t*-test, n = 10, Fig. 3D), suggesting the repeated incisions facilitate HFS-induced spinal LTP. Additionally, weak HFS-induced LTP in nIN-IN rats was not related to gender (Table 1). Together with aforementioned data, we found that repeated incisions facilitate spinal LTP.

3.4. Pretreatment or posttreatment with TrkB-Fc prevents spinal LTP facilitation and alleviates pain hypersensitivity in rats with neonatal and adult incisions

To further elucidate the role of spinal BDNF in neonatal incision-exaggerated adult incisional pain, we used TrkB-Fc, which is a TrkB-immunoglobulin G fusion protein applied to scavenge endogenous BDNF. Pretreatment or posttreatment with TrkB-Fc was examined on BDNF-induced LTP facilitation and pain hypersensitivity in adult rats with repeated incisions. In the pretreatment experiments (Fig. 4A), we found that preincisional TrkB-Fc (350 ng/20 μl) treatment completely inhibited 100 ng BDNF-induced LTP in TrkB-Fc treated nIN-IN rats compared with IgG treated nIN-IN rats (Fig. 4B and C). When compared to the baseline responses, the mean C-fiber-evoked field potentials during 180–240 min

following 100 ng BDNF application were 102.6 ± 3.72% in TrkB-Fc pre-treated nIN-IN rats and 169.7 ± 7.07% in IgG pre-treated nIN-IN rats ($P < 0.001$, two-tailed unpaired *t*-test, n = 7–8, Fig. 4B). Similarly, pretreatment with TrkB-Fc abolished the increase of the magnitude of 300 ng BDNF-induced LTP (Fig. 4C). The mean C-fiber-evoked field potentials during 180–240 min following 300 ng BDNF application were 164.2 ± 3.66% in TrkB-Fc preincisional injected rats and 213.3 ± 5.96% in IgG preincisional injected rats ($P<0.001$, two-tailed unpaired *t*-test, n = 5–7, Fig. 4C). The weak HFS of sciatic nerve at a low intensity (5–10 V) successfully induced LTP in the IgG treated group but not the TrkB-Fc treated group ($P<0.001$, two-tailed unpaired *t*-test, n = 6–7, Fig. 4D). The mean C-fiber-evoked field potentials during 180–240 min following weak HFS application were 103.7 ± 1.86% in TrkB-Fc injected rats and 187.2 ± 10.00% in IgG injected rats ($P<0.001$, two-tailed unpaired *t*-test, n = 6–7, Fig. 4D). And we also found that pretreatment with TrkB-Fc alleviated pain hypersensitivity in adult rats with repeated incisions. Pretreatment with TrkB-Fc restored repeated incisions-induced decrease of the PWT and PWL in nIN-IN rats by 34–61% from day 1 (PWT: 36.04 ± 3.57% TrkB-Fc/nIN-IN *vs.* 12.09 ± 2.60% IgG/nIN-IN, $P<0.001$, PWL: 55.19 ± 4.93% TrkB-Fc/nIN-IN, *vs.* 32.40 ± 3.64% IgG/nIN-IN, $P<0.01$) to day 14 (PWT: 83.64 ± 4.91% TrkB-Fc/nIN-IN *vs.* 69.19 ± 5.60% IgG/nIN-IN, $P<0.01$, PWL: 86.59 ± 5.83% TrkB-Fc/nIN-IN *vs.* 65.84 ± 4.45% IgG/nIN-IN, $P<0.01$) after adult incision (2-way ANOVA, n = 14, Fig. 4E and F). In contrast, pretreatment with TrkB-Fc in nSham-IN rats have no statistically significant effects on pain behaviors compare with IgG treated rats (for example, on day 1, PWT: 28.18 ± 1.79% TrkB-Fc/nSham-IN, *vs.* 28.11 ± 1.78% IgG/nSham-IN, PWL: 59.08 ± 5.43% TrkB-Fc/nSham-IN, *vs.* 58.59 ± 3.45% IgG/nSham-IN, *P>0.05*, 2-way ANOVA, n = 14, Fig. 4E and F). Using higher dose of TrkB-Fc (10 μg/20 μl), pain hypersensitivity was alleviated in both nSham-IN and nIN-IN rats, and TrkB-Fc had a longer effective time in nIN-IN rats compared with nSham-IN rats (6 h *vs.* 2 days, 2-way ANOVA, n = 7, Supplementary data: Figs. S1E–G). Additionally, there was no significant effect of gender on the PWT and PWL in adult rats with repeated incisions treated with TrkB-Fc or IgG (Table 1), according to BDNF protein expression (Fig. 2C and D), suggesting the role of BDNF in neonatal incision-exaggerated adult incisional pain is irrelevant to gender.

In the posttreatment experiments (Supplementary data: Fig. S2A), TrkB-Fc (350 ng/20 μl) also abolished 100 ng BDNF-induced LTP and attenuated the increase of the magnitude of LTP induced by 300 ng BDNF, however, the electrophysiological recording was done on earlier time points compared with pretreatment experiment (day 4 *vs.* day 7) (Supplementary data: Figs. S2B and C). Moreover, although posttreatment with TrkB-Fc also alleviated pain hypersensitivity in adult rats with repeated incisions, posttreatment had a shorter effective time compared with pretreatment experiment (Supplemental data: Figs. S2D and E). Taken together, these data suggest that pretreatment or posttreatment with TrkB-Fc attenuates repeated incisions-induced LTP facilitation and alleviates repeated incisions-induced pain hypersensitivity, implying that the spinal BDNF contributes to the facilitation of spinal LTP and adult incisional pain primed by neonatal incision. Pretreatment of TrkB-Fc are more effective than posttreatment, implying a more important role of BDNF in initiation of neonatal incision-exaggerated adult incisional pain.

3.5. Neonatal priming effects are revealed by intrathecally administration of BDNF in adult rats with only neonatal incision

Furthermore, we detected whether intrathecally injection of BDNF in adult rats with single neonatal incision could mimic the repeated incisions-induced LTP facilitation and pain hypersensitivity. As shown in Fig. 5A, BDNF were intrathecally injected at 20 ng, twice per day at a 30 min-interval for 3 days. Intrathecal injected BDNF at 20 ng before electrophysiological recording was applied to mimic the adult incision, while spinal surface given 100 ng or 300 ng BDNF during electrophysiological studies was used in order to induce LTP.

We found that spinal surface given 100 ng BDNF could successfully induce significant spinal LTP by intrathecal injection of BDNF (20 ng) in nIN rats compared with injection of saline, while 100 ng BDNF could not induce spinal LTP by intrathecally application of 20 ng BDNF in nSham rats. The average C-fiber-evoked field potentials 180–240 min after 100 ng BDNF application were 170.5 ± 5.63% in BDNF injected nIN rats, 99.06 ± 2.22% in saline injected nIN rats, and 102.8 ± 0.54% in BDNF injected nSham rats ($P<0.001$, 1-way ANOVA, n = 5–6, Fig. 5B). Additionally, spinal application with BDNF at 20 ng increased the magnitude of 300 ng BDNF-induced LTP in nIN rats compared with saline, while in nSham rats intrathecally application of BDNF 20 ng do not alter the magnitude of 300 ng BDNF-induced spinal LTP. The average C-fiber-evoked field potentials 180–240 min after 300 ng BDNF application were 214.6 ± 9.05% in BDNF injected nIN rats, 165.7 ± 2.53% in saline injected nIN rats, and 168.6 ± 3.72% in BDNF injected nSham rats ($P<0.001$, 1-way ANOVA, n = 5–6, Fig. 5C).

Spinal treatment with BDNF decreased the PWT and PWL in nIN rats from day 1 (PWT: 43.11 ± 6.43% BDNF/nIN *vs.* 97.40 ± 7.37% Saline/nIN, $P<0.001$, PWL: 52.06 ± 5.03% BDNF/nIN *vs.* 98.31 ± 3.96% Saline/nIN, $P<0.001$) to day 14 (PWT: 84.23 ± 4.89% BDNF/nIN *vs.* 99.05 ± 0.95% Saline/nIN, $P<0.05$, PWL: 84.33 ± 1.61% BDNF/nIN *vs.* 98.94 ± 3.22% Saline/nIN, $P<0.05$) after adult incision (2-way ANOVA, n = 8, Fig. 5D and E). However, spinal injection of BDNF did not significantly affected the PWT and PWL in nSham rats compared with saline in our experimental design (for example, on day 1, PWT: 87.45 ± 5.76% BDNF/nSham *vs.* 93.38 ± 4.47% saline/nSham, PWL: 98.70 ± 1.03% BDNF/nSham *vs.* 99.62 ± 1.48% saline/nSham, *P>0.05*, 2-way ANOVA, n = 8, Fig. 5D and E). Moreover, intrathecal application of TrkB-Fc (350 ng/20 μl) along with BDNF injection abolished the BDNF-induced decrease in the PWT and PWL in nIN rats (2-way ANOVA, n = 8, Fig. 5D and E), confirmed that impacts of pain behaviors in nIN rats were due to BDNF infusion. These data demonstrate that intrathecally administration with BDNF mimics the adult incisional pain in neonatal primed rats, revealing the important role of spinal BDNF in facilitation of the spinal LTP and adult incisional pain primed by neonatal incision.

BDNF intrathecally given for 3 days in nIN rats did not change the concentration of BDNF in the spinal dorsal horn 7 days later (Supplementary data: Fig. S3A) when electrophysiological studies began (Fig. 5A). Moreover, BDNF intrathecally injected for 1 day decreased the PWT and PWL for only 2 days in nIN rats treated with BDNF (Supplementary data: Figs. S3B–D), while additional injection for just 2 days extended the effective time for 12 more days (Fig. 5D and E). These results suggested that the long-lasted effects after 7 days cannot be directly due to the increased BDNF by intrathecal injection, which may be due to the activation of downstream signals. Accordingly, a more important role for BDNF in the initiation of neonatal incision-exaggerated adult incisional pain was implied by the results that pretreatment with TrkB-Fc in nIN-IN rats were more effective than posttreatment (Fig. 4, Supplementary data: Fig. S2), although the protein expression of BDNF is upregulated both in the early and the late stage in nIN-IN rats (Fig. 2C). In addition, when the expression of endogenous BDNF is increased, infusion of BDNF-induced LTP can be impaired for attenuated downstream signals (Gooney et al., 2004), and neonatal incision enhances inhibition of the RVM and shows behavioral hypoalgesia (Walker et al., 2015). However, we observed both upregulation of BDNF and facilitation of BDNF-induced LTP (Figs. 2 and 3), and our results of nIN rats and nSham rats injected with

BDNF suggested that the pain-related signaling may be elevated by neonatal incision in the spinal dorsal horn, according to previous reports (Li et al., 2013, 2015). Additionally, similar as differential consequences led by gradual and acute increases of BDNF in the hippocampus (Balkowiec and Katz, 2002; Canossa et al., 1997; Griesbeck et al., 1999; Hartmann et al., 2001; Ji et al., 2010; Lessmann et al., 2003), the intrathecal BDNF to mimic adult incision and tBDNF application in electrophysiological studies probably activate distinct downstream signals.

3.6. Inhibition of microglia activation by minocycline prevents neonatal incision enhanced upregulation of Iba1 and BDNF, abolishes BDNF-induced LTP facilitation, and alleviates machenical allodynia and heat hyperalgesia in adult rats with repeated incisions

Finally, to further provide direct evidence for the important role of microglia which can synthesize and secret BDNF, we explored whether minocycline, a microglia inhibitor, affected the neonatal priming on the expression of Iba1 and BDNF, BDNF-induced LTP facilitation and pain behaviors in adult rats with repeated incisions. In the pretreatment experiments (Fig. 6A), we found that intrathecal administration of minocycline (105 μg/20 μl) could abolish the upregulation of Iba1 and BDNF induced by repeated incisions by 94% (Fig. 6B–F). The positive staining cells of Iba1 in the ipsilateral superficial dorsal horn were decreased in minocycline treated rats compared with saline treated rats (4.549 ± 0.40% Minocycline/nIN-IN, *vs.* 10.51 ± 1.09% Saline/nIN-IN, $P<0.001$, 1-way ANOVA, n = 3 rats/group, 3–5 tissues per rat, Fig. 6B). Ultimately, pretreatment with minocycline attenuated the increase of gene expression of BDNF in the spinal dorsal horn in nIN-IN rats from day 1 (1.69 ± 0.19 Minocycline/nIN-IN *vs.* 5.35 ± 1.28 Saline/nIN-IN, $P < 0.001$) to day 3 (1.16 ± 0.45 Minocycline/nIN-IN *vs.* 3.48 ± 0.89 Saline/nIN-IN, $P < 0.05$) after adult incision (2-way ANOVA, n = 6, Fig. 6C). However, minocycline at the dose we used did not statistically affect the expression of BDNF gene in nSham-IN rats 8 days after adult incision ($P>0.05$, 1-way ANOVA, n = 9–10, Fig. 6D). Furthermore, spinal administration of minocycline abolished the evaluation of protein expression of BDNF in the spinal dorsal horn in nIN-IN rats from day 1 (128.5 ± 10.97 pg/mg total protein Minocycline/nIN-IN *vs.* 266.9 ± 17.17 pg/mg total protein Saline/nIN-IN, $P < 0.001$) to day 14 after adult incision (127.4 ± 9.84 pg/mg total protein Minocycline/nIN-IN *vs.* 182.9 ± 13.59 pg/mg total protein Saline/nIN-IN, $P < 0.01$, 2-way ANOVA, n = 8, Fig. 6E). In contrast, minocycline injection did not statistically affect the concentration of BDNF protein in nSham-IN rats 8 days after adult incision using our experimental design ($P>0.05$, 1-way ANOVA, n = 8) (Fig. 6F).

Moreover, preincisional treatment with minocycline could inhibit 100 ng BDNF-induced LTP by 90% in nIN-IN rats compared with saline treated rats. When compared to the baseline responses, the mean C-fiber-evoked field potentials during 180–240 min following 100 ng BDNF application were 106.9 ± 2.03% in minocycline treated nIN-IN rats and 167.5 ± 3.76% in saline injected nIN-IN rats ($P < 0.001$, two-tailed unpaired *t*-test, n = 5, Fig. 6G). Similarly, pretreatment with TrkB-Fc abolished the increase of the magnitude of 300 ng BDNF-induced LTP. The mean C-fiber-evoked field potentials during 180–240 min following 300 ng BDNF application were 168.4 ± 5.77% in minocycline injected rats and 207.1 ± 3.60% in saline injected rats ($P<0.001$, two-tailed unpaired *t*-test, n = 5, Fig. 6H).

Furthermore, pretreatment with minocycline alleviated pain hypersensitivity in adult rats with repeated incisions. The decreased PWT and PWL in adult rats with repeated incisions were restored 85% by minocycline from day 1 (PWT: 59.18 ± 7.24% Minocycline/nIN-IN *vs.* 9.551 ± 1.07% Saline/nIN-IN, $P<0.001$, PWL: 76.29 ± 3.87% Minocycline/nIN-IN *vs.* 39.13 ± 8.30% Saline/nIN-IN, $P<0.001$) to day 14 (PWT: 95.98 ± 4.02% Minocycline/nIN-IN *vs.* 72.61 ± 9.26% Saline/nIN-IN, $P<0.01$, PWL: 88.95 ± 3.29% Minocycline/nIN-IN *vs.* 65.84 ± 3.85% Saline/nIN-IN, $P<0.01$) after adult incision, while no significant difference between the minocycline treatment and saline treatment in nSham-IN rats at all the time points detected in our study expects of a slight tendency (for example, on day 1, PWT: 42.07 ± 6.20% minocycline/nSham-IN *vs.* 29.53 ± 3.02% saline/nSham-IN, PWL: 79.66 ± 8.21% minocycline/nSham-IN *vs.* 62.87 ± 5.25% saline/nSham-IN, $P>0.05$, 2-way ANOVA, n = 8, Fig. 6I). These data together indicate that pretreatment with minocycline inhibits Iba-1 and BDNF upregulation, attenuates BDNF-induced LTP facilitation, alleviates mechanical allodynia and thermal hyperalgesia in adult rats with repeated incisions, implying that spinal BDNF, which takes part in neonatal incision-induced exaggeration of adult incisional pain, at least partly comes from microglia.

We also detected that whether posttreatment with minocycline has the similar effects on pain sensitivity in adult rats with repeated incisions (Supplementary data: Fig. S4A). Using the same dose of minocycline with the pretreatment experiment, posttreatment with minocycline did not have significant effects on the neonatal incision-induced evaluation of pain hypersensitivity in adult rats with repeated incisions without motor dysfunction ($P>0.05$, n = 8–10, Supplementary data: Figs. S4B–D), according to previous reports of minocycline in rats with only adult hindpaw incision (Ito et al., 2009), implying a more important role of minocycline in the initiation of neonatal incision-exaggerated adult incisional pain.

Spinal LTP is considered as a synaptic model to pathological pain, for the reasons that they are both induced by noxious stimulations and repressed by the same drugs (Ji et al., 2003; Liu and Zhou, 2015; Ruscheweyh et al., 2011; Sandkuhler, 2009; Sandkuhler and Gruber-Schoffnegger, 2012), and the LTP-inducing stimulations produce long-lasting pain behaviors in intact animals observed by us (Supplementary data: Figs. S5A and B) and others (Bian et al., 2014, 2015; Chu et al., 2012; Liang et al., 2010; Svendsen et al., 1999; Ying et al., 2006; Zhang et al., 2005). Additionally, spinal LTP has been proposed as a mechanism by which excitation in ascending nociceptive pathways can be amplified within the spinal cord (Ruscheweyh et al., 2011; Sandkuhler, 2009; Sandkuhler and Gruber-Schoffnegger, 2012), based on the findings that the LTP-inducing conditioning stimulations facilitate action potential firing of multireceptive neurons in the dorsal horn (Haugan et al., 2008; Qu et al., 2009; Rygh et al., 2006), although other mechanisms of facilitation should not be excluded. However, whether facilitation of spinal LTP and exacerbation of pain are correlated in nIN-IN rats is still unknown.

As shown in Supplementary data: Figs. S5C–G, the LTP-inducible HFS to the sciatic nerve produced an exacerbation of mechanical allodynia and heat hyperalgesia in the nIN-IN rats treated with TrkB-Fc, which were alleviated by the inhibition of the sensory information transmission to the spinal cord, and that lidocaine did not directly change pain sensitivity in our experimental design (Sun et al., 2004; Tobe et al., 2010; Wang et al., 2011) (Supplementary data: Fig. S5). The HFS-induced spinal LTP was facilitated in nIN-IN rats (Fig. 3D). Furthermore, spinal BDNF is involved in the neonatal incision-induced facilitation of LTP and the exacerbation of incisional pain in adult rats (Figs. 4–6). Collectively, these results imply that the facilitation of spinal LTP may contribute to the exaggerated pain sensitivity in nIN-IN rats, although we cannot completely exclude the possibility that the facilitation of spinal LTP and the exaggerated pain sensitivity are just occurred at the same time without any association, for there is no such specific method to induce or attenuate LTP merely. For example, the LTP-inducing HFS also injures peripheral nerves (Liang et al., 2010), which may be the reason why the PWT and PWL were not return to baseline when the conduction to the spinal cord was blocked (Supplementary data:

Figs. S5F–G).

4. Discussion

Several studies suggest a vital role of spinal BDNF in long-term effects of neonatal injury (Beggs et al., 2012; McKelvey et al., 2015). Spinal microglia, which can secrete BDNF, are activated in adult incision rats with previous neonatal incision (nIN-IN rats) (Beggs et al., 2012). Here we provide evidence showing that spinal BDNF plays an important role in neonatal incision-primed adult incisional pain, consistent with previous findings that spinal BDNF contributes to chronic pain in adults (Geng et al., 2010; Liang et al., 2016; Liu et al., 2016).

We observed that only neonatal incision could not impact BDNF expression 10 weeks after neonatal incision, which may be due to the long-time interval, consistent with findings of microglia (Beggs et al., 2012). Another possible reason may be that the neonatal injury to adult sensory system with or without adult incision has different mechanisms. Indeed, rats with only neonatal incision (nIN rats) and nIN-IN rats showed hypoalgesia (Soens et al., 2015; Walker et al., 2015) or hyperalgesia (Gong et al., 2016; Schwaller et al., 2015) respectively, and appear at different time points after the neonatal incision (Walker et al., 2009, 2015). Moreover, BDNF can have distinct roles in uninjured and injured spinal cord (Garraway and Huie, 2016; Huang et al., 2017).

Although whether there are sex differences in the long-term impact of neonatal injury remains unclear (LaPrairie and Murphy, 2010), we and others have found that the elevated pain hypersensitivity (Beggs et al., 2012; Walker et al., 2009) and microglial reactivity as well as the facilitation of HFS-induced spinal LTP in nIN-IN rats occurs without difference between female and male rodents. In addition, we observed that the BDNF upregulation in nIN-IN rats and the alleviated pain hypersensitivity in nIN-IN rats treated with TrkB-Fc are irrelevant to gender. While the report that NGF, which belongs to the same family with BDNF, is involved in neonatal incision-facilitated nociceptive synaptic input in the immature spinal dorsal horn only in female mice (Li and Baccei, 2011) may be possibly explained by the difference between the mature and immature spinal cord.

Evidence has accumulated that the spinal BDNF takes part in the modulation of several protocols-induced LTP in adults (Ding et al., 2015; Zhou et al., 2008). The subthreshold electrical or chemical stimulation can induce LTP in adult rodents with neuropathic pain (Liu et al., 2007; Xing et al., 2007). Similarly, we found that the dose of BDNF to induce spinal LTP and the threshold of the HFS-induced LTP are lower in nIN-IN rats. Furthermore, we also found that the threshold for evoking the C-fiber-evoked field potentials is lower in the nIN-IN group, which is affected by spinal BDNF and microglia (Supplementary data: Fig. S8), indicating a BDNF-involved functional increase in the hyperexcitability in the spinal synaptic transmission.

The magnitude of suprathreshold stimulation-induced LTP can be amplified in different types of pain. The magnitude of HFS-induced LTP is increased at the parabrachial-central nuclei synapses in spared nerve injury-induced neuropathic pain (Li et al., 2017), and at the primary afferent synapses onto spinal neurons in paclitaxel-induced neuropathic pain (Zhu et al., 2015). Especially, neonatal hindpaw incision significantly increases the magnitude of HFS-induced LTP at primary afferent synapses onto spinal projection neurons in adult rats (Li and Baccei, 2016). Here we observed that the amplitude of adequate dose of BDNF-induced spinal LTP is increased in nIN-IN rats. Although we and others have previously documented that the spinal LTP cannot be elicited by HFS in rats with neuropathic pain, for the reason that the spinal nerve ligation-induced sensitized condition (i.e. an LTP-like state) occludes the following HFS-induced spinal LTP (Ding et al., 2015; Ohnami et al., 2011; Rygh et al., 2000) at the time points we used is when pain hypersensitivity is stable and serious. However, in order to show the special mechanisms in the impact of neonatal priming on adult incisional pain, here we chose a late stage (7–10 days) after adult incision to test. In this period of time, we and others have found that the mechanical allodynia and heat hyperalgesia decrease gradually and are approximately cut in half, the PWT is higher than 4 g in nIN-IN rats and there is no pain hypersensitivity in IN rats (Beggs et al., 2012; Gong et al., 2016; Schwaller et al., 2015; Soens et al., 2015; Walker et al., 2009), so it is possible that there is no significant LTP-like state at this time. Also our results showed that the upregulation of BDNF protein decreases gradually and approximately cut in half in nIN-IN rats, and there is no change of BDNF protein in IN rats in this period of time, thus it is likely that the BDNF does not go to saturation.

The nIN rats have long-term alterations in synaptic function of adult spinal nociceptive circuits (Li et al., 2013, 2015). Especially, the neonatal tissue damage affects the adult spinal nociceptive circuits through persistently facilitating LTP at these synapses by broadening the window for spike-timing-dependent LTP and facilitating the HFS-induced spinal LTP (Li and Baccei, 2016). However, here the dose of BDNF to induce spinal LTP is not changed in nIN rats, which may be caused by different LTP induction protocols or electrophysiological recording methods (in vitro or in vivo). Indeed, the nIN rats have an enhanced inhibition of the RVM and show behavioral hypoalgesia (Walker et al., 2015).

Microglia in the mature spinal cord is activated and contributes to the incisional pain (Peters and Eisenach, 2010; Wen et al., 2009). Adverse early life events, such as stress, inflammation or incision, change the role of microglia in pain physiology in adulthood (Beggs et al., 2012; Walker et al., 2016). BDNF in the mature spinal cord participated in neuropathic pain only derives from microglia, not from primary sensory neurons (Coull et al., 2005; Zhao et al., 2006). Here we demonstrated that intrathecally injection of minocycline, a microglia inhibitor, attenuates the microglia activity as well as the BDNF upregulation and the BDNF-induced LTP facilitation, and also, alleviates the mechanical allodynia and thermal hyperalgesia in nIN-IN rats, suggesting that BDNF derived from microglia plays a vital role in the impact of neonatal priming on adult incisional pain.

Increased hyperexcitability in spinal dorsal horn neurons has been reported in IN rats (Nagakura et al., 2008; Pogatzki et al., 2002; Zahn et al., 2005). Here we found that in the early stage (day 0 to day 1) after adult incision the expression of BDNF is increased in nSham-IN rats, which is negatively correlated with pain hypersensitivity, consistent with previous reports (Li et al., 2008; Masaki et al., 2016; Sahbaie et al., 2016; Zhang et al., 2016), while in the late stage (3–21 days), the expression of BDNF and the BDNF-induced LTP are not changed in nSham-IN or IN rats. Also, a high dose of TrkB-Fc alleviates the pain hypersensitivity in nSham-IN rats according to others report (Li et al., 2008), whereas TrkB-Fc has a longer effective time in nIN-IN rats. These results imply that the neonatal priming exacerbates the role of spinal BDNF in adult incisional pain, similar to the previous report that morphine treatment before incision enhances the effects of spinal BDNF in adult incisional pain (Sahbaie et al., 2016).

Hyperalgesic priming is a latent hyperresponsiveness of nociceptors to inflammatory mediators subsequent to an inflammatory or neuropathic injury, which contains a long-lasting neuroplastic change (Sun et al., 2013). Similarly, the neonatal incision enhances glutamatergic transmission regulated by NGF in the immature spinal dorsal horn (Li and Baccei, 2011), and sensitizes synapses (Li et al., 2013, 2015), changes the dependence of LTP on N-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA) receptors in the mature spinal cord

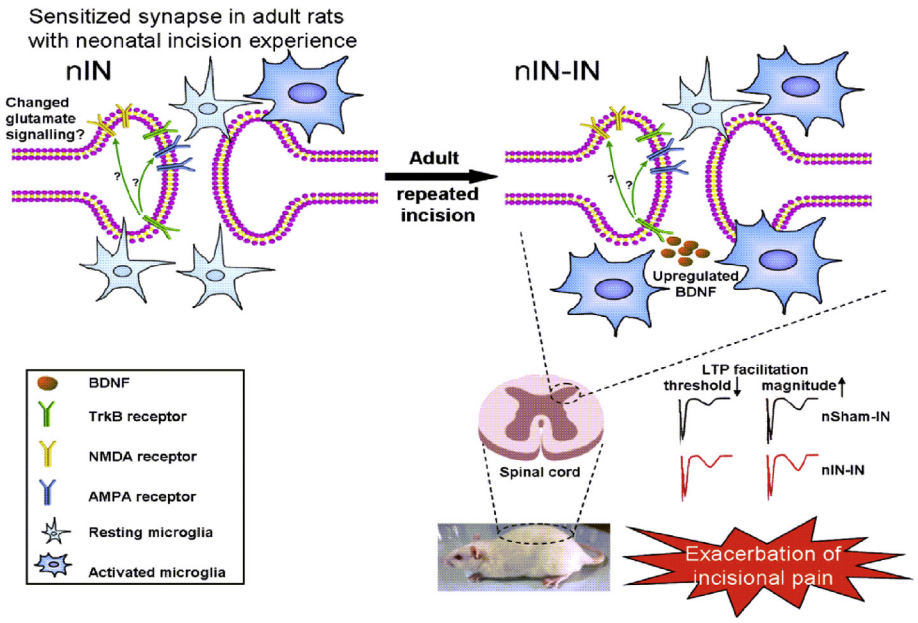

Fig. 7. Schematic diagram illustrating the possible mechanisms underlying neonatal incision-induced exacerbation of adult incisional pain. In adult rats with neonatal incision experience (nIN), spinal synapses are sensitized by changed pain-related signaling. For example, altered glutamate signaling, such as α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptor (?), possibly contribute to sensitization in nIN rats, implied by several previous studies. After adult repeated incision, in adult rats subjected to neonatal incision and adult incision (nIN-IN), spinal microglia are activated and the expression of BDNF is increased. Spinal BDNF may rekindle the downstream signaling which are already developmental changed by neonatal incision, such as NMDA and/or AMPA receptors (?), facilitate spinal LTP, including decreased induction threshold and increased magnitude of LTP, thereby exaggerating incisional pain. In other words, spinal BDNF secreted from microglia in nIN-IN rats serve as a cue of exaggerated incisional pain.

(Li and Baccei, 2016). Incision again in the adulthood induces the activation of microglia and the upregulation of BDNF, which may rekindle the neonatal incision-primed downstream signaling such as NMDA and/or AMPA receptors (Ding et al., 2015; Hayashi et al., 2000; Nakata and Nakamura, 2007) and facilitates the spinal LTP, thereby elevating pain hypersensitivity. In other words, BDNF secreted from microglia in adulthood is likely a cue of exaggerated pain hypersensitivity (Fig. 7). Thus it is reasonable that posttreatment with minocycline did not have significant effects and posttreatment with TrkB-Fc had a shorter effective time compared with the corresponding pretreatment in nIN-IN rats. And application of BDNF at 20 ng of each injection in nIN rats mimics the exaggerated incisional pain in nIN-IN rats 7 days later, when the BDNF is metabolized. It will be of great interest to determine whether the neonatal incision-induced developmental changes of glutamate receptors take parts in the BDNF-mediated exaggeration of adult incisional pain.

In conclusion, we provide solid evidence to show that the repeated incisions in neonatal and adult periods produce a significant upregulation of spinal BDNF, secreted at least partly from microglia, leading to the facilitation of spinal LTP and the exacerbation of pain hypersensitivity. Our results imply that the spinal BDNF combines the alterations in neuroimmune signaling and synaptic plasticity in rats with neonatal incision and adult repeated incisions.

Conflicts of interest

The authors declare that they have no competing interests.

Authors' contributions

X. Ding carried out the ELISA, the real-time PCR and the electrophysiological studies, participated in the design of the study and drafted the manuscript. Y.-J. Liang participated in the immunohistochemical studies. L. Su participated in the statistical analysis and the behavioral tests. F.–F. Liao and D. Fang participated in the behavioral tests. J. Tai participated in the design of the study and the statistical analysis of data. G.-G. Xing contributed to the conception and design of the study, participated in the statistical analysis of data, drafted the manuscript and made final approval of the version to be submitted. All authors have read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2018.04.032.

References

Balkowiec, A., Katz, D.M., 2002. Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. J. Neurosci. 22, 10399–10407.
Beggs, S., et al., 2012. Priming of adult pain responses by neonatal pain experience: maintenance by central neuroimmune activity. Brain 135, 404–417.
Benarroch, E.E., 2015. Brain-derived neurotrophic factor Regulation, effects, and potential clinical relevance. Neurology 84, 1693–1704.
Bian, C., et al., 2014. Up-regulation of interleukin-23 induces persistent allodynia via CX3CL1 and interleukin-18 signaling in the rat spinal cord after tetanic sciatic stimulation. Brain Behav. Immun. 37, 220–230.
Bian, C., et al., 2015. Involvement of CX3CL1/CX3CR1 signaling in spinal long term potentiation. PLoS One 10 e0118842.
Brennan, T.J., et al., 1996. Characterization of a rat model of incisional pain. Pain 64,

493–501.
Canossa, M., et al., 1997. Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. Proc. Natl. Acad. Sci. U. S. A 94, 13279–13286.
Chaplan, S.R., et al., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Meth. 53, 55–63.
Chu, Y.X., et al., 2012. Involvement of microglia and interleukin-18 in the induction of long-term potentiation of spinal nociceptive responses induced by tetanic sciatic stimulation. Neurosci. Bull. 28, 49–60.
Coull, J.A., et al., 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438, 1017–1021.
Ding, X., et al., 2015. BDNF contributes to the development of neuropathic pain by induction of spinal long-term potentiation via SHP2 associated GluN2B-containing NMDA receptors activation in rats with spinal nerve ligation. Neurobiol. Dis. 73, 428–451.
Dixon, W.J., 1980. Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. 20, 441–462.
Garraway, S.M., Huie, J.R., 2016. Spinal plasticity and behavior: BDNF-Induced neuromodulation in uninjured and injured spinal cord. Neural Plast. 9857201.
Geng, S.J., et al., 2010. Contribution of the spinal cord BDNF to the development of neuropathic pain by activation of the NR2B-containing NMDA receptors in rats with spinal nerve ligation. Exp. Neurol. 222, 256–266.
Gong, X., et al., 2016. Exercise preconditioning reduces neonatal incision surgery-induced enhanced hyperalgesia via inhibition of P38 mitogen-activated protein kinase and IL-1beta, TNF-alpha release. Int. J. Dev. Neurosci. 52, 46–54.
Gooney, M., et al., 2004. BDNF-induced LTP in dentate gyrus is impaired with age: analysis of changes in cell signaling events. Neurobiol. Aging 25, 1323–1331.
Griesbeck, O., et al., 1999. Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. Microsc. Res. Tech. 45, 262–275.
Groth, R., Aanonsen, L., 2002. Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia. Pain 100, 171–181.
Hartmann, M., et al., 2001. Synaptic secretion of BDNF after high-frequency stimulation of glutamatergic synapses. EMBO J. 20, 5887–5897.
Haugan, F., et al., 2008. Ketamine blocks enhancement of spinal long-term potentiation in chronic opioid treated rats. Acta Anaesthesiol. Scand. 52, 681–687.
Hayashi, Y., et al., 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287, 2262–2267.
Huang, Y.J., et al., 2017. Complete spinal cord injury (SCI) transforms how brain derived neurotrophic factor (BDNF) affects nociceptive sensitization. Exp. Neurol. 288, 38–50.
Ito, N., et al., 2009. Spinal microglial expression and mechanical hypersensitivity in a postoperative pain model: comparison with a neuropathic pain model. Anesthesiology 111, 640–648.
Ji, R.R., et al., 2003. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 26, 696–705.
Ji, Y.Y., et al., 2010. Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat. Neurosci. 13, 302–309.
Kehlet, H., et al., 2006. Persistent postsurgical pain: risk factors and prevention. Lancet 367, 1618–1625.
Lalo, U., et al., 2016. ATP from synaptic terminals and astrocytes regulates NMDA receptors and synaptic plasticity through PSD-95 multi-protein complex. Sci. Rep. 6, 33609.
LaPrairie, J.L., Murphy, A.Z., 2010. Long-term impact of neonatal injury in male and female rats: sex differences, mechanisms and clinical implications. Front. Neuroendocrinol. 31, 193–202.
Lessmann, V., et al., 2003. Neurotrophin secretion: current facts and future prospects. Prog. Neurobiol. 69, 341–374.
Li, C.Q., et al., 2008. Brain derived neurotrophic factor (BDNF) contributes to the pain hypersensitivity following surgical incision in the rats. Mol. Pain 4, 27.
Li, J., Baccei, M.L., 2011. Neonatal tissue damage facilitates nociceptive synaptic input to the developing superficial dorsal horn via NGF-dependent mechanisms. Pain 152, 1846–1855.
Li, J., Baccei, M.L., 2016. Neonatal tissue damage promotes spike timing-dependent synaptic long-term potentiation in adult spinal projection neurons. J. Neurosci. 36, 5405–5416.
Li, J., et al., 2013. Deficits in glycinergic inhibition within adult spinal nociceptive circuits after neonatal tissue damage. Pain 154, 1129–1139.
Li, J., et al., 2015. Aberrant synaptic integration in adult lamina I projection neurons following neonatal tissue damage. J. Neurosci. 35, 2438–2451.
Li, M.J., et al., 2017. Chronic stress exacerbates neuropathic pain via the integration of stress-affect-related information with nociceptive information in the central nucleus of the amygdala. Pain 158, 717–739.
Liang, L.L., et al., 2010. Involvement of nerve injury and activation of peripheral glial cells in tetanic sciatic stimulation-induced persistent pain in rats. J. Neurosci. Res. 88, 2899–2910.
Liang, Y., et al., 2016. CREB-regulated transcription coactivator 1 enhances CREB-dependent gene expression in spinal cord to maintain the bone cancer pain in mice. Mol. Pain 12.
Liu, X.G., Zhou, L.J., 2015. Long-term potentiation at spinal C-fiber synapses: a target for pathological pain. Curr. Pharmaceut. Des. 21, 895–905.
Liu, Y., et al., 2016. TNF-alpha differentially regulates synaptic plasticity in the hippocampus and spinal cord by microglia-dependent mechanisms after peripheral nerve injury. J. Neurosci. 37, 871–881.
Liu, Y.L., et al., 2007. Tumor necrosis factor-alpha induces long-term potentiation of C-fiber evoked field potentials in spinal dorsal horn in rats with nerve injury: the role of NF-kappa B, JNK and p38 MAPK. Neuropharmacology 52, 708–715.
Lu, Y.F., et al., 1999. Enhanced synaptic transmission and reduced threshold for LTP induction in fyn-transgenic mice. Eur. J. Neurosci. 11, 75–82.
Masaki, E., et al., 2016. Early postoperative nociceptive threshold and production of brain-derived neurotrophic factor induced by plantar incision are not influenced with minocycline in a rat: role of spinal microglia. Neurosignals 24, 15–24.
McKelvey, R., et al., 2015. Neuropathic pain is constitutively suppressed in early life by anti-inflammatory neuroimmune regulation. J. Neurosci. 35, 457–466.
Meis, S., et al., 2012. Postsynaptic BDNF signalling regulates long-term potentiation at thalamo-amygdala afferents. J. Physiol. 590, 193–208.
Nagakura, Y., et al., 2008. The sensitization of a broad spectrum of sensory nerve fibers in a rat model of acute postoperative pain and its response to intrathecal pharmacotherapy. Pain 139, 569–577.
Nakata, H., Nakamura, S., 2007. Brain-derived neurotrophic factor regulates AMPA receptor trafficking to post-synaptic densities via IP3R and TRPC calcium signaling. FEBS Lett. 581, 2047–2054.
Ohnami, S., et al., 2011. Role of voltage-dependent calcium channel subtypes in spinal long-term potentiation of C-fiber-evoked field potentials. Pain 152, 623–631.
Peters, C.M., Eisenach, J.C., 2010. Contribution of the chemokine (C-C motif) ligand 2 (CCL2) to mechanical hypersensitivity after surgical incision in rats. Anesthesiology 112, 1250–1258.
Pogatzki, E.M., et al., 2002. Effect of plantar local anesthetic injection on dorsal horn neuron activity and pain behaviors caused by incision. Pain 97, 151–161.
Pyapali, G.K., et al., 1998. Prenatal dietary choline supplementation decreases the threshold for induction of long-term potentiation in young adult rats. J. Neurophysiol. 79, 1790–1796.
Qu, X.X., et al., 2009. Role of the spinal cord NR2B-containing NMDA receptors in the development of neuropathic pain. Exp. Neurol. 215, 298–307.
Rivlin, A.S., Tator, C.H., 1977. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J. Neurosurg. 47, 577–581.
Ruscheweyh, R., et al., 2011. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol. Pain 7.
Rygh, L.J., et al., 2000. Different increase in C-fibre evoked responses after nociceptive conditioning stimulation in sham-operated and neuropathic rats. Neurosci. Lett. 288, 99–102.
Rygh, L.J., et al., 2006. Local and descending circuits regulate long-term potentiation and zif268 expression in spinal neurons. Eur. J. Neurosci. 24, 761–772.
Sahbaie, P., et al., 2016. Epigenetic regulation of spinal cord gene expression contributes to enhanced postoperative pain and analgesic tolerance subsequent to continuous opioid exposure. Mol. Pain 12.
Sakata, K., et al., 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. Proc. Natl. Acad. Sci. U. S. A 110, 15103–15108.
Sandkuhler, J., 2009. Models and mechanisms of hyperalgesia and allodynia. Physiol. Rev. 89, 707–758.
Sandkuhler, J., Gruber-Schoffnegger, D., 2012. Hyperalgesia by synaptic long-term potentiation (LTP): an update. Curr. Opin. Pharmacol. 12, 18–27.
Schwaller, F., et al., 2015. Targeting p38 mitogen-activated protein kinase to reduce the impact of neonatal microglial priming on incision-induced hyperalgesia in the adult rat. Anesthesiology 122, 1377–1390.
Soens, M., et al., 2015. Systemic progesterone administration in early life alters the hyperalgesic responses to surgery in the adult: a study on female rats. Anesth. Analg. 121, 545–555.
Song, M.K., et al., 2009. The facilitating effect of systemic administration of Kv7/M channel blocker XE991 on LTP induction in the hippocampal CA1 area independent of muscarinic activation. Neurosci. Lett. 461, 25–29.
Sun, X.H., et al., 2004. The effects of pretreatment with lidocaine or bupivacaine on the spatial and temporal expression of c-Fos protein in the spinal cord caused by plantar incision in the rat. Anesth. Analg. 98, 1093–1098.
Sun, Y., et al., 2013. Epigenetic regulation of spinal CXCR2 signaling in incisional hypersensitivity in mice. Anesthesiology 119, 1198–1208.
Suo, Z.W., et al., 2017. Striatal-enriched phosphatase 61 inhibited the nociceptive plasticity in spinal cord dorsal horn of rats. Neuroscience 352, 97–105.
Svendsen, F., et al., 1999. Behavioural effects of LTP-inducing sciatic nerve stimulation in the rat. Eur. J. Pain 3, 355–363.
Thalhammer, J.G., et al., 1995. Neurologic evaluation of the rat during sciatic-nerve block with lidocaine. Anesthesiology 82, 1013–1025.
Tobe, M., et al., 2010. Long-term effect of sciatic nerve block with slow-release lidocaine in a rat model of postoperative pain. Anesthesiology 112, 1473–1481.
Valeri, B.O., et al., 2016. Neonatal invasive procedures predict pain intensity at school age in children born very preterm. Clin. J. Pain 32, 1086–1093.
Walker, S.M., et al., 2016. Persistent changes in peripheral and spinal nociceptive processing after early tissue injury. Exp. Neurol. 275, 253–260.
Walker, S.M., et al., 2015. Surgical injury in the neonatal rat alters the adult pattern of descending modulation from the rostroventral medulla. Anesthesiology 122, 1391–1400.
Walker, S.M., et al., 2009. Hindpaw incision in early life increases the hyperalgesic response to repeat surgical injury: critical period and dependence on initial afferent activity. Pain 147, 99–106.
Wang, C.F., et al., 2011. Prolonged suppression of postincisional pain by a slow-release formulation of lidocaine. Anesthesiology 114, 135–149.

Wen, Y.R., et al., 2009. Activation of p38 mitogen-activated protein kinase in spinal microglia contributes to incision-induced mechanical allodynia. Anesthesiology 110, 155–165.
Xing, G.G., et al., 2007. Long-term synaptic plasticity in the spinal dorsal horn and its modulation by electroacupuncture in rats with neuropathic pain. Exp. Neurol. 208, 323–332.
Ying, B., et al., 2006. Involvement of spinal glia in tetanically sciatic stimulation-induced bilateral mechanical allodynia in rats. Biochem. Biophys. Res. Commun. 340, 1264–1272.
Zahn, P.K., et al., 2005. Spinal administration of MK-801 and NBQX demonstrates NMDA-independent dorsal horn sensitization in incisional pain. Pain 114, 499–510.
Zhang, M.D., et al., 2016. Orthopedic surgery modulates neuropeptides and BDNF expression at the spinal and hippocampal levels. Proc. Natl. Acad. Sci. U. S. A 113, E6686–E6695.
Zhang, X.C., et al., 2005. Involvement of nitric oxide in long-term potentiation of spinal nociceptive responses in rats. Neuroreport 16, 1197–1201.
Zhang, Y., et al., 2012. Estrogen facilitates spinal cord synaptic transmission via membrane-bound estrogen receptors implications for pain hypersensitivity. J. Biol. Chem. 287, 33268–33281.
Zhao, J., et al., 2006. Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. Mol. Cell. Neurosci. 31, 539–548.
Zhou, L.J., et al., 2008. BDNF induces late-phase LTP of C-fiber evoked field potentials in rat spinal dorsal horn. Exp. Neurol. 212, 507–514.
Zhu, H.Q., et al., 2015. Bulleyaconitine A depresses neuropathic pain and potentiation at C-fiber synapses in spinal dorsal horn induced by paclitaxel in rats. Exp. Neurol. 273, 263–272.
Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.
Zimmermann, M., 2001. Pathobiology of neuropathic pain. Eur. J. Pharmacol. 429, 23–37.