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Research article

Brain-derived neurotrophic factor in the infralimbic cortex alleviates inflammatory pain

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HIGHLIGHTS

- Infralimbic BDNF decreases after inflammatory pain.
- Infralimbic BDNF infusion alleviates CFA induced thermal hyperalgesia and mechanical allodynia.
- Consecutive infralimbic BDNF infusion accelerates recovery process of inflammatory pain.

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ABSTRACT

In chronic pain, it has been reported that the medial prefrontal cortex (mPFC) takes important regulatory roles, and may change functionally and morphologically in result of chronic pain. Brain-derived neurotrophic factor (BDNF) is well known as a critical modulator of neuronal excitability and synaptic transmission in the central nervous system. The aim of the present study is to investigate the role of BDNF in the infralimbic cortex and the prelimbic cortex of the mPFC in complete Freund's adjuvant (CFA)-induced inflammatory pain. We found that the BDNF level decreased in the infralimbic cortex, but not in the prelimbic cortex, 3 days after the CFA induction of the inflammatory pain. BDNF infusion into bilateral infralimbic cortices to activate neuronal activities could alleviate inflammatory pain and accelerate long-term recovery from pain. In conclusion, BDNF in the infralimbic cortex of the mPFC could accelerate recovery from inflammatory pain.

1. Introduction

Increasing evidence indicates that the medial prefrontal cortex (mPFC) is involved in pain processing [5,9,29]. The mPFC shows abnormal morphological and functional changes in patients with chronic pain. Morphologically, fMRI studies found that the prefrontal cortex displayed decreased gray matter in patients with complex regional pain syndrome (CRPS); functionally, the connectivity between the mPFC and several associated regions (ie. hippocampus and nucleus accumbens) contributed to the prediction of pain chronicity [2,7,30]. The prefrontal changes were also reported in pain models of rats, including decreased intrinsic excitability and dendritic length of neurons [3,8,15].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. It exerts extensive effects in neurogenesis, neuron survival and learning and memory [16,38]. BDNF maintains pyramidal neuron firings [19] and induces long-term potentiation [13], thus acting as a potent modulator of neural plasticity. In the infralimbic cortex of the mPFC, BDNF is involved in the fear-memory extinction [42].

We speculate that BDNF in the infralimbic cortex is involved in pain development. In the present study, we measured the BDNF level in the infralimbic cortex of the mPFC by ELISA in the inflammatory pain model of rats, and BDNF was infused into the bilateral infralimbic cortices to examine BDNF function in inflammatory pain.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g) were supplied by the Department of Experimental Animal Sciences Peking University Health Science Center. To protect cannulas, the rats were housed individually in a constant temperature of 23 °C under a 12 h

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light/dark cycle with food and water available *ad libitum*. All experimental procedures complied with Guidelines of the Animal Care and Use Committee of our University. Rats were handled for at least three days before any experiments were conducted.

2.2. Establishment of CFA-induced inflammatory pain model of rats

100 μl of complete Freund's adjuvant (CFA, Sigma-Aldrich, St Louis, USA) was intraplantarly injected into the left hind paw to induce inflammatory pain [11]. The equal volume of normal saline (NS) was injected as a control.

2.3. ELISA measurement for BDNF in the infralimbic cortex

The prelimbic cortex tissue was dissected and homogenized in ice-cold lysis buffer containing protease inhibitor cocktail. Protein levels of BDNF were examined with ELISA method using the BDNF Emax® ImmunoAssay System kit (Promega, WI, USA) according to the manufacturer's instructions. Measurements were performed in duplicate. Total protein was measured by BCA method using bovine serum albumin as a standard. Data was presented as a ratio between BDNF and total protein level.

2.4. Assessment of thermal hyperalgesia and mechanical allodynia

Before behavioral testing, the rat was allowed to acclimate to the experimental environment for 20 min. For thermal hyperalgesia assessment, the power of the radiant heat source was adjusted to obtain averaged baseline paw withdrawal latencies (PWLs) to 15–18 s, with a cut-off time of 30 s to avoid any possible tissue damage. PWLs were measured three times with a 5-min interval, and the mean value was used to represent the thermal hyperalgesia. For mechanical allodynia assessment, *von* Frey filaments (0.41–15.1 g; North Coast, Gilroy, CA) were applied to the hind paws. The 50% paw withdrawal threshold (50% PWT) was calculated by the "up and down" method as described by Chaplan et al.[21,28,39]. The behavioral tests were performed with single-blinded manner.

2.5. Surgery for cannula implantation in the infralimbic cortex

The rat was anesthetized with 1% pentobarbital sodium (0.1 g/kg, *i.p.*) and positioned in a stereotaxic instrument (RWD, Shenzhen, China). Stainless steel, 22-gauge guide cannulas were bilaterally implanted 1-mm above the infralimbic cortex according to the atlas of Paxinos and Watson (1997) (AP: +2.8 mm, ML: ±0.5 mm, DV: −4.4 mm). The cannula was secured with screws and dental acrylic cement on the skull. A stainless steel stylet was placed in the guide cannulas to prevent clogging. The rat was returned to its home cage for one-week recovery before subsequent experiments.

2.6. BDNF infusion into the infralimbic cortex

BDNF (Sigma-Aldrich, St Louis, USA) was dissolved in artificial cerebrospinal fluid (aCSF) to a concentration of 1 μg/μL, and 0.5 μl in volume was administered per side through the inner stylets 1 mm over the cannula within 3 min. This dose of infusion followed previous studies that BDNF at such a concentration could induce behavioral changes [20,25]. Behavioral tests were performed after BDNF administration for about 30 min.

2.7. Immunofluorescent staining for BDNF-induced c-Fos expression

BDNF (0.5 μg, 1 μg/μl) was infused unilaterally after the rat was deeply anesthetized with 1% sodium pentobarbital (50 mg/kg, *i.p.*), and saline infused into the contralateral side was used as a control. One hour after the infusion, the rat was perfused transcardially with 4% paraformaldehyde. The brain was removed, post-fixed overnight at 4 °C, and kept in 30% sucrose in 0.10 mol/L phosphate-buffered saline (PBS) for dehydration. Coronal sections (30 μm) were cut on a cryostat and kept in anti-freezing fluid at −20 °C. Sections were washed three times in 0.01 mol/L PBST for 5 min each and incubated in 0.3% Triton X-100 for 30 min at room temperature. Then the sections were blocked for 1 h in 3% bovine serum albumin (0.01 mol/L PBS with 0.3% Triton X-100). All sections were incubated overnight at 4 °C with a mixture of rabbit anti-c-Fos antibody (1: 300, Santa Cruz, California, US) and mouse anti-neuronal specific nuclear protein (NeuN, a neuronal marker, 1: 500, Millipore, Temecula, CA). After washing in PBST for three times, the sections were then incubated with a mixture of FITC- and Cy3-conjugated secondary antibody for 1 h at room temperature. The stained sections were examined under a fluorescence microscope (Leica DMI 4000B, Wetzlar, Germany), and images were captured with a CCD spot camera.

2.8. Data analysis

Data were presented as means ± SEMs. Statistical analysis was performed using Student's unpaired or paired *t*-test between groups, one-way or two-way ANOVA with Bonfferoni post hoc test among groups. The P value of less than 0.05 was considered as statistically significant.

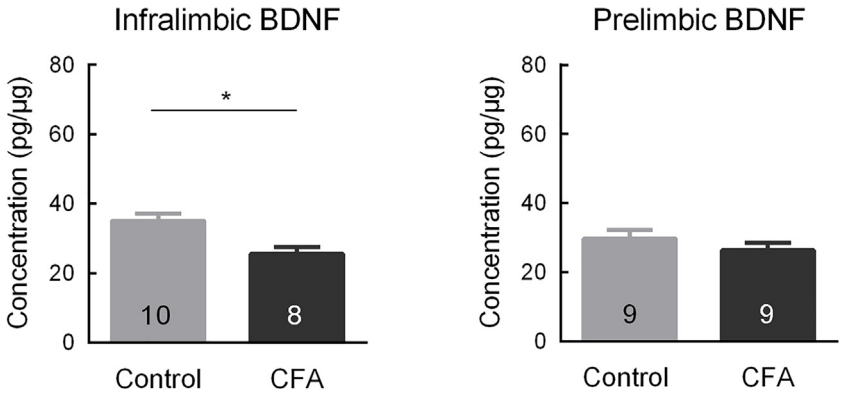

Fig. 1. BDNF levels in the infralimbic cortex and the prelimbic cortex in inflammatory pain rats 3 days after intraplantar CFA injection (A) BDNF levels in the infralimbic cortex (IL). BDNF level decreased in inflammatory pain rats 3 days after intraplantar injection of CFA compared with that in the control rats with intraplantar injection of saline. Control group: n = 10; CFA group: n = 8; * P = 0.0051, t = 3.243, unpaired *t*-test,(B) BDNF levels in the prelimbic cortex (PL). No significant changes between CFA inflammatory pain group and saline control group. Control group: n = 9; CFA group: n = 9.

3. Results

3.1. BDNF level was decreased in infralimbic cortex in CFA inflammatory pain rats

Firstly, in the CFA inflammatory pain rats, we observed whether the BDNF levels changed in two sub-regions of the medial prefrontal cortex, the infralimbic cortex and the prelimbic cortex. As shown in Fig. 1A, BDNF level in the infralimbic cortex decreased significantly in CFA-induced inflammatory pain rats 3 days after CFA injection. As a control, no obvious changes were observed in BDNF levels in the prelimbic cortex (Fig. 1B). These results indicate that BDNF in the infralimbic cortex, but not the prelimbic cortex, decreased in rats with CFA-induced inflammatory pain.

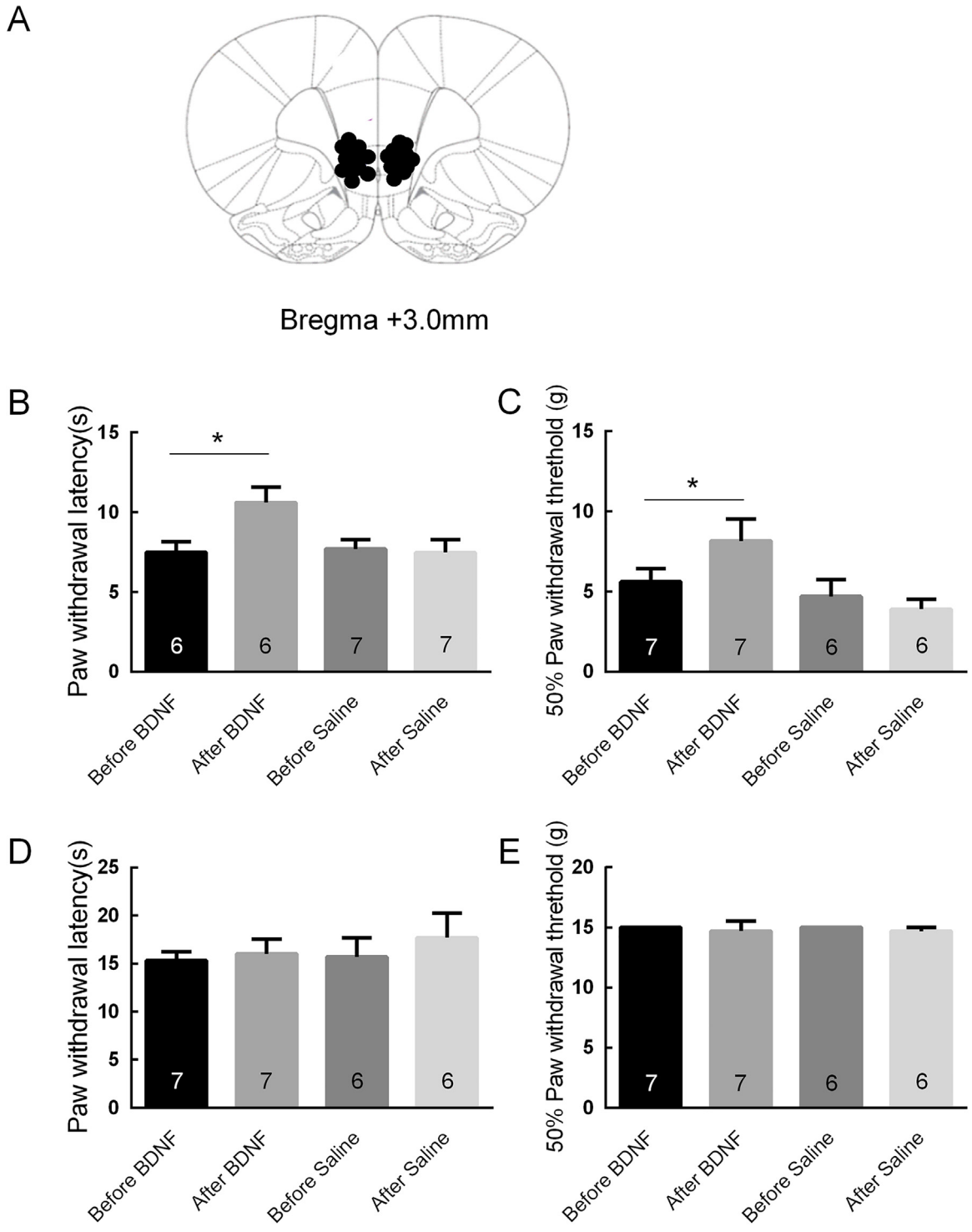

Fig. 2. BDNF infusion into bilateral infralimbic cortices in inflammatory pain rats at day 3 after intraplantar injection of CFA alleviated thermal hyperalgesia and mechanical allodynia (A) Diagrammatic illustration showing BDNF injection sites (filled circles) in bilateral infralimbic cortices. (B-C) BDNF infusion alleviated thermal hyperalgesia (B) and mechanical allodynia (C) in CFA inflammatory pain rats. Compared with saline infusion, single infusion of BDNF into infralimbic cortex increased paw withdrawal latencies (*P = 0.0431, t = 2.694, paired *t*-test) and 50% PWT (*P = 0.0469, t = 2.469, paired *t*-test) in rats with CFA inflammatory pain on CFA 3d. No changes were found after the infusion of saline. (D-E) BDNF infusion did not change the thermal and mechanical pain thresholds in physiological conditions. No obvious changes were observed after the infusion of BDNF or saline in non-CFA rats.

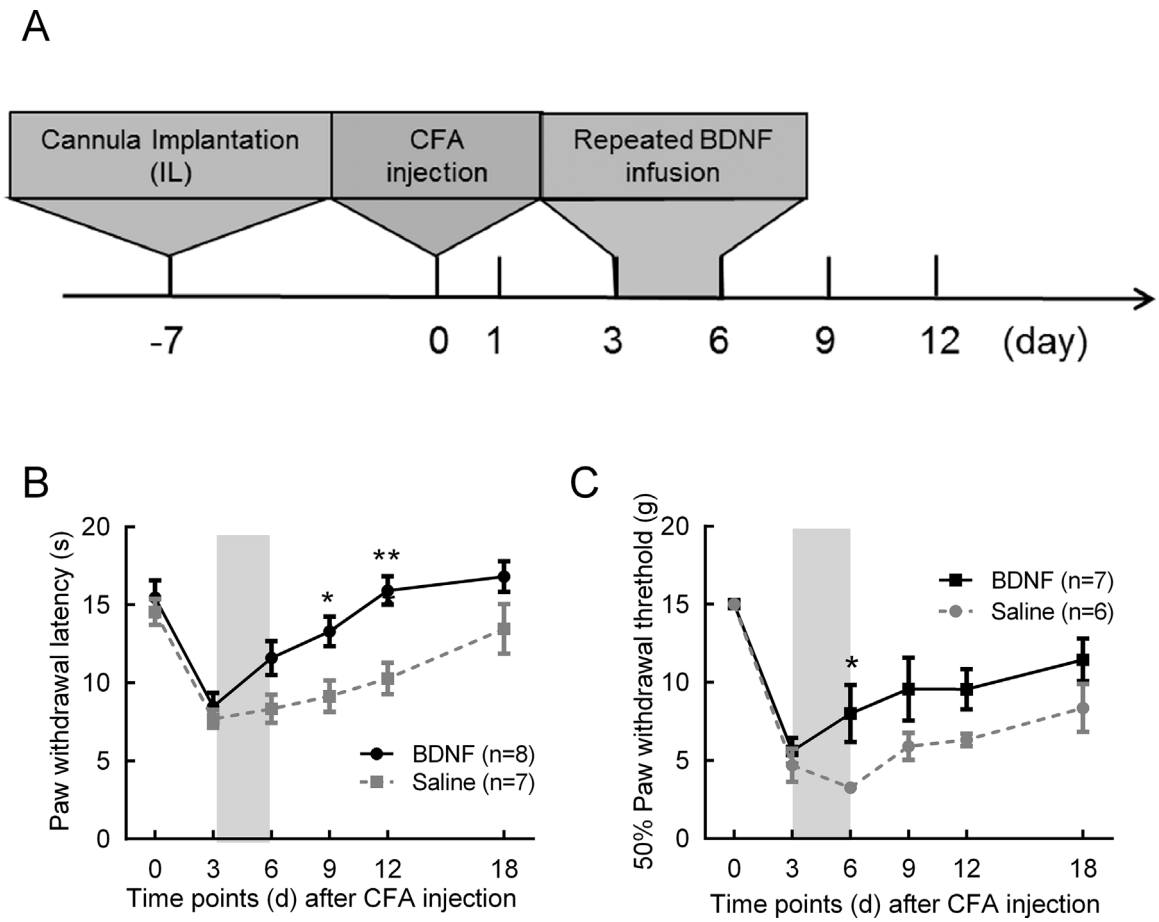

Fig. 3. Consecutive BDNF infusion into infralimbic cortex accelerated long-term recovery from inflammatory pain (A) Schematic experimental design. BDNF was infused into bilateral infralimbic cortices, once per day, from day 3–6 (gray area) after intraplantar injection of CFA. (B) Consecutive BDNF infusion accelerated recovery from inflammatory pain. Rats with consecutive BDNF infusion showed higher paw withdrawal latencies on CFA 12 d and 18 d compared with those rats in the control group with saline infusion. *P < 0.05, two-way ANOVA with Bonferroni *post-hoc* test. Time: P < 0.0001, F (5, 65) = 23.94; Group: P = 0.0066, F (1, 13) = 10.43; Time*Group: P = 0.0394, F (5, 65) = 2.468. (C) Consecutive BDNF infusion accelerated recovery from mechanical allodynia. Rat with consecutive BDNF infusion showed higher 50% PWT on CFA 6d compared with those rats in the control group with saline infusion. *P < 0.05, two-way ANOVA with Bonferroni post-hoc test. Time: P < 0.0001, F (5, 55) = 20.59; Group: P = 0.0143, F (1, 11) = 8.439; time × Group: P = 0.2878, F (5, 55) = 1.275.

3.2. Infralimbic BDNF infusion alleviated thermal hyperalgesia and mechanical allodynia in CFA-induced inflammatory pain rats

The BDNF decrease in the infralimbic cortex indicates the dysfunction of infralimbic cortex. To find whether BDNF in the infralimbic cortex is involved in the CFA-induced inflammatory pain, we infused BDNF protein to bilateral infralimbic cortices via cannula injection. Fig. 2B and C show that BDNF infusion alleviated the CFA-induced thermal hyperalgesia and mechanical allodynia, respectively. After infusion of BDNF into bilateral infralimbic cortices, CFA inflammatory pain rats showed increased paw withdrawal latencies and higher 50% PWTs. As a control, after infusion of saline, inflammatory pain rats showed no obvious changes in either measurements. These results indicate that BDNF infusion could reverse thermal hyperalgesia and mechanical allodynia. The BNDF infusion did not affect thermal and mechanical pain thresholds under physiological conditions (Fig. 2D, E).

3.3. Continuous BDNF infusion in infralimbic cortex accelerated recovery from inflammatory pain

We further tested whether continuous infusion of BDNF in the bilateral infralimbic cortices after CFA injection could promote long-term recovery from inflammatory pain. The experiment design is shown in Fig. 3A. BDNF was infused in the bilateral infralimbic cortices from 3 d to 6 d after CFA injection into the hind-paw, once per day. Paw withdrawal latencies were measured at different time points during inflammatory pain development. As shown in Fig. 3B, it was found that CFA inflammatory pain rats with continuous BDNF infusion showed higher paw withdrawal latencies at 12 d to 18 d after CFA injection than in those rats with saline infusion control. At the same time, mechanical allodynia showed a similar trend of accelerated recovery after BDNF infusion. Higher 50% PWTs were found on CFA 6d, but not significantly different at the later time points compared to the saline control. These results suggest that rats with BDNF infusion into bilateral infralimbic cortices at CFA 3–6 d showed accelerated recovery from thermal and mechanical hyperalgesia.

3.4. BDNF infusion elevated infralimbic neuronal c-Fos expression

To test whether BDNF infusion could activate neuronal activities during its analgesic effects, we examined the local neuronal c-Fos expression after BDNF infusion in the rats on day 3 after CFA injection. Double immunofluorescent staining of c-Fos with NeuN (a marker of neurons) was performed in rats with unilateral infralimbic BDNF infusion and contralateral saline infusion (Fig. 4A). One hour after BDNF infusion, the percentage of neurons expressing c-Fos was elevated compared with that after the contralateral saline

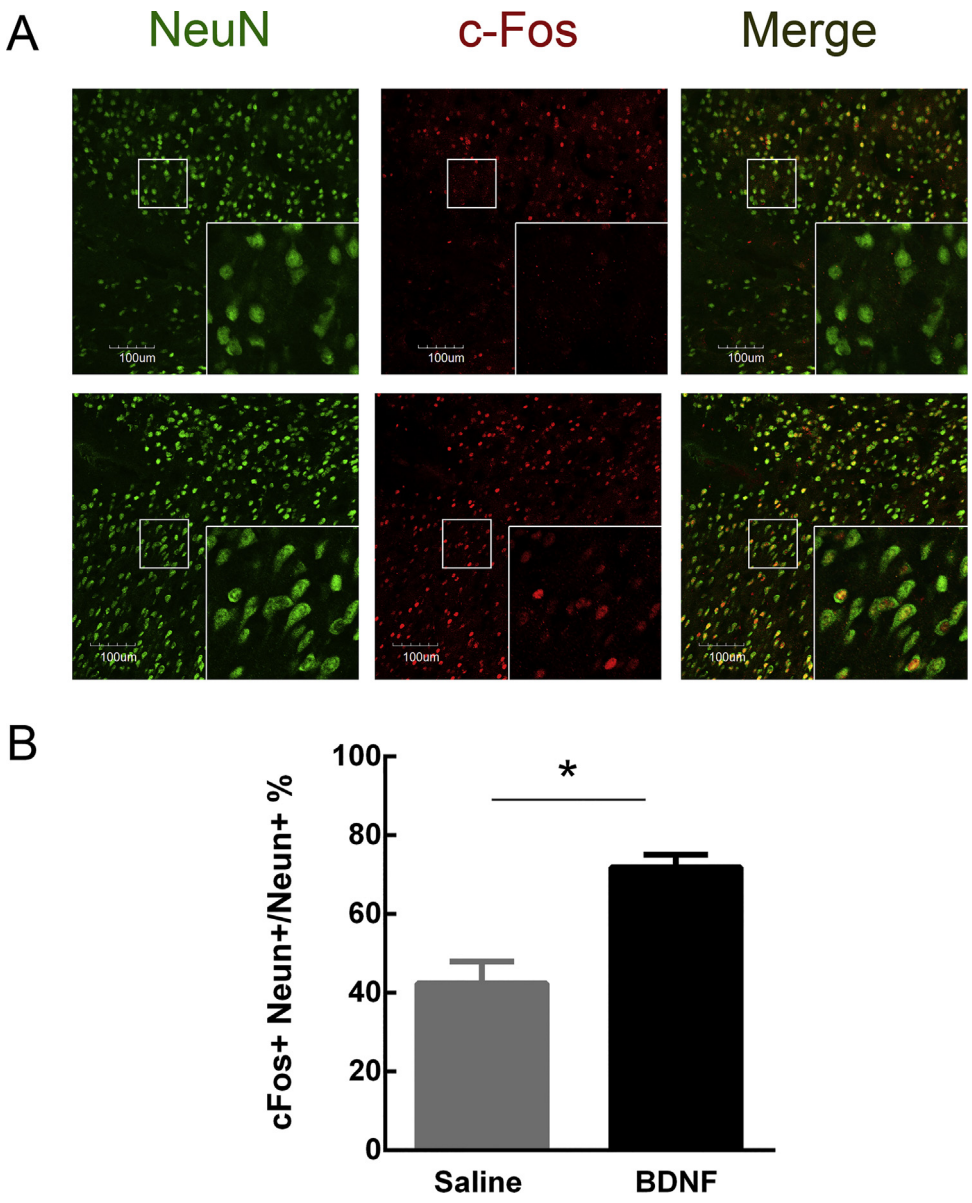

Fig. 4. BDNF infusion into the infralimbic cortex induced neuronal c-Fos expression on rats with CFA injection (A) Immunoflurorescent staining of c-Fos protein in the infralimbic cortex. The left row: NeuN (green); the middle row: c-Fos (red); the right row: Merge. Top: Saline side; Bottom: BDNF side. (B) Percentage of c-Fos positive neurons to total neurons. n = 5, *P = 0.0196, t = 3.769, paired *t*-test.

infusion (Fig. 4B-D). These results suggest that infralimbic BDNF infusion could induce local neuronal activities.

4. Discussion

The mPFC has been reported to encode pain perception, and modulate pain behaviors. Its evoked firings were reported to encode the intensity of a nociceptive stimulus [32], and prefrontal dysfunction was involved in emotional and cognitive deficiency of pain [27]. The underlying molecular mechanism is of great interest. Our results suggest that the BDNF level in the infralimbic cortex decreased during inflammatory injury (Fig. 1), and BDNF supplement could inhibit thermal hyperalgesia, mechanical allodynia, which can thus accelerate a long-term effect of recovery from inflammatory pain (Fig. 2).

Several studies reported the deactivation of the prefrontal cortex during chronic pain. mPFC pyramidal neurons showed decreased intrinsic excitability during arthritic pain and neuropathic pain [12,15]. Moreover, it was reported that the mPFC contains a decreased amount of dendrites and reduced glutamate currents during chronic pain [8]. Our results suggest that the decreased BDNF level might induce the prefrontal synaptic and functional loss, and may also induce medial prefrontal deactivation in chronic pain.

The infralimbic cortex and prelimbic cortex are two regions of the mPFC. Interestingly, BDNF levels in the infralimbic cortex, but not prelimbic cortex, showed a significant decrease. The local BDNF level may be regulated by or originate from different afferents. Several studies have reported that the infralimbic cortex can be innervated by the ventral hippocampus, and that the BDNF level in the infralimbic cortex could be regulated during fear extinction [20,25]. However, there is little projection from the ventral hippocampus to the prelimbic cortex. This may be similar during inflammatory pain.

These distinct changes might reflect the dichotomous function of these two subregions of the mPFC in chronic pain. In fact, the

infralimbic and prelimbic cortices have dichotomous functions in various cognitive and emotional processes such as fear memory, addiction and stress [10,17,22,33]. Chronic pain also induces cognitive and emotional variation. Therefore, it is reasonable to speculate that the infralimbic cortex and the prelimbic cortex perform distinct functions during chronic pain.

BDNF-induced pro-nociceptive effect has been well known in the spinal cord and the dorsal root ganglion (DRG) after nerve injury or peripheral inflammation [31,35]. BDNF levels were increased in both neuropathic and inflammatory pain models, and were involved in neuronal plasticity and pain persistence [38,41]. Several evidence showed that the over-expressed BDNF contributed to the spinal central sensitization and thus facilitated pain [23,36], and blockade of the BDNF signaling induced analgesia [18,26,40]. Unlike its pro-nociceptive effect in the spinal cord or DRG, we found in the present study that BDNF exerted analgesic effects in the infralimbic cortex, as well as a long-term effect of accelerating pain recovery. This difference may reflect the diverse plasticity between the peripheral and the central nervous systems under chronic pain status.

In chronic pain, it has been reported that the BDNF-enhanced synaptic transmission is associated with the activation of NR2 B receptors in the spinal cord [26,37] and in the anterior cingulate cortex [14,24]. In our present study, a possible mechanism underlying the analgesic effects induced by BDNF in the infralimbic cortex also depends on the NR2 B receptor activation.

It has been reported that the infralimbic cortex facilitated nociception through a descending pathway via mGluR5 signaling in normal and arthritic rats, but it exerted analgesic effect through mGluR1 activation in arthritic rats [1]. In our present study, the exogenous BDNF in the infralimbic cortex could alleviate CFA-inflammatory pain, but not physiological pain. So, it seems that the infralimbic BDNF takes its analgesic effects through the mGluR1 activation, rather than the mGluR5-mediated descending facilitation.

We observed that consecutive BDNF infusion into the infralimbic cortex had long-term effects in alleviating pain (Fig. 3). The BDNF induced infralimbic plasticity in inflammatory pain was similar to the memory extinction process. BDNF in hippocampal inputs to the infralimbic cortex was reported to induce fear extinction [20,25]. Infralimbic BDNF also induced an extinct effect from sustainable pain. A similarity of memory formation and pain chronicity has been assumed [4,6,34]. Persistent pain could be regarded as a sustainable memory of initial injury, or the inability of extinction [6]. Our results suggest that the BDNF supplement enhances the ability of extinction from the pain memory.

In summary, in the present study, we found that BDNF decreased in the infralimbic cortex in inflammatory pain rats. BDNF supplement in the infralimbic cortex could alleviate pain and accelerate long-term recovery from pain. In addition, these results may also suggest different roles of the infralimbic cortex and prelimbic cortex in the mPFC in pain processing.

Competing financial interests

The authors declare no competing financial interests.

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