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# Modulating cAMP responsive element binding protein 1 attenuates functional and behavioural deficits in rat model of neuropathic pain

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**Abstract. – OBJECTIVE: Chronic neuropathic pain (NP) has become a worldwide public health problem. This study was aimed to establish graded NP model to investigate the effect of CREB1 on nerve repair and NP after peripheral nerve injury.**

**MATERIALS AND METHODS: Based on NP model, we measured the 50% paw withdrawal threshold (PWT) of rat hind paws and sciatic functional index (SFI). Luxol fast blue staining was performed to measure the ratio of distal myelin sheath to proximal (DPR). The c-Fos, GFAP, CX3CR1 and IBA-1 expressions in spinal cord were measured by Western blot. The expression levels of CREB1 and ATF-3 in dorsal root ganglion (DRG) were both measured. Intrathecal injection was performed by using recombinant CREB, or anti-CREB antibody for NP model, respectively. The above indexes were detected.**

**RESULTS: In NP model, the 50% PWTs and DPR were gradually reduced and SFI was increased. The c-Fos, GFAP, CX3CR1 and IBA-1 expressions were increased compared to control group. The CREB1 and ATF-3 expressions in DRG showed gradually increase. With the injection of recombinant CREB, the similar changes were found in rats compared with NP model. While after anti-CREB1 antibody injection, all effects of CREB1 were impaired. Likewise, anti-CREB1 antibody treatment increased 50% PWT and DPR, decreased SFI, decreased expressions of c-Fos, GFAP, CX3CR1 and IBA-1. Besides, ATF-3 expression was inhibited by CREB1 suppression.**

**CONCLUSIONS: CREB1 involved in the regulation of NP and nerve repair process, suggesting that CREB1 has potential as a new target for the treatment of chronic NP.**

Key Words

Nerve injury, Neuropathic pain, Nerve regeneration, Astrocyte, CREB1.

### Abbreviations

NP, neuropathic pain; PWT, paw withdrawal threshold; SFI, sciatic functional index; GFAP, glial fibrillary acidic protein; CX3CR1, CX3C chemokine receptor 1; IBA-1, ionized calcium binding adaptor molecule-1; ATF-3, activating transcription factor 3; DRG, dorsal root ganglion; IASP, International association for the study of pain; CREB, cAMP-response element binding; CCI, chronic constriction injury.

## Introduction

Chronic pain has become a serious problem in people's lives due to the great economic costs[1]. This situation reflects that there are still some limitations on the treatment of chronic pain. Neuropathic pain (NP) and inflammatory pain are two of the main kinds of chronic pain. Inflammatory pain often can be recovered after tissue damage or inflammation which has been controlled. In contrast, NP may persist after patients recovered from other disease, and it is hard to cure[2]. International Association for the Study of Pain (IASP) named the pain caused by a lesion or disease of the somatosensory nervous system as NP according to Classification of Chronic Pain, Second Edition (Revised). There were about 10-15% of patients undergoing surgery such as mastectomy, amputation, heart bypass surgery and etc., who might have chronic NP due to neurological injury caused by surgery[3]. After peripheral nerve injury, nerve regeneration and NP often coexist. The interaction of neurons, glial cells and immune cells are involved in regulating both nerve regeneration and NP process, as well as many cytokines[4]. Therefore, in treatment of NP, it should also be considered the impact on nerve repair and regeneration.

The cAMP-response element binding protein (CREB) is a nuclear transcription factor and is the downstream factor of the extracellular signal-regulated kinase ERK1/2[5]. CREB regulates many genes transcriptions including c-Fos, BDNF, tyrosine hydroxylase, numerous neuropeptides (such as somatostatin, enkephalin, VGF, and corticotropin-re-



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leasing hormone)[6], and genes involved in the mammalian circadian clock (PER1 and PER2)[7]. CREB proteins in neurons are thought to regulate learning and long-term memories[8]. It modulates several signaling pathways concerned with neuronal plasticity[9]. CREB is also important for the survival of neurons[10]. REB is up-regulated in DRG cells following nerve injury[11] and some studies have implicated CREB in nerve repair and structural plasticity[12,13]. In addition, CREB-dependent gene expression is found to contribute to NP in rats suffered with chronic constriction injury (CCI)[14]. Considering these multiple roles, CREB may have potential on modulating nerve regeneration and NP.

In this study, we established a graded NP rat model according to the CCI of the sciatic nerve model, and evaluated the degree of sciatic nerve injury and sensitization of the central nervous system in the spinal cord of NP model. Also, expressions of activating transcription factor 3 (ATF-3) and CREB1 in dorsal root ganglion (DRG) were detected. In the next drug intervention trial, the effects of DREB1 on the activation of DRG neurons, peripheral nerve repair and pathologic NP were observed by injection of recombined CREB1 or anti-CREB1 antibodies. This study might provide a new CREB strategy in neural repair and NP treatment.

## Materials and Methods

### Construction of Graded Chronic NP Animal Model

Adult male Sprague-Dawley (SD) rats (weighted 250-350 g) were purchased from Experimental Animal Center of Zhejiang Chinese Medical University and housed in constant temperature (about 22°C), humidity (about 60%), 12/12 h light/dark cycling. Rats were free to get water and rodent chow. All the experiments were approved by Local Animal Care and Users Committee. The Specific Pathogen-Free (SPF) nature of the rats was demonstrated by sentinel animals testing. All efforts were made to minimize the number and suffering of rats involved in our study. All the surgical operations were conducted by the same person.

The CCI of the sciatic nerve model was used to study NP following the method described in experiments about rats[15]. The rats were randomly allocated into five groups namely N0, N1, N2, N4 and control group (n=9 per group). The randomization was performed using sealed envelopes and the randomization code was only known to the chamber operator. All researchers were blinded to the treatment. After anesthesia with 40-50 mg/kg sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA), rats in group N0, N1, N2 and N4 received 0, 1, 2 and 4 loose ligations with 1 mm spacing on the trunk of the right sciatic nerve with 4-0 chromic gut, respectively. The control group was not taken this surgical treatment.

### Detection of 50% Paw Withdrawal Threshold (PWT)

Mechanical allodynia was measured by using a series of calibrated von Frey filaments (Stoelting, Bioseb, France), which range in bending force from 0.008 to 300 gr (typical force range used in rat testing is 0.4-25 g). Control, N0, N1, N2 and N4 groups were assessed before operation or on the 3rd, 7th, 10th and 14th day postoperatively. Each rat was tested five times on right hind paw. The time interval between consecutive filament administrations was 2-3 minutes. The 50% PWT was calculated using the up-down iterative method.

### Assessment of Sciatic Functional Index (SFI)

SFI was detected according to a method described by Muthuraman et al[16] and Arruri et al[17]. Rat hind paws were dipped in Indian ink and the rats were free to walk in the enclosed walking track. After that, the following measurements were conducted from the footprints of the rat, (I) distance from the heel to the third toe, the print length (PL); (II) distance from the first to the fifth toe, the toe spread (TS); and (III) distance from the second to the fourth toe, the intermediary toe spread (ITS). All three measurements were taken from the operation side (O) and normal (N) side. The factors were calculated as follows: (I) Print length factor (PLF) = (OPL - NPL)/NPL; (ii) Toe spread factor (TSF) = (OTS – NTS)/NTS; (iii) Intermediary toe spread factor (ITF) = (OIT – NIT)/NIT. These factors were then incorporated into the Bain-Mackinnon-Hunter (BMH) formula: SFI = -38.3 × PLF + 109.5 × TSF + 13.3 × ITF – 8.8. A SFI value of 0 indicates the normal foot and a SFI value of 100 indicates the total impairment, which indicates a complete transection of the sciatic nerve.

### Detection of Ratios of Distal Myelin Sheath to Proximal (D/P ratio, DPR)

On the 15th day after operation, a total of 15 rats in each NP model group were sacrificed (n=3), and the right sciatic nerve was taken out to make transverse slicing and strained with Luxol fast blue (LFB) to count the ratios of Don the nerve cross section.

### Detection of Expressions of c-Fos, GFAP, CX3CR1 and IBA-1

The lumbar expanded part of 15 rats from NP model groups (n=3) was used to test the c-Fos, glial fibrillary acidic protein (GFAP) expression in the superficial laminae of spinal cord L3-L6 by Western blotting. Expressions of CX3CR1 and IBA-1 were also detected by Western blotting.

### Detection of Neuronal Activity in Graded Chronic NP Animal Model

Besides, the DRG, taken from lumbar spinal cord levels L3-L6 from NP model group rats (n=3), was fixed with 4% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) to make frozen sections. The ratio of ATF-3 positive neurons in DRG was investigated by immunofluorescent double staining (ATF/NeuN). All the tests were conducted in blind by a separate experimenter. The expression of CREB1 in DRG neurons from lumbar levels L3-L6 of rats (n=3) was detected by Western blotting.

### The Effect of CREB1 on NP and DRG Neuronal Activation

Normal adult male Sprague Dawley (SD) rats were used to construct intrathecal catheterization model according to the standard method[18]. Five days later, a total of 48 rats with intrathecal catheter and no nerve damage symptoms were selected. Twelve of them were randomly divided into four groups, and single intrathecal injected with 0, 0.1, 1 or 100 ng recombined human CREB1 (Creative BioMart, Shirley, NY, USA), respectively (n=3). The von Frey filaments were used to measure the changes of 50% PWT at 6 h, 1 d, 4 d, and 7 d after injection, respectively. The rats were sacrificed on the 7th day after injection to analyze the expression changes of c-Fos, GFAP, CX3CR1 and IBA-1 in spinal dorsal horn of rat lumbar spinal cord levels L3-L6. According to the injury response of rats in NP model, N2 model was selected as the appropriate condition for next experiments. A total of 36 rats were randomly divided into four groups (n=9), namely control without injection (Control); anti-CREB1 injection (Control + anti-CREB1); N2 of NP model (N2); N2 model with anti-CREB1 injection (N2 + anti-CREB1). Rats of groups Control + anti-CREB1 and N2 + anti-CREB1 were intrathecal injected with 5 μg CREB1 antibody (sc240, Santa Cruz Biotechnology, Santa Cruz, CA, USA) every day and IgG was used as the control antibody. Next, 50% PWT and SFI of each group was measured (a total of 12 rats, n=3). On the 14th day after corresponding treatment, DPR of sciatic nerve on the surgery side was determined by LFB straining method as previously mentioned (a total of 12 rats, n=3). Ipsilateral dorsal rat spinal cords L3-L6 were used to measure the c-Fos, GFAP, CX3CR1 and IBA-1 levels and the ratio of ATF-3 positive neurons in DRG was determined by immunofluorescent (a total of 12 rats, n=3).

### Western Blot Assay

c-FOS, GFAP, CX3CR1 and IBA-1 levels in spinal dorsal horn of rat lumbar spinal cord levels L3-L6 and CREB1 level in DRG were analyzed by Western blot. The tissues samples were lysed in Radio Immunoprecipitation Assay (RIPA) Lysis buffer (Beyotime, Shanghai, China) and the protein concentrations were measured by BCA protein assay kit (Pierce, Appleton, WI, USA). Approximately 20 μg protein was subjected to 12% sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE), and the electrophoresis and immunoblotting procedures were performed according to the previous report[19]. The membranes carrying blots were incubated overnight at 4°C with primary antibodies (1:1,000): c-FOS (ab209794); GFAP (ab7260), CX3CR1 (ab8021), IBA-1 (ab15690), CREB1 (ab7540), GAPDH (ab8245) (all from Abcam, Cambridge, MA, USA). The rinsed membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The blots were visualized by using PowerOpti-ECL (BioNote, Inc., Korean) detection system according to the manufacturer's instructions and analyzed by Image LabTM Software (Bio-Rad, Hercules, CA, USA).

### Immunohistochemistry Assay

The ratio of ATF-3 positive neurons in DRG was determined by immunofluorescent double staining (ATF-3/NeuN). ATF-3 immunostaining was performed as described[20]. Briefly, 4 mm segment of lesion-dorsal spinal cords were processed as above, and serial sections were cut with 10 μm thickness and collected on coverslips, blocked with 5% goat serum for 20 min at room temperature, followed by primary antibody incubation. The primary antibodies were rabbit anti-rat ATF-3 (ab216569, 1:200, Abcam, Cambridge, MA, USA) and mouse anti-rat NeuN (ab104224, 1:200, Abcam, Cambridge, MA, USA). Secondary antibodies were CY3-conjugated goat anti-rabbit

IgG (1:1000, Beyotime, Shanghai, China) and FITC-conjugated goat anti-mouse IgG (1:1000, Beyotime, Shanghai, China). Fluorescence images were captured using an epifluorescence microscope (Nikon 80i, Nikon, Tokyo, Japan). Sections were 5 random sagittal sections through central canal in each rat (n=3).

### Statistical Analysis

Data were presented as mean ± standard deviation (SD) and analyzed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). The *p*-values were calculated using Student's *t*-test for pairwise comparisons and a two-way analysis of variance (ANOVA) followed by Tukey post-hoc test for multi-group comparisons. Statistical significance was defined as $p < 0.05$.

## Results

### Establishment of the Graded NP Model in SD Rats

As shown in Figure 1A, 50% PWT values of the right hind foot were dropped dramatically in N1, N2 and N4 groups compared to control group on the 7th and 14th days (all $p < 0.001$). While there were no significantly changes between control and N0 groups before or after operation. During the whole period, 50% PTW values presented a grade manifestation as N1 > N2 > N4. SFI reveals the function of sciatic nerve after CCI injury. As displayed in Figure 1B, SFI was significantly decreased after CCI treatment in N1, N2, and N4 models (all $p < 0.001$). Also, compared to control group, the DRP values were significantly

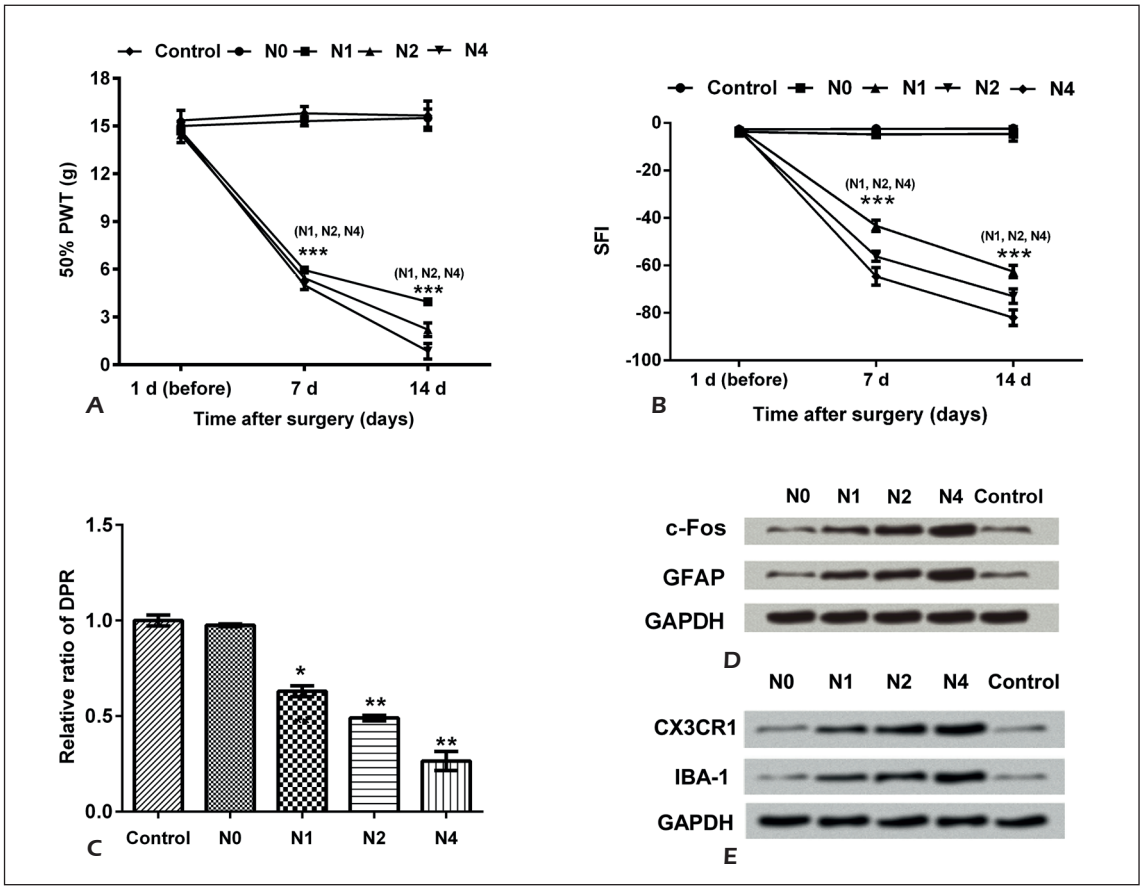


**Figure 1.** The assessment results of the graded NP model. **A**, The 50% PWT of NP model rats were measures by up-and-down test using von-Frey filaments. **B**, The level of SFI was detected for analyzing the sciatic functional loss. **C**, The DPR in the sciatic nerve cross section was tested by counting the myelin sheaths. **D**, The expressions of c-Fos and GFAP as well as **E**, expressions of CX3CR1 and IBA-1 in the superficial laminae of spinal dorsal horn of NP model were analyzed by Western blot. GAPDH acted as an internal control. NP, neuropathic pain; PWT, paw withdrawal threshold; SFI, sciatic functional index; DPR, distal myelin sheath to proximal (D/P) ratio; GFAP, glial fibrillary acidic protein; CX3CR1, CX3C chemokine receptor 1; IBA-1, ionized calcium binding adaptor molecule-1; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

reduced in N1, N2 and N4 groups ($p < 0.05$ or $p < 0.01$), presenting a graded manifestation as N1 > N2 > N4 (Figure 1C). Western blot analysis revealed the significant increase of c-Fos, GFAP, CX3CR1 and IBA-1 expressions in the lumbar enlargement of spinal cord L3-L6 on the 15th day after operation (Figure 1D and E). Results indicate that CCI induced functional and behavioral changes of sciatic nerve, and might affect activations of microglia and astrocytes.

### CREB1 and Positive ATF-3 Expression Were Increased in the Lumbar DRG of the Graded NP Model

CREB1 expressions of N1, N2, and N4 groups were significantly up-regulated in the lumbar DRG neurons in graded NP model as shown in Figure 2A. In Figure 2B, the rate of ATF-3 positive neurons in ipsilateral L3-L6 DRGs showed significant increase as N0 < N1 < N2 < N4 ($p < 0.05$, $p < 0.01$ or $p < 0.001$). It suggested that ATF-3 was up-regulated in DRG neurons of spinal cord L3-L6 following injury.

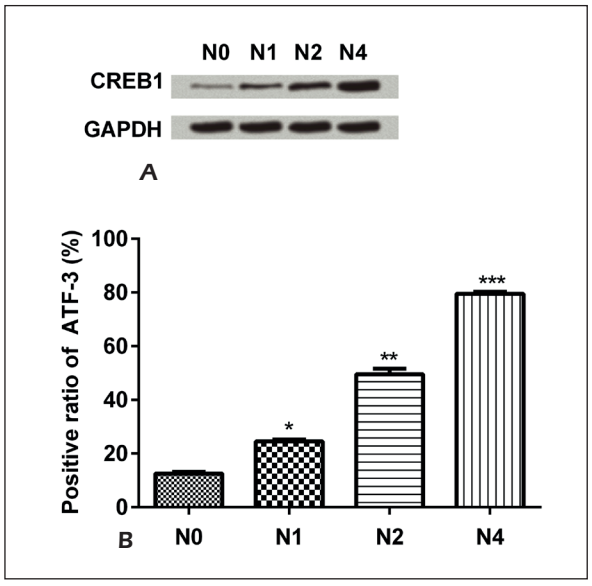


**Figure 2.** The CREB1 and ATF-3 expressions were up-regulated in the DRG neurons of the graded NP model. (A) Protein immunoreaction of CREB1 in DRG of NP model. GAPDH acted as an internal control. (B) The positive ATF-3 in DRG was counted after immunofluorescent double staining. CREB1, cAMP responsive element binding protein 1; ATF-3, activating transcription factor 3; DRG, dorsal root ganglion; NP, neuropathic pain; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

### CREB1 was Involved in the Development of NP in Sciatic Nerve

A previous study[21] has reported that NP induced by peripheral nerve injury could be modulated at least partly by CREB. Thus, herein we examined signs of mechanical allodynia in recombined CREB1-injected rats. After rats were intrathecal injected with deferent doses of recombined CREB1, the 50% PWT of the right hind foot was dramatically dropped at 6 h, 1 d, 4 d and 7 d (all $p < 0.001$, Figure 3A). The expression levels of c-Fos

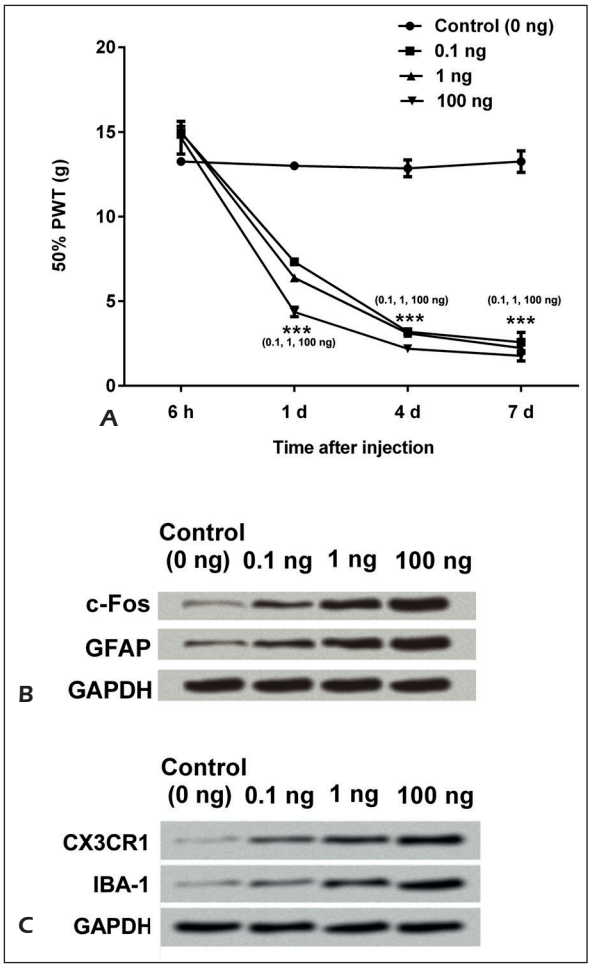


**Figure 3.** Effect of recombined CREB1 injection with different concentrations on NP of SD rats. **A**, The 50% PWT of SD rats injected with 0, 0.1, 1, or 100 ng recombined CREB1 was measures by up-and-down test using von-Frey filaments. **B**, The expressions of c-Fos and GFAP as well as (**C**) expressions of CX3CR1 and IBA-1 in the superficial laminae of spinal dorsal horn in CREB1 injected SD rats were analyzed by Western blot. GAPDH acted as an internal control. CREB1, cAMP responsive element binding protein 1; PWT, paw withdrawal threshold; GFAP, glial fibrillary acidic protein; CX3CR1, CX3C chemokine receptor 1; IBA-1, ionized calcium binding adaptor molecule-1; ***, $p<0.001$.

and GFAP were both upregulated after CREB1 injection and gradually enhanced with increased concentration of CREB1 injection (Figure 3B). Similar changes were observed on the expressions of CX3CR1 and IBA-1 (Figure 3C). It suggested that intrathecal injection of deferent doses of CREB1 evoked significant allodynia in healthy rats and caused overexpression of c-FOS, GFAP, CX3CR1 and IBA-1 in the lumbar spinal cord.

### *Effect of CREB1 Suppression on Development of NP and Nerve Regeneration After Sciatic Constriction*

Injection of recombined CREB1 induced decrease of 50% PWT, whereas the effect was inhibited by anti-CREB1 antibody injection (Figure 4A, B, and C). GFAP and c-FOS expressions were enhanced by CREB1 but were then suppressed by anti-CREB1 treatment (Figure 4D). These data indicate that decreasing CREB1 expression inhibited CREB1-induced effect on sciatic nerve. Thus, we speculated that decreasing CREB1 could suppress NP, since CREB1 was involved in NP in sciatic nerve. As shown in Figure 5A and B, compared with N2 model group, the 50% PWT value was significantly increased and SFI was significantly decreased after injection of anti-CREB1 for 1 d, 4 d, and 7 d (all $p < 0.001$) even not as much as control. And the DPR value in N2 + anti-CREB1 group was significantly increased compared to N2 model rats without anti-CREB1 injection ($p < 0.05$, Figure 5C). These results suggest that CREB1 plays important roles for recovery of sciatic nerve function after injury.

We also found that the c-Fos, GFAP, CX3CR1 and IBA-1 expression levels in the lumbar DRG neurons of N2 model was higher than those in control group, while this promoting effect was reversed by

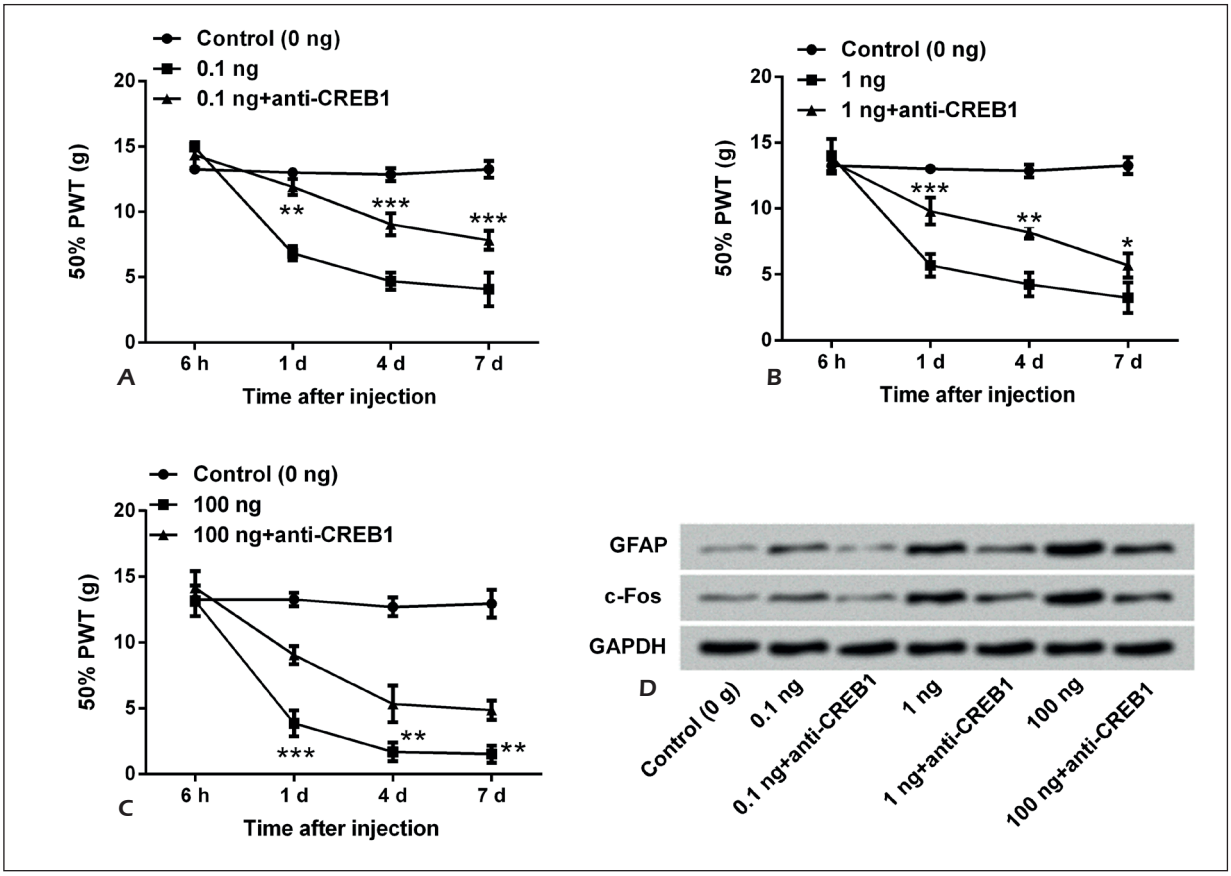


**Figure 4.** Effect of anti-CREB1 injection on CREB1-induced sciatic nerve injury of SD rats. The 50% PWT of SD rats injected with (**A**) 0.1 ng recombined CREB1 and anti-CREB1 antibody, (**B**) 1 ng recombined CREB1 and anti-CREB1 antibody, as well as (**C**) 100 ng recombined CREB1 and anti-CREB1 antibody was measured by up-and-down test using von-Frey filaments. (**D**) The expressions of c-Fos and GFAP in the superficial laminae of spinal dorsal horn in different concentrations of CREB1 and anti-CREB1 antibody co-injected SD rats were analyzed by Western blot. GAPDH acted as an internal control. CREB1, cAMP responsive element binding protein 1; PWT, paw withdrawal threshold; GFAP, glial fibrillary acidic protein; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

anti-CREB1 injection (Figure 5D and E). The ATF-3 expression in DRG neurons was also inhibited by anti-CREB1 injection in N2 model rats ($p < 0.05$, Figure 5F). As it was increased in N2 model, whereas it was decreased after anti-CREB1 injection. Together, these data indicate that CREB1 is important in the development of NP and the suppression of CREB1 has potentials on the recovery of sciatic nerve function after peripheral nerve injury.

## Discussion

As the pathogenesis of NP is not clear due to the etiology and clinical manifestations of the diversity, the current treatment and prevention for NP are still limited[22]. A variety of animal models have been used to study the mechanism of NP caused by different reasons, such as trauma, infection, tumor or autoimmune diseases, in which the NP model caused by peripheral nerve injury is the most widely studied peripheral neuropathy[23]. Commonly used models are CCI, partial sciatic nerve ligation (PSNL), spinal nerve ligation (SNL), spared nerve injury (SNI) etc.[24]. However, these animal models can only respond to pain or not rather than grading, that affect the next study about mechanism of pain and drug intervention[25]. Based on the CCI model, a hierarchical biblical disease model has been established by Grace et al[26], which could reveal correlation between pain behavioral changes and the activation state of glial cells in dorsal horn of spinal cord.

In this study, we constructed a NP animal model in SD rats. We found that in N1, N2, N4 groups, the 50% PET of the hind paw in surgical side after surgery showed varying degrees of decline and SFI showed different degrees of rise, indicating

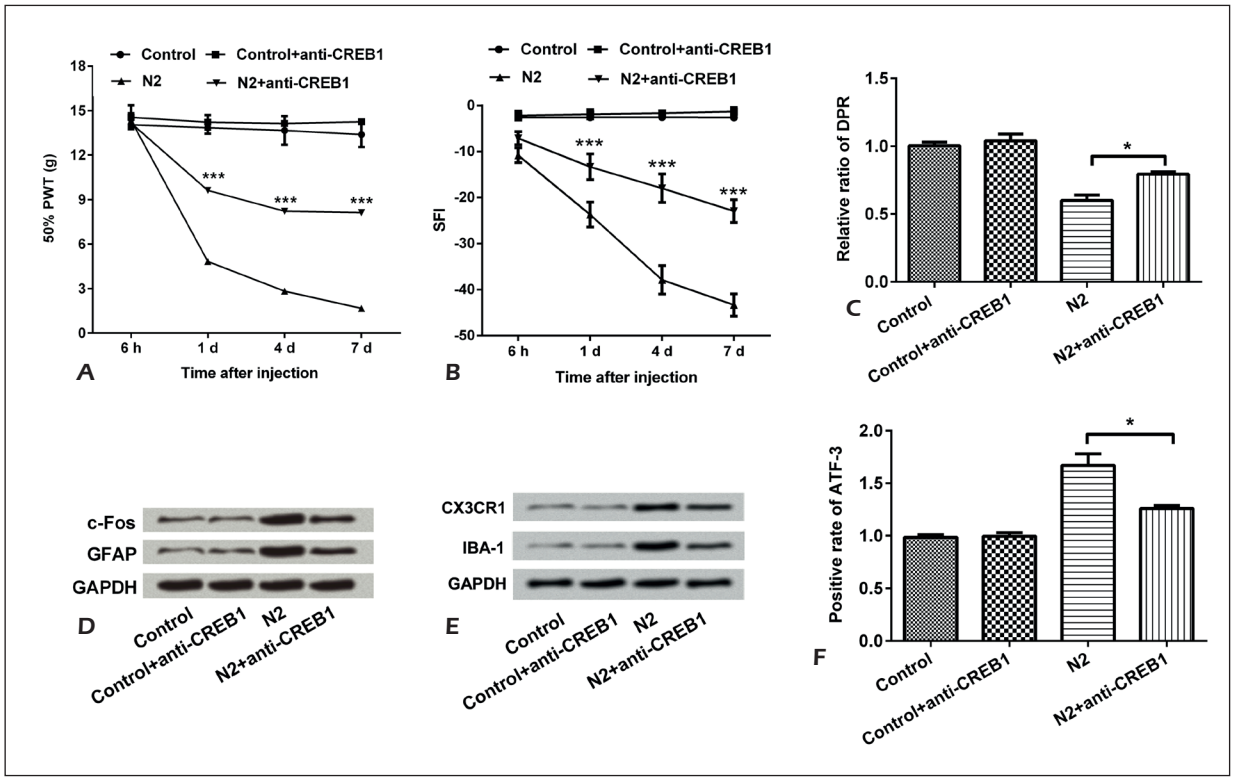


**Figure 5.** Effect of anti-CREB1 injection on NP model. SD rats and N2 model rats (received 2 loose ligations) were injected with anti-CREB1, respectively. **A**, The 50% PWT of rats injected with anti-CREB1 were measured by up-and-down test using von-Frey filaments. **B**, The level of SFI was detected for analyzing the sciatic functional loss. **C**, The DPR in the sciatic nerve cross section of rats was tested by counting the myelin sheaths. **D**, The expressions of c-Fos and GFAP as well as (**E**) expressions of CX3CR1 and IBA-1 in the superficial laminae of spinal dorsal horn of anti-CREB1 injected rats were analyzed by Western blot. GAPDH acted as an internal control. **F**, The ATF-3 in DRG of rats was counted after immunofluorescent double staining (ATF/NeuN). CREB1, cAMP responsive element binding protein 1; NP, neuropathic pain; PWT, paw withdrawal threshold; SFI, sciatic functional index; DPR, distal myelin sheath to proximal (D/P) ratio; GFAP, glial fibrillary acidic protein; CX3CR1, CX3C chemokine receptor 1; IBA-1, ionized calcium binding adaptor molecule-1; ATF-3, activating transcription factor 3; DRG, dorsal root ganglion; *, $p<0.05$; ***, $p<0.001$.

that different numbers of sciatic nerve ligation could cause varying degrees of pain behaviors and also produced different effects on nerve function. However, no significant pain sensory responses were observed in the N0 group with no sciatic nerve ligation. In most previous investigations, chromic catguts were placed next to the nerve stem to cause significant pain, while in this study, it was placed in the subcutaneous tissue around the incision that could cause different level of pain[27]. Previous research regarding the CCI model found fibers demyelination phenomenon in the distal myelin sheath of the spinal cord cerclage[28]. In this work, we found the variation trend of DPR value was coincided with 50% PWT, indicating that the different degrees of distal nerve demyelinating lesions might be related with sciatic nerve narrowing. Current studies suggest that sensitization of the central nervous system plays an important role in the development and progression of chronic NP, whereas nerve cell and astrocyte are the hallmark of central nervous sensitization[29,30]. In this study, the expressions of c-Fos and GFAP in NP model were increased, possibly indicating different activation degrees of spinal cord central neurons or astrocytes in this model, further confirming the establishment of grading NP model. Additionally, the expressions of CX3CR1 and IBA-1 were elevated in rats suffered with NP, possibly indicating the activation of microglia induced by NP. CREB has a well-documented role in neuronal plasticity and long-term memory formation in the brain. It has been shown that there are activator and repressor forms of CREB in the formation of spatial memory science[31]. CREB1, a kind of CREB protein, regulates gene transcription and is also involved in the exchange of neuronal cells. Peripheral nerve injury triggers a series of reactions within the body to repair nerve. In this work, we found the increased expression of CREB1 in DRG of NP model. And expression of ATF-3 was also enhanced, which is used as a marker for DRG activation in recent years[32]. These increased expression levels corresponded to the degree of nerve injury and pain sensitivity, indicated that the NP model could initiate different degrees of neural repair process, which is closely related to the development of pathologic pain. We found that intrathecal injection of different concentrations of recombinant CREB1 could trigger persistent pain sensitivity and activation of astrocyte and microglia. However, NP was significantly alleviated and the astrocyte activity was inhibited by injection of anti-CREB1 antibody compared with the NP model group. These results descripted that CREB1 might be one of the key regulatory factors in inducing NP. The sustained activation of astrocytes and microglia is the reason for central nervous sensitization and chronic pain[33,34]. In recent years, researchers believed that cytokines could regulate protein synthesis through MAPK, PI3K or some else signaling pathways, which will further impact the sensitization of neurons[35]. Therefore, after injury, the neurons and glial cells in the spinal cord interact with each other and activated. In this investigation, CREB1 antibody injection significantly inhibited this activity, indicating that CREB1 might be one of the key regulatory factors in inducing astrocyte and microglia activation; moreover, blocking CREB1 might alleviate NP. ATF-3 is a key factor to maintain neuron survival and promote nerve regeneration after peripheral nerve injury[32,36] In recent years, the expression of ATF-3 has been found to be significantly increased after nerve injury[37]. In this study, we found ATF-3 expression was significantly increased in the NP model, and after injection of CREB1 antibody, the expression of ATF-3 in DRG neurons was significantly reduced, suggesting that CREB1 played an important role in the activation of DRGs after nerve injury. However, the action mechanism of CREB1 in the expression of ATF-3 needed to be further studied in the cellular and molecular levels.

## Conclusions

We established a graded NP rat model according to the CCI model, and found that the expression of CREB1 was increased and correlated with the degree of pain. Blocking the action of CREB1 improved pain and affected the activation of DRG neurons. These results suggested that CREB1 might be a new strategy of chronic NP therapy and provide a new direction for clinical treatment.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

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