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Experimental Trial

Trigeminal Neuralgia Induced by Cobra Venom Leads to Cognitive Deficits Associated with Downregulation of CREB/BDNF Pathway

Li Zhang, MD[1], Xinli Ding, PhD[3], Zhe Wu, MD[2], Xiaoyan Qian, CRNA[2] , Jianxiong An, PhD[2], and Ming Tian, PhD[1]

From: [1]Department of Anesthesiology, Beijing Friendship Hospital, Capital Medical University, No.95, Yong'an Road, Xicheng District, 100050 Beijing, China; [2]Department of Anesthesiology, Pain Medicine & Critical Care Medicine, Aviation General Hospital of China Medical University, No.3, Beiyuan Road, 100012 Beijing, China; [3]Department of Rehabilitation Medicine, Shandong Provincial Hospital Affiliated to Shandong University, No.324, Jingwu Road, Jinan, Shandong Province, 250021 Jinan, China

Address Correspondence: Jianxiong An, PhD Department of Anesthesiology, Pain Medicine & Critical Care Medicine, Aviation General Hospital of China Medical University, No.3, Beiyuan Road, 100012 Beijing, China E-mail: anjianxiong@yahoo.com

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Background: Chronic pain often results in cognitive impairment. Our previous study showed that trigeminal neuralgia induced by cobra venom leads to spatial learning and memory deficits, although the underlying mechanism remains unclear. However, recent evidence indicates that the c-AMP-responsive element binding protein (CREB)/brain derived neurotrophic factor (BDNF) pathway plays a critical role in various etiologies of cognitive deficits.

Objectives: Our aim was to explore the CREB/BDNF pathway to determine the molecular mechanisms involved in the pathogenesis of cognitive impairment caused by cobra venom-induced trigeminal neuralgia.

Study Design: A randomized, controlled animal study.

Setting: Department of Anesthesiology, Beijing Friendship Hospital, Capital Medical University.

Methods: Fifty male Sprague–Dawley rats were randomly divided into 3 groups: cobra venom group, sham group, and control group. Cobra venom or saline was injected into the sheath of the infraorbital nerve (ION), respectively. Video recordings and mechanical thresholds were used to analyze changes in behavioral activity 3 days before surgery and 4, 7, 14, 21, 28, and 56 days after surgery. Morris water maze tests were conducted at 4- and 8-week time points after surgery to evaluate spatial learning and memory. We also investigated expression changes of phosphorylated CREB (p-CREB) and BDNF in the hippocampus and prefrontal cortex (PFC) using western blotting and immunohistochemistry.

Results: Cobra venom-treated rats exhibited significant changes in face grooming, as well as exploratory and resting behaviors, compared with the control group and sham group (both $P < 0.001$). Rats in the cobra venom group exhibited slightly impaired acquisition ($P < 0.05$) without memory deficits ($P > 0.05$) in the first water maze protocol. In the second water maze test, rats in the cobra venom group exhibited spatial learning and memory deficits, with fewer platform site crossings during the probe trial ($P < 0.05$). Moreover, results showed decreased p-CREB and BDNF expressions in the hippocampus and PFC in the cobra venom group, with significant differences at 9 weeks post-surgery ($P < 0.05$).

Limitations: No signaling inhibitor or genetic manipulation was administered to further confirm upstream factors of the CREB/BDNF pathway in cognitive deficits caused by chronic trigeminal neuralgia.

Conclusions: The findings suggest that cognitive impairment caused by cobra venom-induced trigeminal neuralgia is associated with downregulation of the CREB/BDNF pathway in the hippocampus and PFC.

Key words: Cognitive impairment, the CREB/BDNF pathway, cobra venom, trigeminal neuralgia, hippocampus, prefrontal cortex, free behavior, Morris water maze

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Cognitive impairment in chronic pain has become a significant medical problem (1). Chronic pain patients often complain of memory deterioration that accompanies other alterations, such as attention deficit and faulty decision-making, which diminishes their already compromised quality of life (2). Although several studies have proposed anatomical (3), neurochemical (4), and psychological causes (5) to be the basis of cognitive deficits, the exact mechanisms underlying the cognitive deficits in chronic pain remain to be elucidated. More clinical and pre-clinical investigations should be promoted.

To mimic persistent pain manifestations in humans, our research group developed a new rat model of trigeminal pain induced by administration of cobra venom to the infraorbital nerve (ION) (6), and indicated that trigeminal neuralgia impairs spatial learning and memory function using Morris water maze (MWM) (7). But, brain regions which are responsible for cognitive deficits have not be fully defined. Recent research indicates that the hippocampus is particularly relevant to cognitive impairment in animal models of neuropathic pain, such as L5 spinal nerve transection (8) and spared nerve injury (9). Moreover, in a persistent inflammatory pain model, the prefrontal cortex (PFC) has been shown to play a major role in spatial cognition and memory (10). Furthermore, reduced hippocampus-prefrontal cortex connectivity has been revealed to account for impaired spatial memory performance in a rat model of peripheral neuropathic pain (11). As is well known, the hippocampus is responsible for rapid acquisition of new memories and retrieval of stored memories during the spatial learning and memory task (12), and PFC, which is reciprocally connected to the hippocampus, contributes to memory retrieval and long-term memory storage (13). However, whether the hippocampus and PFC are associated with cognitive deficits in a rat model of trigeminal neuralgia induced by cobra venom remains unknown. Given that the hippocampus and PFC play a critical role in learning and memory (8,9,10,11), the present study adopted this new model to further analyze the underlying molecular mechanisms of chronic trigeminal neuralgia involved in learning and memory, especially in the hippocampus and PFC.

A growing body of evidence has shown that various etiologies of cognitive deficits are associated with synaptic plasticity change (14), decreased neurogenesis (15), and reduced production of neurotrophic factors (16) in the central nervous system (CNS). The c-AMP-responsive element binding protein (CREB) is a critical factor in synaptic remodeling and memory formation (17). Activation of CREB through Ser133 phosphorylation controls expression of synaptic or memory-related genes, such as brain derived neurotrophic factor (BDNF) and postsynaptic density protein 95 (PSD95). Otherwise, CREB has been shown to contribute to cognitive deficits in various models, such as Alzheimer's disease (18), vascular dementia (19), stress-induced cognitive deficits (5), age-related cognitive deficits (20), schizophrenia-related cognitive deficits (21), and diabetes mellitus-induced dementia (22). Despite all the knowledge gathered recently, it remains unclear whether CREB participates in cognitive deficits induced by trigeminal neuropathic pain.

BDNF, which is an important member of the neurotrophin family involved in neural plasticity, synaptogenesis, neurogenesis, and cell survival (20,23), plays a critical role in regulating the induction and maintenance of long-term potentiation (LTP) and memory formation (24). BDNF-knockout mice exhibited poor spatial learning and memory performance in water maze tasks (25). Additionally, BDNF is reported as one of downstream genes modulated by CREB. Accumulating evidence suggests that the CREB/BDNF pathway plays a pivotal role in regulating synaptic activity, as well as memory consolidation (1,26,17). Accordingly, we hypothesize that the CREB/BDNF pathway may be involved in pathogenic mechanisms of cognitive impairment in trigeminal neuralgia induced by cobra venom.

Based on the above-mentioned hypothesis, we investigated whether chronic pain could influence the function of the hippocampus and PFC through CREB/BDNF pathway by employing trigeminal neuralgia induced by cobra venom.

Methods

Animals

Male Sprague–Dawley rats weighing 200 – 260 g were used in this study. All animal procedures were approved by the Ethical Committee of the Beijing Friendship Hospital, Capital Medical University (Beijing, China), and were performed in accordance with the guidelines of the International Association for the Study of Pain. Rats were housed at 22 – 24°C on a 12-hour light/dark cycle, with food and water available ad libitum. Fifty rats were randomly divided into 3 groups: (1) cobra venom group (n = 26), (2) sham group (n =

12), and (3) control group (n = 12). Rats in the cobra venom group received a cobra venom injection into the left sheath of the ION. A total of 26 rats were chosen in case of accidental deaths or surgical failure to ensure at least 20 rats in the cobra venom group. Twenty-four rats remained in the cobra venom group. Rats in the sham group received a 0.9% sterile saline injection. No surgical procedure or injection was performed on the control rats. A total of 12 rats from the cobra venom group were randomly sacrificed after the first MWM test (5 weeks post-surgery), and all remaining rats were sacrificed after the second MWM test (9 weeks post-surgery).

Drug Preparation

The lyophilized cobra venom (Formosan cobra; Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline. Rats in the cobra venom group were injected with 4 µL saline containing 0.4 mg lyophilized whole venom (6), while rats in the sham group were injected with 4uL 0.9% saline only.

Surgery Procedure

The cobra venom model was previously established and described (6,7). Briefly, a 1-cm radical incision was made above the superciliary arch with sodium pentobarbital anesthesia (40 mg/kg intraperitoneally). The left ION trunk was exposed and cobra venom was injected into the ION sheath. The incision was then closed. Sham-surgery rats underwent all procedures, but were only injected with saline. No procedure was performed on the control rats.

Behavioral Testing

Free behavioral testing and mechanical allodynia were evaluated to investigate whether the trigeminal neuralgia model was established successfully.

Free Behavioral Testing

Free behavioral testing was performed as described in detail previously (7,27). Rats were placed in a 24 cm × 35 cm × 18 cm transparent plastic cage. Testing was conducted in a darkened room with a video camera placed 1 m in front of the cage. Rats were tested 3 days before surgery and 4, 7, 14, 21, 28, and 56 days after surgery. After 15 minutes in the cage, a 7-minute video was recorded to observe rat behavior using an IBM computer placed outside of the room. Behavioral changes were analyzed by an experimenter blinded to the surgery paradigm. The number and duration of face grooming (movement patterns in which paws contact facial areas), exploratory (walking, running, climbing, or rearing), body grooming (movement patterns with paws, tongue, or incisors contacting body areas other than the face or the forepaws), and resting (head resting on flexed forepaws) behaviors were recorded to confirm the presence of pain behavior by playing videotapes frame-by-frame.

Evaluation of Mechanical Allodynia

Rats were placed in a plastic chamber (20 cm ×25 cm × 15 cm) and habituated for 15 minutes before testing. Mechanical withdrawal thresholds (MWT) were evaluated with von Frey filaments (Stoelting, Chicago, IL) using the up-down method as described previously (6). Each filament was applied perpendicularly to the ipsilateral ION territory, near the center of the vibrissal pad. Avoiding further contact with filament, turning head away quickly, scratching the stimulated area, or attacking the filament was considered a positive response. Observation times coincided with free behavioral testing.

Morris Water Maze: Learning and Memory Testing

The Morris water maze (MWM) consists of a circular pool (180 cm diameter,60 cm high) filled with opaque water (22 ± 1°C) and located in a well-illuminated room with external cues visible from the inside of the pool. The pool was divided into 4 equivalent quadrants. A 2-cm submerged escape platform (10 cm diameter) was placed in the middle of one of the 4 quadrants.

Rats underwent 4 acquisition trials per day (limit 2 minutes per trial to find the hidden platform) for 4 consecutive days with a 30-second inter-trial interval on the platform. For each trial, the rat was placed facing the pool wall at different start locations, while the platform remained in the same location. Escape latency and swimming speed were collected. On day 5 (24 hours following the last acquisition trial), the hidden platform was removed, and a probe trial was conducted in which rats were allowed to swim freely for 90 seconds. The percentage of time spent in the previous target quadrant and the number of crossings over the previous platform location were recorded.

The WMW task was performed twice at 4 and 8 weeks after surgery. During the second WMW, all procedures were the same as before except for the platform location, which was moved to the opposite quadrant.

Western Blotting

After decapitation, the left hippocampus and PFC from each group (n = 6) were rapidly dissected and

stored in liquid nitrogen. Tissues were homogenized and centrifuged at 5000 × g for 10 minutes at 4°C. Protein concentrations were measured using a bicinchoninic acid kit. Equal amounts of protein were resolved by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat milk at room temperature and incubated overnight at 4°C with primary antibodies (rabbit anti-pCREB, 1:1000, rabbit anti-CREB, 1:1000, Cell Signaling Technology, Beverly, MA, USA; rabbit anti-BDNF, 1:600, Abcam, Cambridge, MA, UK). Then, the membranes were incubated with peroxidase-conjugated anti-rabbit secondary antibodies (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature for 2 hours. Finally, the bands were detected using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA). Band intensity was quantified using Image J (National Institutes of Health, Bethesda, MD, USA); β-actin (1:5000, Abcam, Cambridge, UK) was used as the loading control.

Immunohistochemistry

Six rats in each experimental group were deeply anesthetized and transcardially perfused with 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde. The brains were removed, post-fixed in 10% formalin solution for 24 hours. Collected tissues were embedded by paraffin, and 5 μm coronal sections were taken using microtome. After deparaffinization and gradual hydration through graded alcohols, the sections were washed with distilled water. Then the slides were incubated with 0.01 M citrate buffer (pH 6.0) in the microwave oven for 10 minutes. A 3% H2O2 solution was applied to inhibit endogenous peroxidase activity. The sections were blocked in 5% normal goat serum for 30 minutes and incubated with primary antibodies (rabbit anti-pCREB, 1:200, rabbit anti-CREB, 1:200, Cell Signaling Technology, UK; rabbit anti-BDNF, 1:250, Abcam, USA) at 4°C overnight, then with goat anti-rabbit secondary antibody at 37°C for 2 hours. The DAB staining was performed using the ABC kit (Boster, Wuhan, China). CA1, CA3, and dentate gyrus (DG) subfields of the hippocampus and PFC were observed and photographed under an optical microscope (Olympus, Tokyo, Japan) (magnification × 200). Brown punctate staining was observed in the positive cells labeled with p-CREB, CREB, and BDNF, and the staining density was measured in mean optical density (OP) by Image Pro Plus software (IPP 6.0, Media Cybernetics).

Statistical Analysis

Analysis of time-courses of behavioral alterations, MWT, and latencies to reach the platform in the MWM between groups were performed by repeated measures two-way ANOVA followed by Bonferroni test. Swimming speed, percentage of time spent in target quadrant, and platform crossings between groups were performed with one-way ANOVA followed by Bonferroni multiple comparison tests. Differences between 2 MWM protocols were calculated using Student t-test for independent samples to analyze percentage of time spent in the target quadrant and platform crossings between 2 probe trials. Spearman's correlation analysis was performed to analyze the correlation between the MWT and MWM tests. In the western blotting and immunohistochemistry experiments, we used one-way ANOVA followed by LSD multiple comparison tests to compare protein expressions of p-CREB, CREB, and BDNF between groups. Data are expressed as mean ± standard deviation (SD) and were analyzed by SPSS 19.0 (SPSS Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Cobra Venom Induces Behavioral Changes in Rats

To evaluate whether a trigeminal neuralgia model was successfully established, changes in behavioral activity were determined using a 7-minute video observation. Rats injectied with cobra venom spent significantly more time ($F = 73.02$, $P < 0.01$) and frequency ($F = 100.7$, $P < 0.01$) face grooming, as well as more resting time ($F = 204.41$, $P < 0.001$) and frequency ($F = 121.08$, $P < 0.001$), compared with preoperative, control, or sham-operated rats (Fig. 1A, B, G, H). Moreover, duration ($F = 203.22$, $P < 0.01$) and frequency ($F = 28.07$, $P < 0.01$) of exploratory behavior significantly decreased from day 4 to 56 post-surgery (Fig. 1C, D). However, duration ($F = 0.033$, $P = 0.968$) and frequency ($F = 0.196$, $P = 0.823$) of body grooming behavior did not significantly change after surgery (Fig. 1E, F).

Cobra Venom Induces Mechanical Allodynia in Rats

The MWT of rats was further assessed following the injection of cobra venom. The rats in the cobra venom group, but not the control or sham groups, exhibited significant mechanical allodynia ($F = 342.51$, $P < 0.001$).

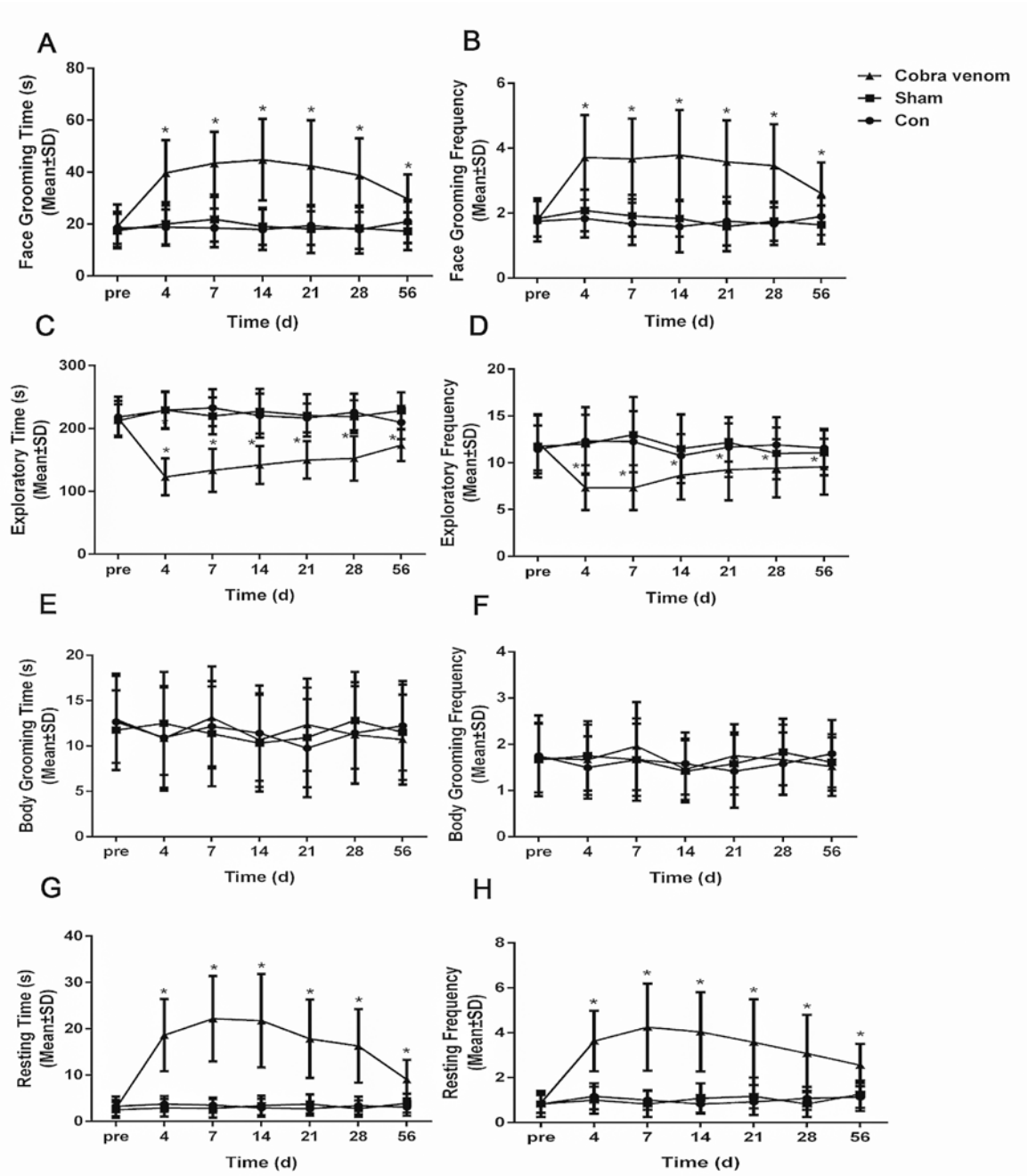

Fig. 1. *Duration and frequency of face grooming, exploratory behavior, body grooming, and resting behavior during a 7-minute observation session prior to surgery (pre) and at 6 post-operative time points. (A–H) The behavioral activity of rats in cobra venom group was different from preoperative, control group, or sham group levels after surgery. No significant differences between different time points were found in the control group and sham group ($P > 0.05$). No significant differences between groups were found prior to surgery ($P > 0.05$). Error bars indicate the SD. Data are presented as mean ± SD. *$P < 0.05$ vs. sham.*

The time course of ipsilateral MWT is presented in Fig. 2. The attenuation of facial allodynia persisted through the observation period of 56 days. The results were consistent with free behavioral changes.

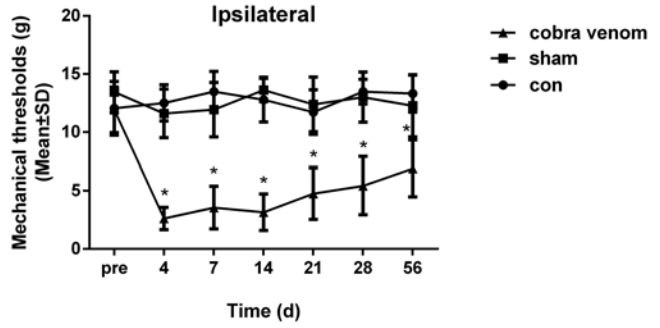

Fig. 2. *Time course of ipsilateral mechanical withdrawal threshold (MWT) in control, sham and cobra venom groups. Cobra venom produced significant mechanical allodynia in rats. No significant differences between different time points were found in the control group and sham group ($P > 0.05$). Data are presented as mean ± SD. *$P < 0.05$ vs. sham.*

Spatial Learning and Memory Are Impaired in Cobra Venom-induced Trigeminal Neuralgia Rats

To test whether chronic neuropathic pain impairs cognitive function, we compared behavioral performance in MWM tests. The protocol contained 2 phases: acquisition and probe trial. During the acquisition trial, all rats reliably learned to locate the platform throughout 4 days of acquisition training. The cobra venom group exhibited a slightly longer latency curve (F = 15.23, P = 0.011), demonstrating that at 4 weeks after surgery neuropathic pain impaired learning function (Fig. 3A). However, no significant difference was found in swimming speed (F = 2.089, P = 0.136) (Fig. 3C). During the second water maze test, rats in the cobra venom group displayed a significantly longer latency in reaching the platform than the control and sham groups, indicating that long-term existence of neuropathic pain aggravated cognitive impairment (F = 68.71, P < 0.001) (Fig. 3B). Swimming

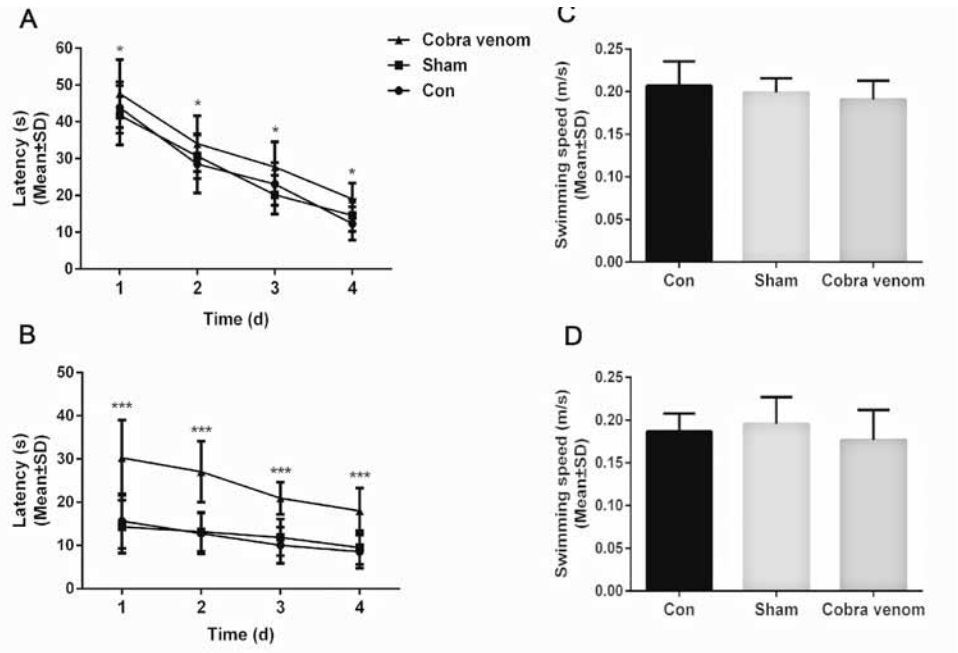

Fig. 3. *Chronic trigeminal neuralgia led to learning deficits in rats on the Morris water maze task. (A) At 4 weeks after surgery, the cobra venom group exhibited a slightly longer latency to reach the platform after training ($P < 0.05$). (B) At 8 weeks after surgery, the cobra venom group displayed a significantly lower latency curve compared with the control group and sham group ($P < 0.001$). (C, D) No significant differences were found in swimming speed between the experimental groups ($P > 0.05$). *$P < 0.05$, ***$P < 0.001$ vs. sham.*

speed between the experimental groups was similar (F = 1.209, $P = 0.311$) (Fig. 3D).

On day 5 (24 hours following the last acquisition trial), the platform was removed and rats were tested on a probe trial. Rats in the cobra venom group exhibited significantly lower percentages of total probe time in the target quadrant (F = 11.54, $P < 0.001$) and fewer numbers of platform crossings (F = 9.18, $P < 0.01$) only in the second probe trial compared with control rats and sham-surgery rats (Fig. 4). Platform site crossings of the cobra venom group decreased significantly between probe trials ($P < 0.05$) (Fig. 4B). No difference existed between probe trials in the control and sham groups. The reduction of numbers of platform crossings and time in the target quadrant at the second MWM test were positively associated with the changes of mechanical responses at 8 weeks post-surgery ($r = 0.803$, $P < 0.001$ for crossings and $r = 0.842$, $P < 0.001$ for time in target quadrant).

The CREB/BDNF Pathway Was Down-regulated in the Hippocampus and PFC in Cobra Venom-induced Trigeminal Neuralgia Rats

The CREB/BDNF pathway has been shown to play an important role in synaptic activity, as well as learning and memory processes (15). Therefore, we examined whether the CREB/BDNF pathway is involved in cognitive impairment caused by cobra venom-induced trigeminal neuralgia. Levels of phosphorylated CREB (p-CREB) and BDNF in the hippocampus and PFC were detected by western blotting and immunohistochemistry. Analysis of western blotting showed that p-CREB was decreased in both the hippocampus and PFC; that was shown by a decreased ratio of p-CREB to CREB in the cobra venom group, with a significant difference at 9 weeks post-surgery (F = 6.85, $P = 0.013$ for the hippocampus and F = 15.08, $P < 0.01$ for the PFC) (Fig. 5A, B). Similar pattern changes occurred in BDNF expression (F = 10.93, $P < 0.01$ for the hippocampus and F = 6.919, $P = 0.013$ for the PFC) (Fig. 5C, D). Consistent with data collected by western blotting analysis, immunohistochemistry results showed that immunoreactivity of p-CREB (F = 12.961, $P < 0.01$ for CA1, F = 5.887, $P = 0.025$ for CA3, F = 12.101, $P < 0.01$ for DG and F = 42.236, $P < 0.001$ for the PFC) (Fig. 6) and BDNF (F = 47.967, $P < 0.001$ for CA1, F = 94.095, $P < 0.001$ for CA3, F = 14.173, $P = 0.017$ for DG and F = 6.367, $P = 0.021$ for the PFC) (Fig. 8) were dramatically weaker in each subfield of the hippocampus and PFC in 9-week rats with cobra venom injection, compared with control and sham groups. The decrease in the 5-week cobra venom group was slight ($P > 0.05$); there was no obvious change in CREB levels between different experimental groups ($P > 0.05$) (Fig. 7). Taken together, these results suggested that the cobra venom-induced trigeminal neuralgia model resulted in reduced expressions of p-CREB and BDNF in both the hippocampus and the PFC, without altering CREB levels.

Discussion

This study demonstrated that cobra venom administration to the ION sheath induces changes in free behavioral activity and MWT. Moreover, we found that in the water maze task, chronic trigeminal neuralgia in rats leads to spatial learning and memory dysfunction.

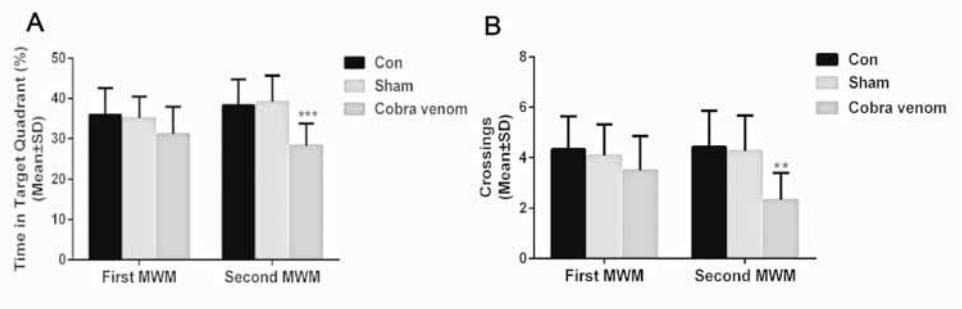

Fig. 4. *Chronic trigeminal neuralgia caused memory deficits in rats on the Morris water maze task. Two probe trials were respectively performed following the learning trial at 4 and 8 weeks after surgery. (A) Rats in the cobra venom group displayed significantly reduced percentages of time spent in the target quadrant on probe trial 2 compared with control rats and sham-surgery rats. (B) Platform site crossings of the cobra venom group showed a similar pattern. No difference was found between probe trials in the control group and sham group. **P < 0.01, ***P < 0.001 vs. sham.*

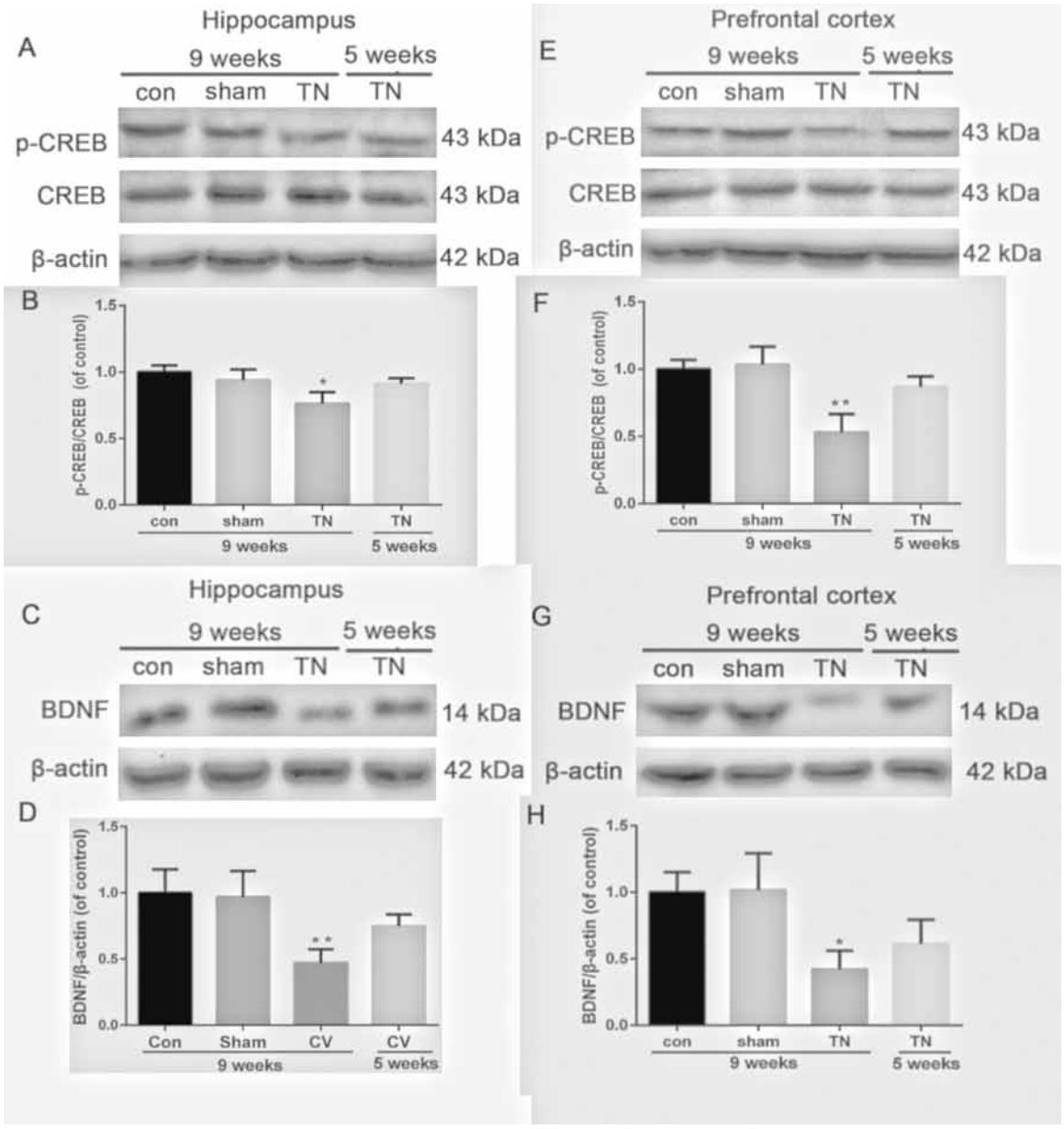

Fig. 5. *Western blot analysis of p-CREB and BDNF levels in the hippocampus and prefrontal cortex (PFC). Cobra venom-induced trigeminal neuralgia attenuated protein expression of p-CREB and BDNF in the hippocampus and PFC. (A, B) p-CREB protein expressions in the hippocampus (A) and PFC (B) were decreased in cobra venom-induced trigeminal neuralgia rats, with significant differences at 9 weeks post-surgery. (C, D) BDNF protein expressions in the hippocampus (C) and PFC (D) displayed similar changes to p-CREB. Upper panels are representative immunoblots of measures detected by western blotting; lower panels represent quantification of p-CREB/CREB and BDNF. No significant differences were found between control and sham group ($P > 0.05$). Values show mean ± SD from 6 rats per group (*$P < 0.05$, **$P < 0.01$ vs. sham). Western blotting data are expressed as relative values of corresponding optical density in control samples (9 weeks).*

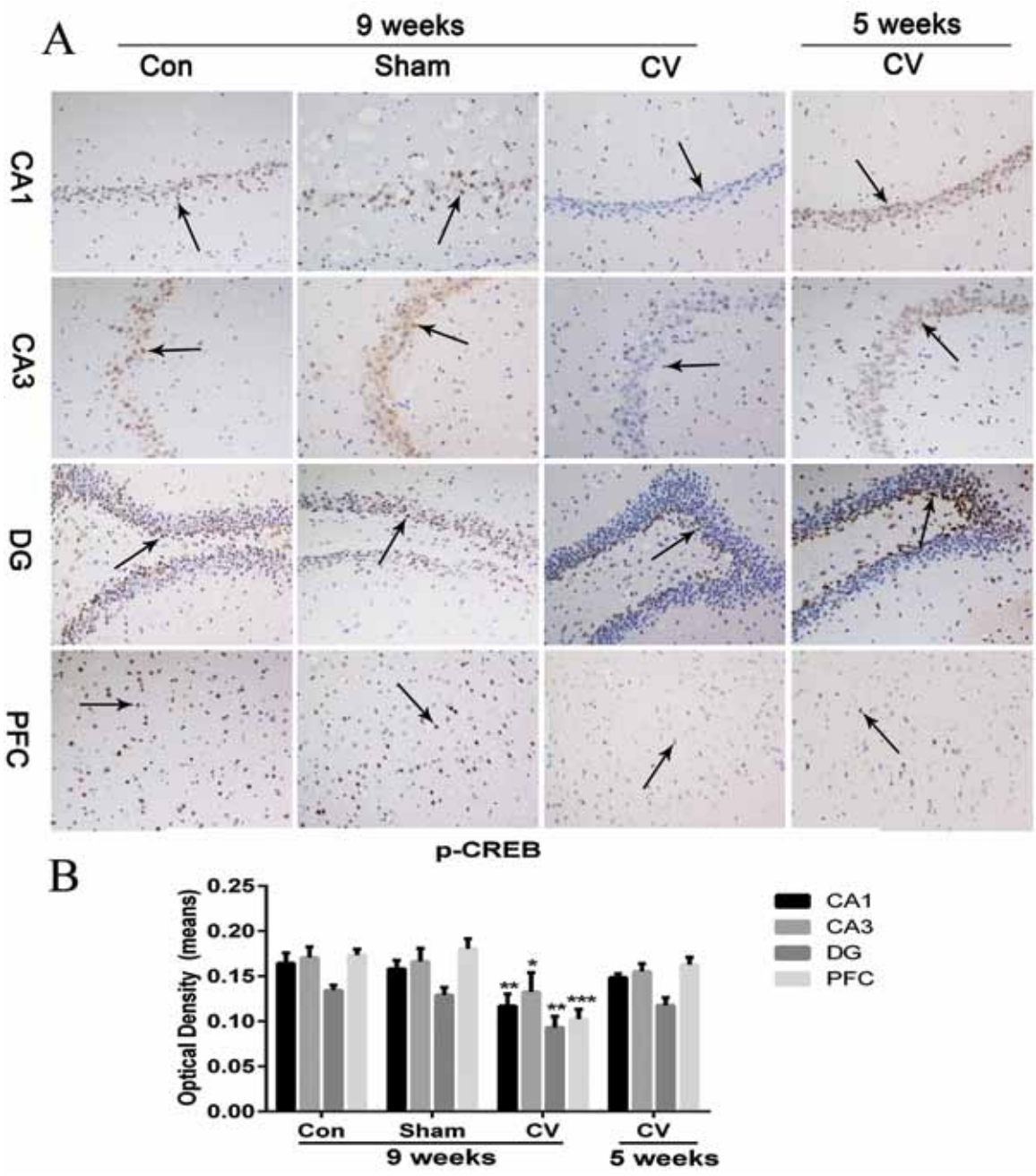

Fig. 6. *Immunohistochemistry analysis of p-CREB levels in the hippocampus and prefrontal cortex (PFC). Cobra venom-induced trigeminal neuralgia reduced p-CREB immunoreactivity in CA1, CA3, dentate gyrus (DG) subfie of the hippocampus and PFC, especially in cobra venom rats at 9 weeks post-surgery. (A) Representative images o he hippocampus and PFC for p-CREB immunoreactivity from control, sham and cobra venom groups (magnification 200). p-CREB-positive cells are indicated by arrows. (B) The mean optical density in each region of the hippocampus and PFC. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 vs. sham.*

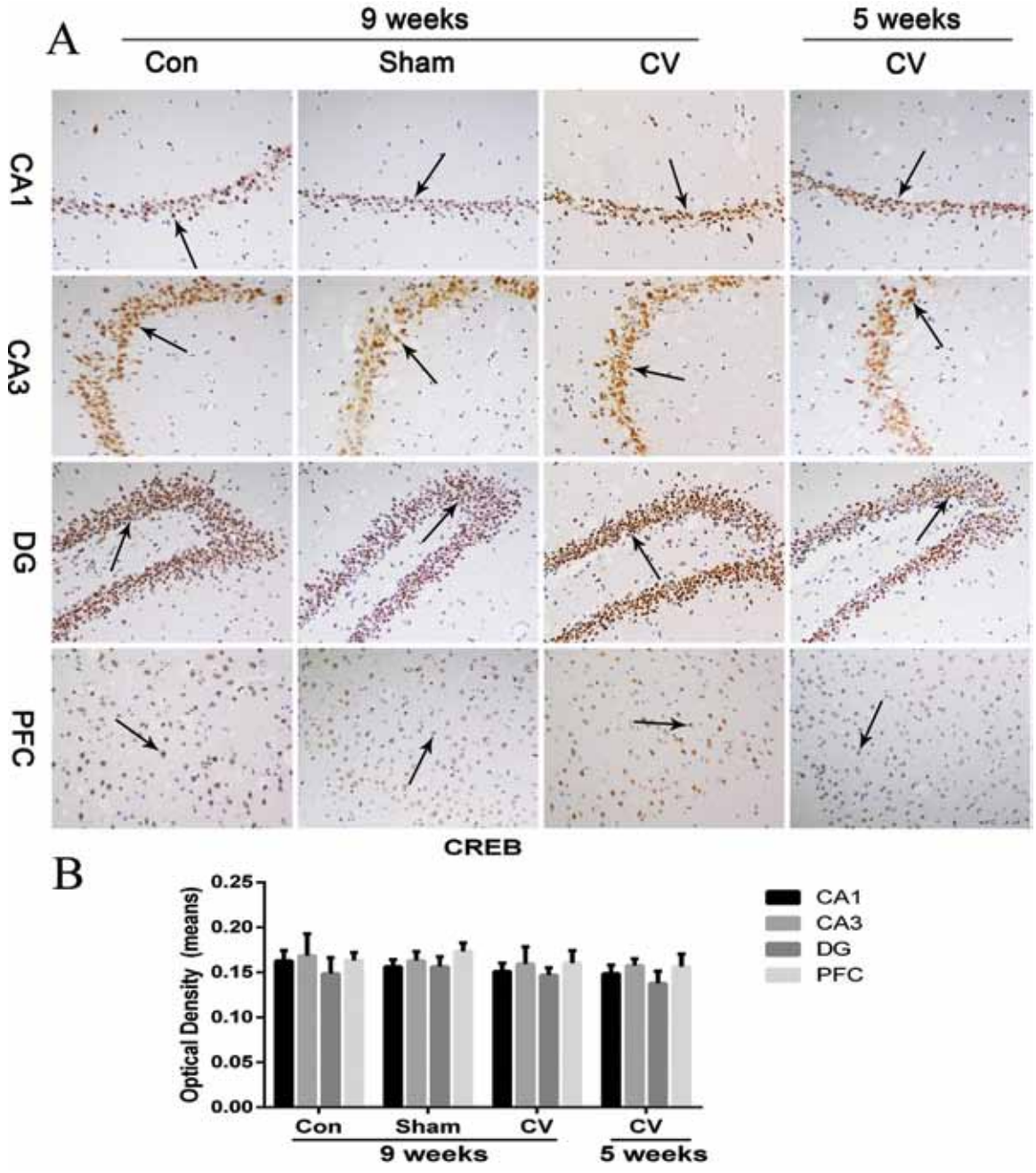

Fig. 7. *Immunohistochemistry analysis of CREB levels in the hippocampus and prefrontal cortex (PFC). There were no obvious CREB level changes betw the different experimental groups (P > 0.05). (A) Representative images of CA1, CA3, dentate gyrus (DG) sub s of the hippocampus and PFC for CREB immunoreactivity from different experimental groups (magnification 200). CREB-positive cells are indicated by arrows. (B) The mean optical density in each region of the hippocampus and PFC. Data are expressed as mean ± SD (n = 6).*

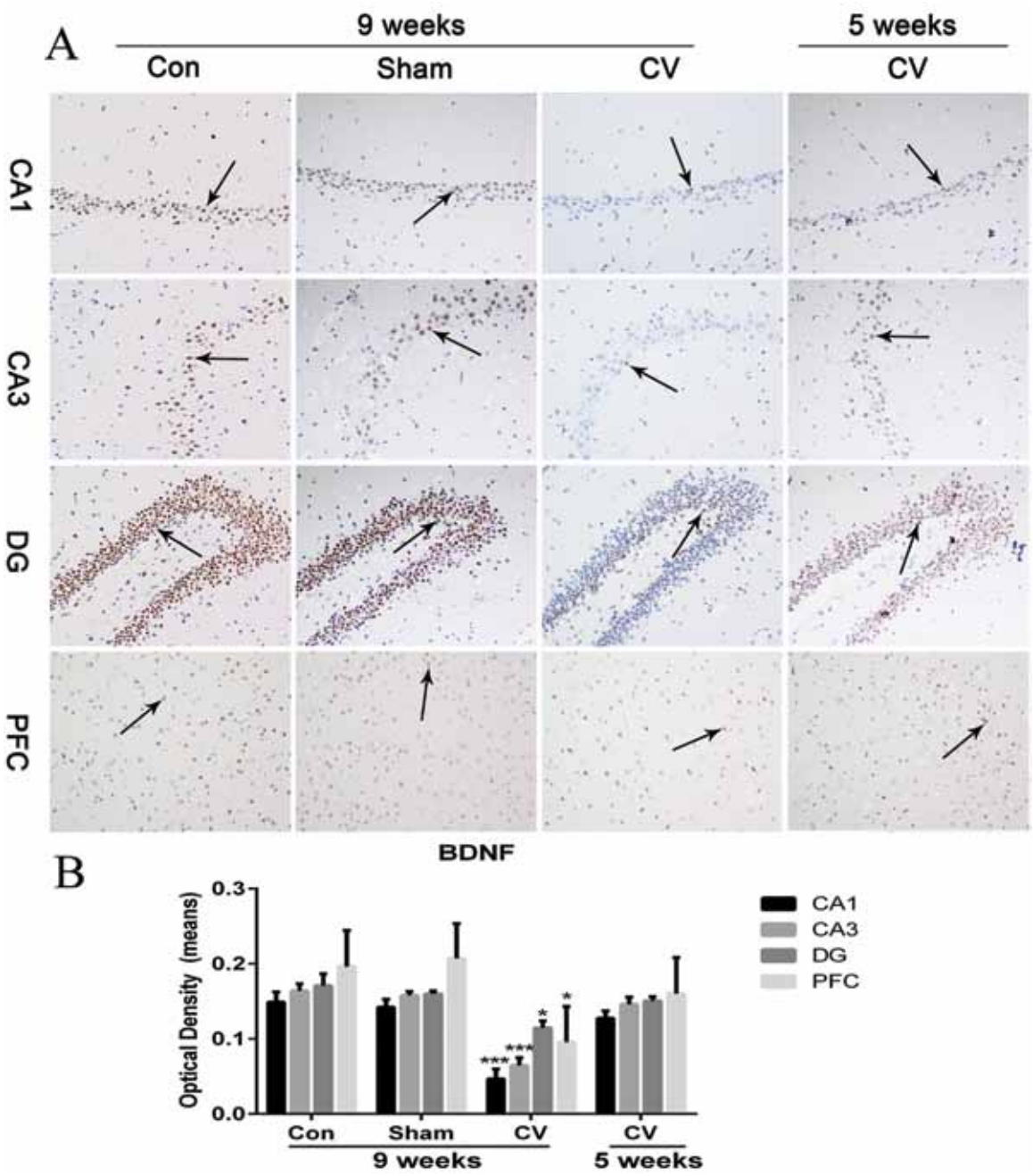

Fig. 8. *Immunohistochemistry analysis of BDNF levels in the hippocampus and prefrontal cortex (PFC). Cobra venom-induced trigeminal neuralgia reduced BDNF immunoreactivity in CA1, CA3, dentate gyrus (DG) subfiel of the hippocampus and PFC, especially in cobra venom rats at 9 weeks post-surgery. (A) Representative images of hippocampus and PFC for BDNF immunoreactivity from control, sham and cobra venom groups (magnification 200). BDNF-positive cells are indicated by arrows. (B) The mean optical density in each region of the hippocampus and PFC. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 vs. sham.*

Additionally, we demonstrated the significance of the hippocampal and prefrontal CREB/BDNF pathway in cognitive deficits caused by chronic pain. These results are in strong agreement with previous data from human patients showing that chronic pain induces cognitive deficits (1), and sheds lights on the molecular mechanisms underlying cognitive impairment observed in chronic pain sufferers with trigeminal neuralgia (28).

Chronic pain is processed by a complex neuronal network involving many structures associated with cognitive capacities. Several studies indicate an overlap between the areas involved in chronic pain processing and cognitive changes (29,30). Imaging studies have demonstrated that nociceptive stimulation in chronic pain sufferers activates brain areas associated with memory acquisition, such as the hippocampus and PFC (31). Moreover, pain-induced chronic stress leads to changes in synaptic connectivity between the PFC and hippocampus (32). Given the remarkable and well-established role that the hippocampus and PFC play in the onset of some pain-related cognitive alterations, we raised the hypothesis that cognitive deficits might be due to abnormal changes in the hippocampus and PFC.

Previous studies on the mechanisms of cognitive impairment in chronic pain have mostly focused on anatomy, neurochemistry, and psychology. Anatomical studies conducted in chronic pain patients showed reductions in grey matter in the PFC, insular cortex, and parahippocampal gyrus (33-35). Several studies have also reported neurochemical changes in chronic pain patients, such as reduced levels of endogenous opioids in the nucleus accumbens (36), such as N-acetylaspartate, the derivative of aspartate, in the PFC (37). Furthermore, some authors raised the hypothesis that cognitive deficits are due to the effects of depression, stress, and anxiety concomitant with pain. So far, these results do not illustrate a clear mechanism for pain-induced cognitive deficits, thereby highlighting the importance of molecular-level studies using animal models of chronic pain.

Recent evidence supports the importance of synaptic plasticity, genesis, and production of neurotrophic factors in cognitive processes, such as memory, learning, and adaptation (38,39). CREB, as an important cellular transcription factor, plays critical roles in synaptic plasticity and long-term memory formation processes. Following ser133 phosphorylation, CREB may bind to cyclic-AMP response element (CREs) sequences and induce transcription of synaptic or memory-related genes, such as BDNF and PSD95. Several studies have reported on the modulating memory role of p-CREB in various diseases. One group reported that hippocampal p-CREB decreased in a rat model of Aβ-induced cognitive deficits (40), while another group suggested that age-related impairment in memory was associated with decreased p-CREB levels and hippocampal plasticity deterioration (20). Our study detected a similar reduction in the hippocampus and PFC. At 9 weeks after surgery, p-CREB expression in the cobra venom group was significantly decreased compared with control and sham groups. Moreover, the 9-week cobra venom group had lower p-CREB expression than the 5-week cobra venom group. These results are consistent with behavioral changes.

BDNF has pleiotropic effects on synaptic plasticity, thereby enhancing neuronal long-term potentiation (LTP), neurogenesis, and cognitive function. Animal experiments demonstrated that the cognitive decline in aging correlated with decreased BDNF expression and hippocampal neurogenesis. Postmortem studies have documented low expression levels of BDNF, pro-BDNF, and BDNF mRNA in the brains of patients diagnosed with Alzheimer's disease and mild cognitive impairment (41,42), supporting a role for cognitive functions of BDNF. In our study, BDNF expression was lower in the cobra venom group, with the largest difference at 9 weeks post-surgery, which coincided with previous studies. Thus, BDNF may play a critical role in trigeminal neuralgia-induced cognitive deficits. It is noteworthy that our study showed behavioral alterations recovered earlier than changes in the hippocampus and PFC. Decreased p-CREB and BDNF were observed respectively at 9 weeks post-surgery. However, at the same time, behavioral activities showed a recovering tendency. The causes of this phenomenon may be that peripheral nerve injury was easier to recovery than CNS. After nerve injury, in the peripheral nerve system (PNS), the Schwann cells create a milieu supporting axons regrowth and synapses reestablishment. By contrast, in the CNS, the gial cells lead to an environment inhibiting axons regeneration due to a deficiency of neurotrophic factors and presence of myelin-associated inhibitory molecules (43).

Recent studies showed that p-CREB and BDNF levels increased in spinal dorsal horn in chronic pain conditions (44,45), while in our research we found that p-CREB and BDNF levels decreased in the hippocampus and PFC. Persist pain may cause differential regional regulation of the CREB/BDNF pathway in the spinal cord and higher brain centers, and this phenomenon

has been reported in earlier reports (44). The mechanisms responsible for differential regional regulation are not fully understood. It is possible that different kinases activate in the periphery and CNS, or this may be the effect of neurotransmitter transport between spinal dorsal horn and CNS. In our study, hippocampal BDNF experienced a more significant decrease than hippocampal CREB, which might be due to BDNF transport from the CNS to the periphery, as BDNF was urgently needed by injured peripheral nerves owing to its eminent properties of promoting axonal regeneration and neuronal survival (46,47). Further studies are needed to identify the exact mechanisms.

BDNF is a CREB target gene. The increased BDNF expression via promoting activation of CREB by oral administration of arabinoxylan (48) and the decreased BDNF expression after inhibiting the phosphorylation of CREB by A βexposure (49) strongly suggest that the activation of CREB phosphorylation contributes to the synthesis of BDNF. These results are supported by previous findings that CREB/BDNF signaling was downregulated in the experimental models of Alzheimer's disease and vascular dementia (19,17), indicating that hippocampal and prefrontal expressions of CREB/BDNF may be implicated in behavioral and cognitive dysfunction. Our data support the effects of the CREB/BDNF pathway on learning and memory dysfunction, as shown by findings that p-CREB and BDNF expressions were lower in the hippocampus and PFC of rats with cobra venom injection. Given that trigeminal neuropathic pain contributes to synaptic plasticity alterations in the hippocampus and PFC known to be involved in pain processing that accompanies cognitive deficits in the present study, we suggest that the hippocampal and prefrontal CREB/BDNF pathway could be implicated in cognitive impairment.

It should be emphasized that in the current study changes in protein expressions were much more dramatic in the PFC than in the hippocampus. This inconsistency of change levels in different brain regions has not been illuminated precisely and may be relevant to some factors, such as individual differences in the reorganized ability of the CNS, pain type, and pain intensity (50,51). Besides spatial learning and memory, the PFC is also involved in working memory, motivation, and execution. Our results indicate that other cognitive deficits might also occur under chronic trigeminal neuralgia conditions. Further investigations should be conducted to clarify this hypothesis.

Conclusions

In conclusion, this study confirmed our previous work that cobra venom-induced trigeminal neuralgia in rats leads to spatial learning and memory dysfunction with a significant time effect. The cognitive deficits induced by this model reliably mimic manifestations of cognitive impairment in human chronic pain patients. The downregulation of the CREB/BDNF pathway in the hippocampus and PFC could be related to the mechanisms involved in this event. Our results may provide a potential interventional target for future treatment.

Limitations

CREB can be activated by multiple kinases, including protein kinase A (PKA), protein kinase C(PKC), Ca2+-calmodulin-dependent protein kinases II and IV (CaMK II and IV), and the Ras-MAPK signaling pathway, in response to various physiological and pathological stimuli (52,53). In spatial learning and memory deficits caused by cobra venom-induced trigeminal neuralgia, the upstream factors of the CREB/BDNF pathway remain currently unknown. This necessitates further investigation using signaling inhibitors or genetic manipulation to confirm specific involvement.

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