Experimental Study

A New Rat Model of Thalamic Pain Produced by Administration of Cobra Venom to the Unilateral Ventral Posterolateral Nucleus

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Background: Thalamic pain is a neuropathic pain syndrome that occurs as a result of thalamic damage. It is difficult to develop therapeutic interventions for thalamic pain because its mechanism is unclear. To better understand the pathophysiological basis of thalamic pain, we developed and characterized a new rat model of thalamic pain using a technique of microinjecting cobra venom into the ventral posterolateral nucleus (VPL) of the thalamus.

Objectives: This study will establish a new thalamic pain rat model produced by administration of cobra venom to the unilateral ventral posterolateral nucleus.

Study Design: This study used an experimental design in rats.

Setting: The research took place in the laboratory at the Aviation General Hospital of China Medical University and Beijing Institute of Translational Medicine.

Methods: Male Sprague-Dawley rats were subjected to the administration of cobra venom or saline into the left VPL. The development of mechanical hyperalgesia and changes in pain-related behaviors and motor function were measured after intrathalamic cobra venom microinjection using the von Frey test, video recording, and cylinder test, respectively. On postoperative days 7 to 35, both electroacupuncture and pregabalin (PGB) were administered to verify that the model reproduced the findings in humans. Moreover, the organizational and structural alterations of the thalamus were examined via transmission electron microscopy (TEM).

Results: The threshold for mechanical stimuli in the left facial skin was significantly decreased on day 3 after thalamic pain modeling as compared with pre-venom treatment. Furthermore, the ultrastructural alterations of neurons such as indented neuronal nuclei, damaged mitochondria and endoplasmic reticulum, and dissolved surrounding tissues were observed under TEM. Moreover, electroacupuncture treatment ameliorated mechanical hyperalgesia, pain-like behaviors, and motor dysfunction, as well as restore normal structures of neurons in the thalamic pain rat model. However, no such beneficial effects were noted when PGB was administered.

Limitations: The pathophysiological features were different from the present model and the patients in clinical practice (in most cases strokes, either ischemic or hemorrhagic).

Conclusion: The cobra venom model may provide a reasonable model for investigating the mechanism of thalamic pain and for testing therapies targeting recovery and pain after thalamic lesions.

Key words: Thalamic pain, cobra venom, electroacupuncture, pregabalin, indented neuronal nuclei, damaged mitochondria, dissolved endoplasmic reticulum, golgi body

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Thalamic pain, also described as central poststroke pain (CPSP), is a severe and treatment-resistant type of neuropathic pain that can occur after thalamic lesions (1). There are only a few epidemiological examinations of thalamic pain, probably because of the difficulty in interpreting its clinical characteristics; further, it is easy to misdiagnose (2). The prevalence of thalamic pain, however, is as high as 12% in patients following stroke (1). A variety of psychoactive medications such as anticonvulsants, antidepressants, and corticosteroids, and some nonpharmacological treatments such as deep brain stimulation (DBS), cingulotomy and repetitive transcranial magnetic stimulation (rTMS) have been found to be useful for the treatment of thalamic pain. However, the efficacies of such methods are debatable and further studies are warranted (3). Additionally, the pathophysiological features of thalamic pain remain to be revealed and the therapeutic interventions are still difficult at present because of the lack of clinically relevant animal models.

Recently, several animal models of CPSP have been developed to examine the exact pathophysiology. In these models, various lesions of the thalamus were produced, including microinjection of collagenase, endothelin-1, autologous blood or kainite to the ventral posterolateral nucleus (VPL) or ventral posterior medial (VPM) nucleous, and occlusion of the bilateral carotid arteries in mice (4-8). Although these models have increased our understanding of the mechanisms of thalamic pain, no single model can mimic all of the sensory, motor, and molecular changes seen. In our previous studies, we have established several neuropathic pain models in rats including brachial plexus and trigeminal neuralgia following the administration of cobra venom (9,10). Cobra venom is a natural substance that contains phospholipase A2 (PLA2), cardiotoxin (CTX), and neurotoxin (cobrotoxin). Lewinska et al (11) reported that cobra venom leads to changes in cell cycle regulators, limitations in cell proliferation and migration, and the induction of cellular senescence.

In the present study, we attempt to establish a thalamic pain rat model by cobra venom injection. In addition, we have also assessed the effects of electroacupuncture and pregabalin (PGB) therapy to verify that the model was reproducible and similar to the findings seen in humans.

Methods

The entire experimental procedure on conscious animals was approved by the Ethical Committee of Aviation General Hospital of China Medical University and was in accordance with the Guidelines for the Care and Treatment of Laboratory Animals of the US National Institutes of Health.

Animals

Thirty-six male Sprague-Dawley rats weighing between 200 and 220 g were provided by the Laboratory Animal Center of the Academy of Military Medical Science (Beijing, China). The animals were housed 4 per cage and maintained on a 12/12-hour light/dark cycle with food and water available ad libitum before surgery for 2 weeks under such controlled conditions. All experiments were performed in accordance with the guidelines set by the Animal Care and Use Committee (Beijing, China). Animals were randomly divided into 4 groups: sham-operated (n = 9), cobra venom (n = 9), electroacupuncture (n = 9), and PGB (n = 9).

Surgery

The surgery was performed according to the techniques described previously (4). Rats were anesthetized with 4% chloral hydrate (0.04 g/mL, 0.8 mL/100 g body weight intraperitoneally, Sigma-Aldrich, St. Louis, MO) and then fixed on an operating table in the prone position. The hair was clipped and skin cleaned with an iodine solution and then a skin incision was made about one cm in length at the approximate midpoint between both ears and eyes. The periosteum was gently retracted to reveal the recognizable landmark of the bregma. An intrathalamic microinjection of cobra venom (Venom Research Institute of Guangxi Medical University) was made into the left VPL of the thalamus according to the stereotaxic coordinates (bregma -3 mm anteroposterior; -3 mm lateral to the midline, and 6 mm ventral to the brain surface) after making a burr hole using a stereotaxic drill. A 2-μL microinjection syringe containing one μL of cobra venom (mixture of 0.4 mg lyophilized cobra venom) prepared in sterile saline was injected into the region mentioned previously over a period of 10 minutes and the syringe remained for 10 minutes after each injection. Then the skin incision was closed using 5-0 absorbable sutures after pulling out the needle. Sham-operated group injections consisted of an equal volume of the saline vehicle.

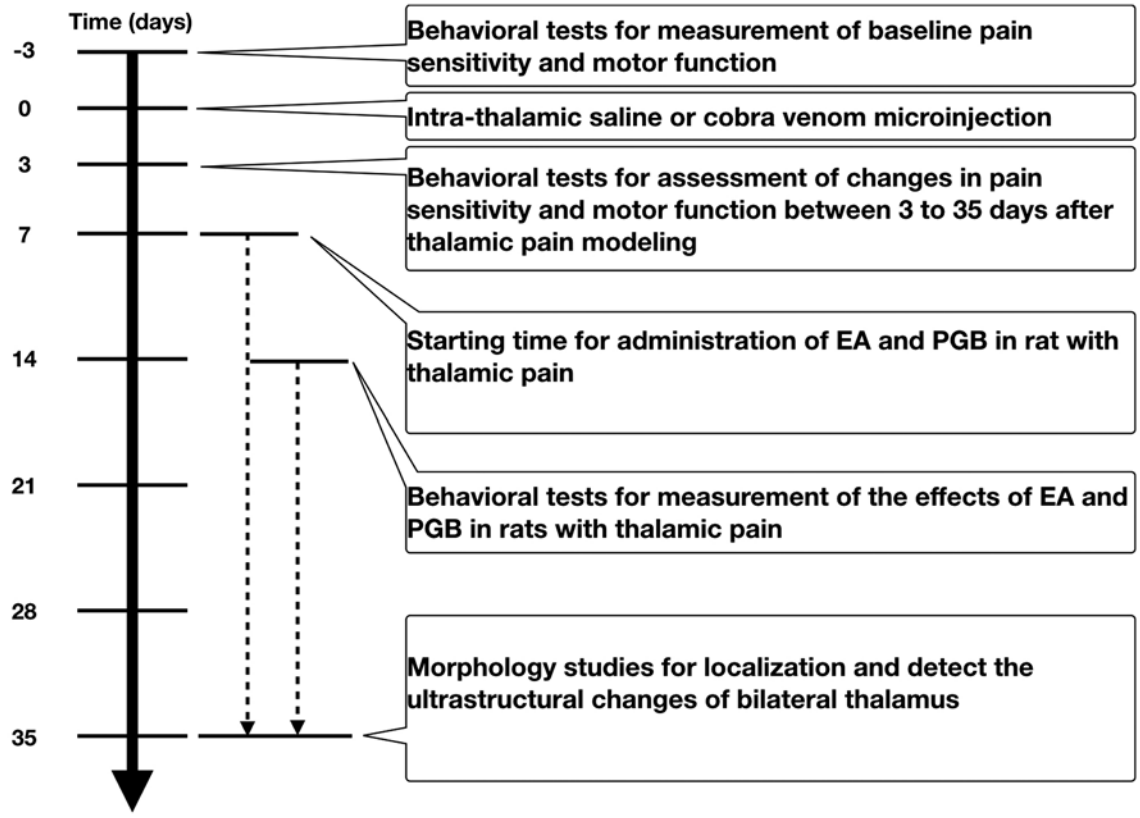

Fig. 1. *Timeline of the experimental procedure.*
Abbreviations: EA, electroacupuncture; PGB, pregabalin.

Experimental Design

The experimental procedure is shown in Fig. 1. Behavioral tests with von Frey filaments, video recordings, and cylinder tests were performed before and on days 3, 7,14, 21, 28, and 35 after cobra venom was injected in the 4 groups. After injection, repeated administrations of PGB (once a day for 4 weeks) or electroacupuncture (once every 2 days for 4 weeks) were made between 7 and 35 days after thalamic pain modeling. On postoperative day 35, morphology studies were performed to detect the ultrastructural changes among all groups.

Electroacupuncture Stimulation

On the 7th day after modeling, a 30-minute electroacupuncture treatment was applied in the electroacupuncture group once every 2 days according to the procedure reported by Tao et al (12). Briefly, the rats were restrained in an immobilization apparatus without anesthesia, and the investigator quickly inserted disposable acupuncture needles (gauge #32, 0.5 inch in length) into the left "Quchi" (LI11) and "Shousanli" (LI10) at a depth of 2 to 3 mm after cleaning the rat's skin with alcohol swabs. The needles were fixed with adhesive tape. LI11 is anatomically located at the depression medial to the extensor carpi radialis, at the lateral end of the cubital crease, and LI10 is anatomically located on the dorsum of the forearm, 2 inches below the elbow in humans. In rodents, LI11 is located at the depression in the lateral front of the elbow joint proximal to the radius and L10 is located at the wrinkles formed by muscles about 10 mm below the "Quchi" L10. The whole procedure lasted less than 15 seconds and the rats were hardly restrained during needle insertion. The rats were then transferred in a small transparent plastic cage and treated with electroacupuncture stimulation using a Han's Acupoint Nerve Stimulator (HANS, LH series, Peking University). The frequency of electroacupuncture stimulation was held to 2 and 100 Hz shifting automatically, and the current intensity was maintained at 1 mA for 10 minutes, then increased to 1.5 mA for 10 minutes

and finally increased to 2 mA for 10 minutes; the total procedure time was 30 minutes. If the needles dropped during electroacupuncture, they were reinserted again as quickly as possible. The electroacupuncture treatment was administered once every 2 days for 15 consecutive courses of treatment until day 35.

Drug Administration

On postoperative day 7, animals in the PGB group were tested following the administration of PGB (13). For drug treatment, PGB (75 mg; Pfizer, New York, NY) was dissolved in 7.5 mL of saline and orally administered (30 mg/kg) once a day consecutively for 28 days from postoperative day 7 to day 35.

Von Frey Mechanical Sensitivity Test

Seven days after surgery, tactile withdrawal responses were measured by mechanically stimulating the bilateral face and 4 limbs using von Frey hairs (Stoelting, Chicago, IL) as described by Chaplan et al (14). Briefly, animals were placed individually in transparent plastic cages (27 × 25 × 20 cm) and allowed to habituate for a minimum of 15 minutes until cage exploration and major grooming activities ceased. von Frey filaments were applied to the middle glabrous area of limbs between the footpads of the plantar surface and the bilateral face near the center of the vibrissal pad on hairy skin surrounding the mystacial vibrissae. Mechanical stimuli were applied using a logarithmic series of 8 von Frey filaments with approximately exponentially incremental bending forces (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g). The surface of each site was stimulated to cause slight filament bending for approximately 5 seconds. A positive withdrawal response was recognized as follows: (1) rat turns head quickly away scratching the stimulated area, or attacks the monofilaments when stimulating the face; and (2) withdraws paw during stimulation of the limbs.

Video Recording

For neurobehavioral assessments, the rats were tested prior to initiating treatment on preoperative days 3 and 3, 7, 14, 21, 28, and 35 days after the surgery. Rats were placed in a transparent plastic cage (24 x 35 x 18 cm) with a mirrored back and a video camera placed 1 m in front of the cage. The rat's behavior was videotaped for a duration of 7 minutes. Behavioral changes of rats such as frequency and length of exploratory (climbing) and grooming (face) behaviors were analyzed after video recording (15). Behavioral changes of the rats observed by video recordings were analyzed offline by 2 investigators who were blind to the condition.

Cylinder Test

The cylinder test was used to assess forelimb use asymmetry described by Schallert et al (16). Briefly, the rat was placed in a transparent plexiglas cylinder (20 × 30 cm) with mirrors placed behind the cylinder to observe the rats fully. They were then videotaped at the same time on preoperative day 3 and 3, 7, 14, 21, 28, and 35 days after modeling. The number of contacts of the ipsilateral, contralateral, and bilateral forepaw with the cylinder wall were recorded for 3 minutes using a high-definition camera. The formula for calculating the percent use of the affected limb was taken from Woodlee et al (17): Asymmetry Score = (I - C)/(I + C + B) × 100%, where I is the number of left forelimb contacts with the cylinder wall, B is the number of simultaneous bilateral foreleg contacts with the cylinder wall, and C is the number of right forelimb contacts with the cylinder wall. The cylinder tests were analyzed offline by 2 experimenters who were blind to the condition.

Transmission Electron Microscopy

For electron microscopic examination, the rats were perfused with saline, followed by a mixed solution of 4% paraformaldehyde and 2% glutaraldehyde (Sigma, St. Louis, MO) after being anesthetized with chloral hydrate on postoperative day 35. The bilateral prefrontal cortex (PFC), thalamus, and hippocampus were dissected and immersed in 3% glutaraldehyde for 24 hours, then rinsed with 0.1 M phosphate buffer 3 times. Then the tissues were fixed with 1% osmium tetroxide (Sigma, St. Louis, MO) for 2 hours, dehydrated, embedded in araldite for 24 hours, and cut into 1-μm plastic sections. The sections were observed under a transmission electron microscope (TEM; H-9000NARIbaraki, Hitachi Ltd., Tokyo, Japan) after staining in uranile acetate. The experimenters were blind to the experimental groups during the reading of TEM images.

Statistical Analysis

All statistical analyses were performed using SPSS Version 23.0 (IBM Corporation, Armonk, NY). Data from the von Frey mechanical sensitivity test were not normally distributed, and the Kruskal-Wallis H test was performed to assess the changes over time within groups. Within-group differences across time were evaluated by the Dunn-Bonferroni test for post

hoc comparisons. Results are presented as the median and ranges. Data for the cylinder test and video recording were normally distributed and therefore analyzed with one-way analysis of variance followed by the Tukey test for post hoc comparison. Results are presented as the mean ± standard deviation. $P < .05$ was considered as statistically significant.

Results

General Observations

There was no mortality after cobra venom or vehicle injection during the 35-day monitoring, and body weight did not differ among the groups. Immediately after venom microinjection, circling or falling to the contralateral side was observed until postoperative day 2. Beginning on day 3 after injection, no limping, limb-dragging, or uneven gait were observed in rats with thalamic pain until the end of the experiment. The sham-operated rats did not show any signs of gait and posture changes. Baseline assessments did not reveal any difference among the 4 groups or between the ipsilateral and contralateral sides. The rats exhibited mechanical allodynia in the left facial skin and motor dysfunction in the contralateral forelimb since day 3 after injection and were stable for approximately 33 days, whereas there were no changes in mechanical thresholds on the bilateral limbs among all groups.

VPL Microinjection Induced Behavioral Signs of Motor Dysfunction in Rats with Thalamic Pain

To assess the effect of exercise on motor function in thalamic pain rats, we tested forelimb-use asymmetry using the cylinder test. Figure 2B shows that the contralateral forelimb asymmetry scores of both the thalamic pain and PGB-treated rats were increased at 7, 14, 21, 28, and 35 days after cobra venom microinjection compared to those in the sham-operated group, indicating a tendency of thalamic pain rats to use the ipsilateral forelimb. However, there was no difference in this variable between the sham-operated group and electroacupuncture-treated rats at 28 and 35 days after thalamic pain modeling.

Signs of Pain in Ipsilateral Facial Skin

As shown in Fig. 3, there were no significant differences between the ipsilateral- and contralateral-side limbs among the 4 groups at any time point.

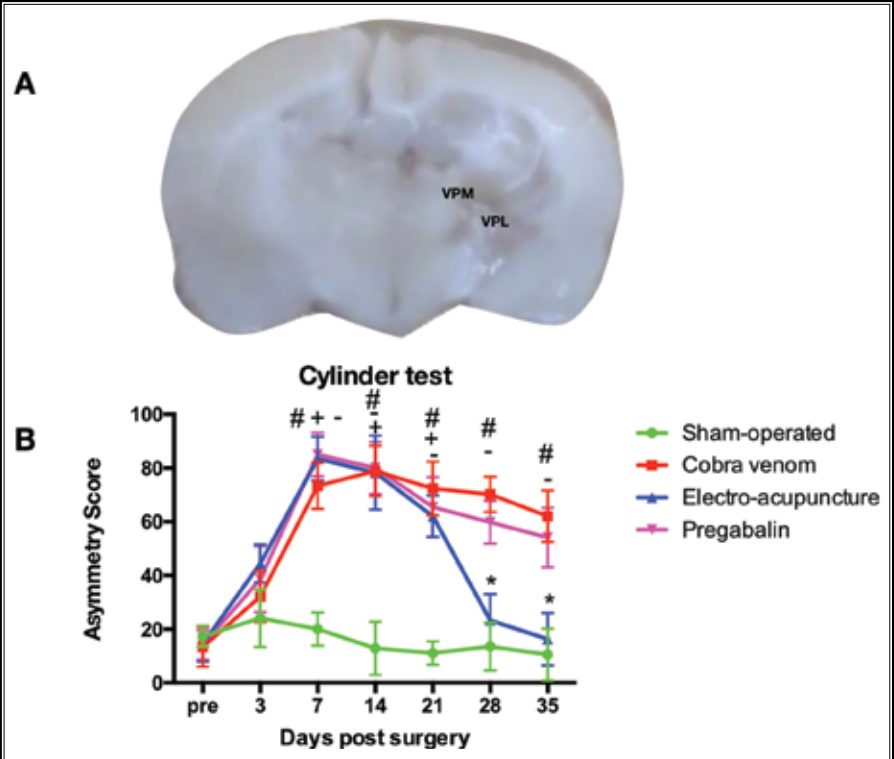

Fig. 2. *(A) Photomicrograph showing the damaged lesion site in the thalamus following unilateral intrathalamic cobra venom microinjection. (B) Cylinder test. The cylinder tests were done both before (baseline) and 3, 7, 14, 21, 28, and 35 days after cobra venom microinjection. On postoperative day 7, the asymmetry score in the cobra venom group showed an obvious decrease compared to the rats in the sham-operated group (comparison between cobra venom and sham-operated groups: #P < .001; comparison between electroacupuncture and sham-operated groups: +P < .001; comparison between PGB and sham-operated groups: -P < .001). The motor dysfunction could improve by electroacupuncture-treated but not PGB-treatment at postoperative day 28 (comparison between cobra venom and sham-operated groups: #P < .001; comparison between electroacupuncture and cobra venom groups: *P < .001; comparison between PGB and sham-operated groups: -P < .001). Data represent mean ± standard deviation (SD). *, +, -, #P < .001.*
Abbreviations: EA, electroacupuncture; PGB, pregabalin.

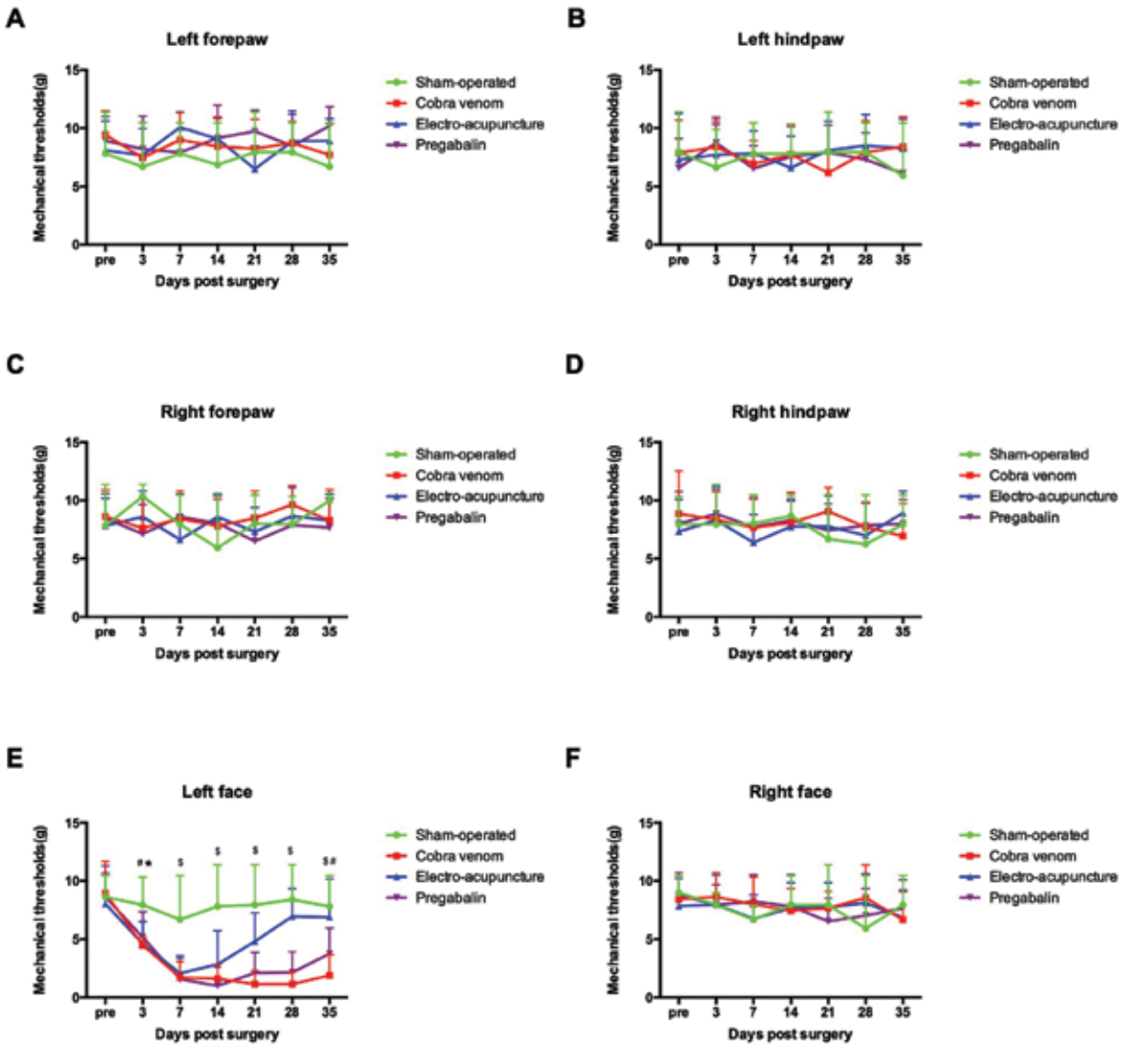

Fig. 3. *Measurements were taken at the left forepaw, right forepaw (B), left hindpaw (C), right hindpaw (D), left face (E), and right face (F). There were no significant differences among the 4 groups in the bilateral limb and contralateral face at any time point. The mechanical withdrawal latency of the left facial skin in the cobra venom group showed remarkable decrease after operation in comparison to the sham-operated group at postoperative day 3 (H = 13.333, adjusted P = .043). At postoperative day 21 to day 35, only electroacupuncture treatment improved the ipsilateral facial skin (postoperative day 21: H = 13.778, adjusted P = .033; postoperative day 28: H = 18.056, adjusted P = .002; postoperative day 35: H = 16.611, adjusted P = .005). Data are presented as median and ranges. *, #, $P < .05*

After cobra venom microinjection, however, the mechanical withdrawal latency of the ipsilateral facial skin progressively decreased compared to the sham-operated group, suggesting a reduced mechanical pain threshold on postoperative day 7. In the electroacupuncture and PGB group, significant recovery was observed after electroacupuncture treatment for 2 weeks. Conversely, no such beneficial effects were noted when PGB was administered. In addition, similar changes were observed in the results of video recording. There were no significant differences among the 4 groups in exploratory frequency and length or

grooming frequency and length before the operation and on postoperative day 3. Rats microinjected with cobra venom demonstrated a significant reduction in exploratory frequency and length compared to the sham-operated group; however, the grooming frequency and length in the cobra venom group were only increased on postoperative day 7 compared to the sham-operated group. In the electroacupuncture group, the exploratory frequency and length were increased compared to the cobra venom group and the grooming frequency and length were reduced on postoperative day 28. However, no such beneficial effects were noted when PGB was administered as shown in Fig. 4.

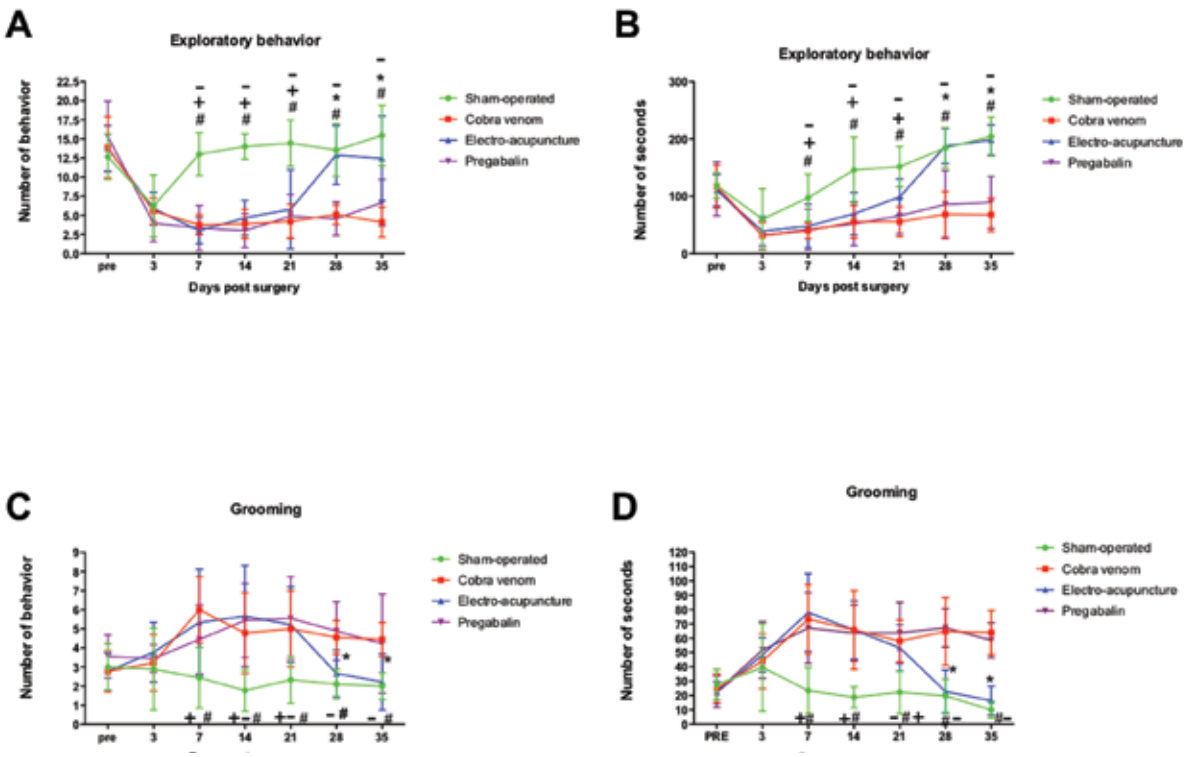

Fig. 4. *Frequency and length of observed face-grooming and exploratory behavior were observed before the operation (pre) and at 7 postoperative time points. Note: (A-D) No significant baseline differences between groups were found before the 3 days after the operation ($P > .05$). On postoperative day 7, the frequency and length of exploratory behavior of rats in the cobra venom group showed an obvious decrease compared to the rats in the sham-operated group (comparison between cobra venom and sham-operated groups, frequency: #$P = .006$, length: #$P < .001$; comparison between EA and sham-operated groups, frequency: +$P = .022$, length: +$P < .001$; comparison between PGB and sham-operated groups, frequency: -$P = .008$, length: -$P < .001$), while the frequency and length of grooming behavior showed an increase in comparison to the rats in the sham-operated group (comparison between cobra venom and sham-operated groups, frequency: #$P = .004$, length: #$P < .001$; comparison between EA and sham-operated groups, frequency: +$P = .025$, length: +$P < .001$; comparison between PGB and sham-operated groups, frequency: -$P = .002$, length: -$P = .180$). On postoperative day 28 after EA and PGB treatment, significant differences were observed compared to the cobra venom group (comparison between cobra venom and sham-operated groups, frequency of exploratory behavior: #$P = .002$, length of exploratory behavior: #$P < .001$, frequency of grooming behavior: #$P < .001$, length of grooming behavior: #$P < .001$; comparison between EA and cobra venom groups, frequency of exploratory behavior: *$P = .007$, length of exploratory behavior: *$P < .001$, frequency of grooming behavior: *$P < .001$, length of grooming behavior: *$P < .001$). Data represent mean ± standard deviation. *, -, +, #$P < .05$.*
Abbreviations: EA, electroacupuncture; PGB, pregabalin.

Cobra Venom Microinjection Caused Ultrastructural Changes at Ventral Posterolateral Thalamic Nucleus

As shown in Fig. 5 and Fig. 6, normal neuronal cell body structures were observed under transmission electron microscopy (TEM) with clear endoplasmic reticulum, golgi body, intact outer nuclear membranes, and mitochondria on both the ipsilateral (Fig. 5A) and contralateral (Fig. 6A) thalamic nuclei in the sham-operated group. However, obviously abnormal indented neuronal nuclei, damaged mitochondria and endoplasmic reticulum, and dissolved surrounding tissues were observed on the ipsilateral ventral posterolateral thalamic nucleus (Fig. 5B) compared to the sham-operated group. Additionally, no abnormal changes were observed in the contralateral thalamic nucleus (Fig. 6B) compared to the sham-operated group mentioned previously. As for the ultrastructural changes in the PFC and hippocampus, there were no obvious differences in neurons compared to the normal tissues as shown in Fig. 7.

Electroacupuncture But Not Pregabalin Treatment Reversed Ultrastructural Alterations in Ventral Posterior Thalamic Nucleus After Local Venom Injection

In electroacupuncture-treated rats, TEM observation showed complete and normal neuronal cell bodies as well as intact endoplasmic reticulum and mitochon-

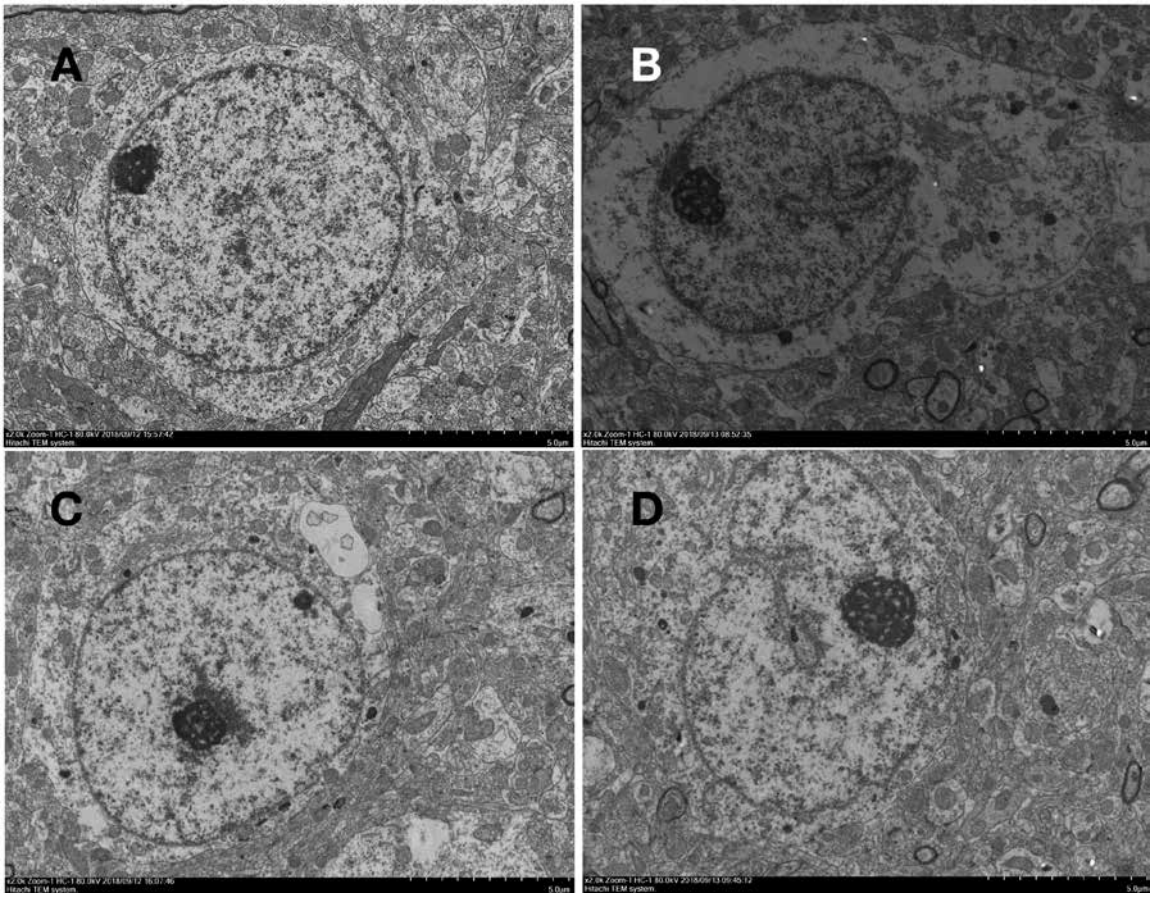

Fig. 5. *Ultrastructural changes of thalamic nucleus in 4 groups. Notes: A: Complete and clearly visible cell body, endoplasmic reticulum, and mitochondria in the left VPL in the sham-operated group. B: The indented cell body (marked by arrows) and damaged surrounding tissues (marked by stars) were observed in the left VPL in the cobra venom group. Similarly, C: Normal cell body in the EA group, and mild damage in the endoplasmic reticulum were also observed. D: PGB group displayed smaller lesions compared to the cobra venom group. Scale bars = 2.0 μm.*
Abbreviations: EA, electroacupuncture; PGB, pregabalin; VPL, ventral posterolateral nucleus.

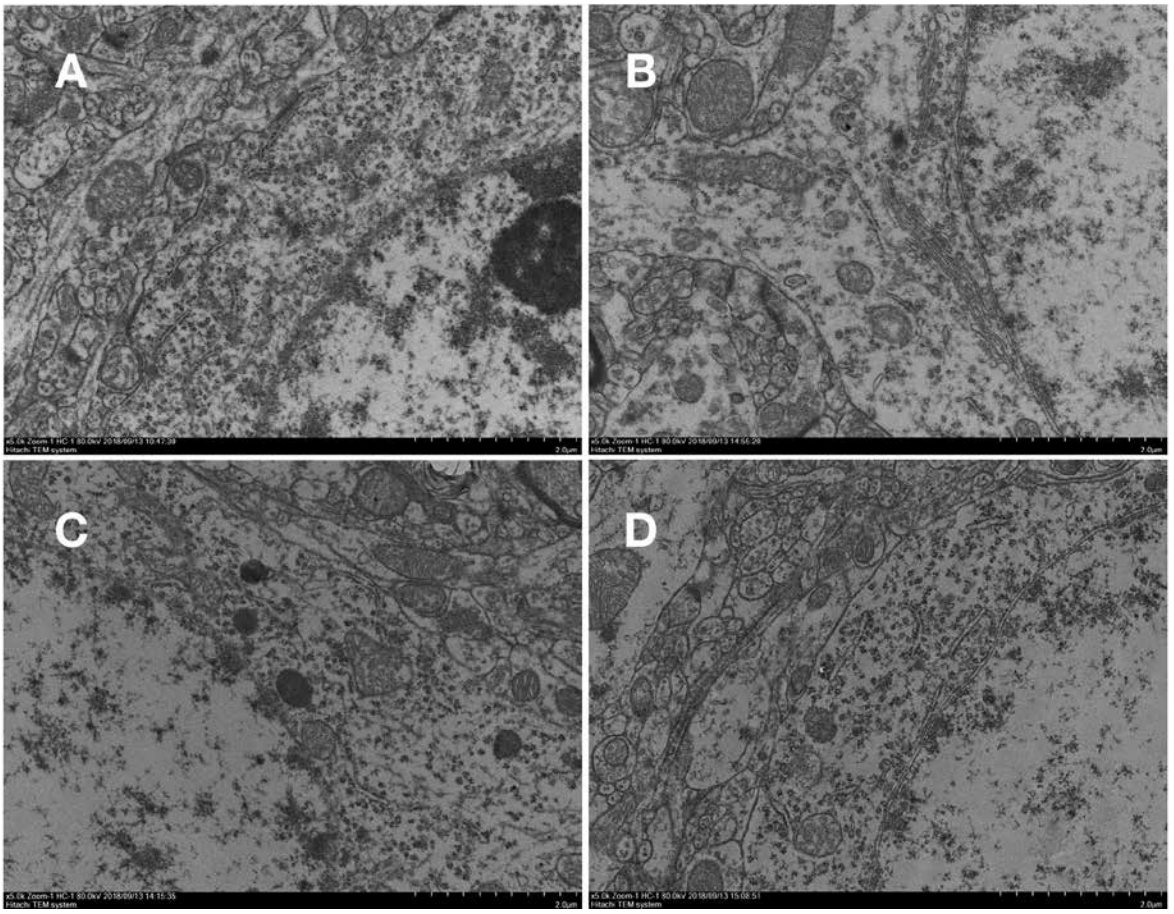

Fig. 6. *Normal cell body and surrounding tissues were observed in the contralateral VPL among all groups. In addition, A, B, C, and D show the ultrastructural changes and represent the contralateral thalamic nucleus in the sham-operated, cobra venom, EA, and PGB groups, respectively. Scale bars = 2.0 μm.*
Abbreviations: EA, electroacupuncture; PGB, pregabalin; VPL, ventral posterolateral nucleus.

dria on the ipsilateral (Fig. 5C) ventral thalamic nucleus on postoperative day 35. The PGB-treated rats did not show any improvement (Fig. 5D). Partial cytoplasm dissolution and alteration of the neuronal cell body and mitochondria were still visible.

Discussion

In the present study, we developed a novel experimental model of thalamic pain following the microinjection of cobra venom to the left VPL in the rat. Thalamic pain rats, but not saline-injected rats, exhibited mechanical allodynia in the left facial skin that was progressive over 35 days after injection, with detectable motor dysfunction.

There are other recently developed animal models of thalamic pain. In 2009, the hemorrhage model was reported by Wasserman and Koeberle (4). This collagenase-injected model was the most widely accepted to understand the mechanisms of thalamic pain and demonstrated the limited efficacy of many potential therapeutic drugs (18-22). In those studies, the pain threshold was significantly decreased in response to mechanical stimuli to the bilateral hind paws compared with a sham-operated group. Similarly, the paw withdrawal threshold in response to mechanical stimuli of bilateral hind paws were also significantly decreased in global cerebral ischemic (BCAO) (8) and autologous blood-injected models (6). The results of thermal and

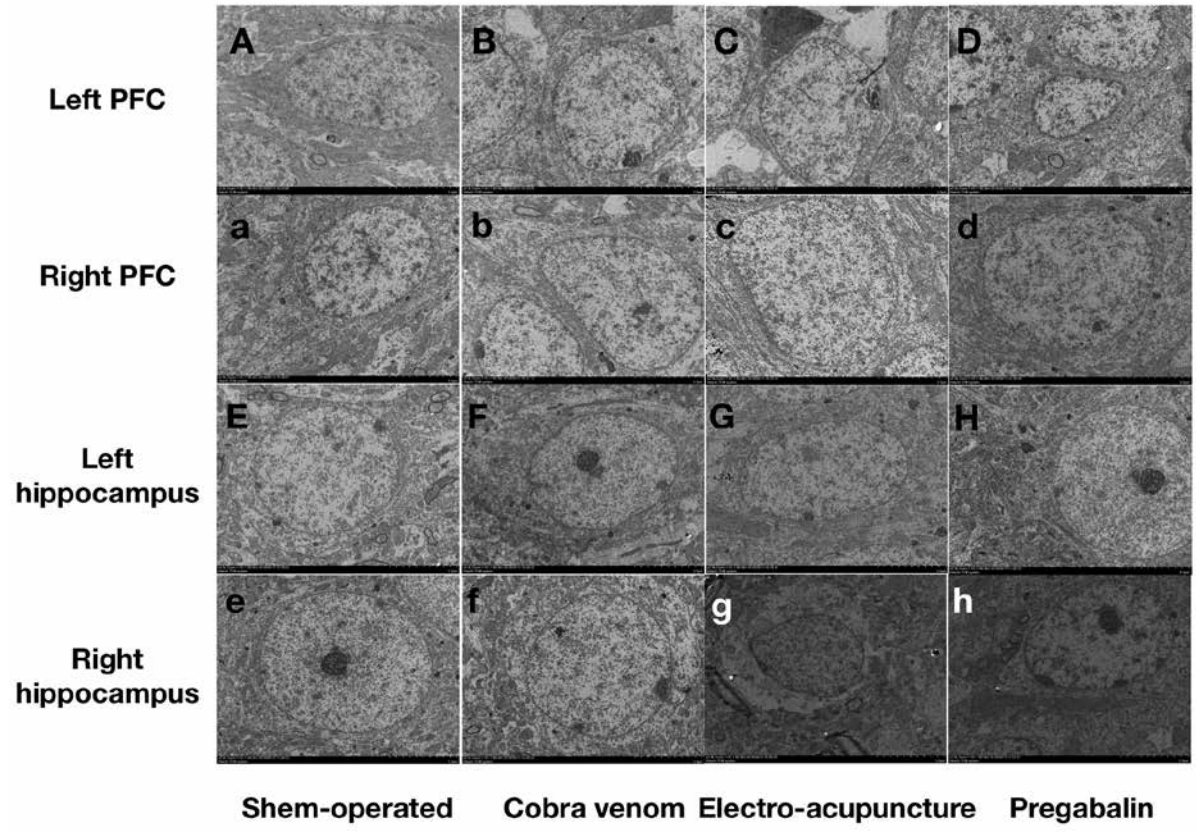

Fig. 7. *Ultrastructural changes in the PFC and hippocampus in the 4 groups. There are no obvious differences in the PFC and hippocampus compared to the ultrastructural changes in neurons of normal tissues. Scale bars = 5.0 μm.*
Abbreviation: PFC, prefrontal cortex

cold withdrawal latency demonstrated a variety of responses across different laboratories, although cold allodynia is one of the few diagnostic criteria for CPSP in patients.

The distribution of pain in patients with thalamic pain can range from a small, discrete lesion to large areas, and the neuropathic pain symptoms usually occur on one side of the body (3). Therefore, results of previous studies mentioned above may not be fully congruent with typical clinical observations. The endothelin-1-injected ischemic model (5) had the curtate thermal withdrawal latency on the contralateral hind paw, and the middle cerebral artery occlusion (MCAO) model (23) had the decreased mechanical withdrawal thresholds on the ipsilateral hind paw. Although these models exhibited typical pain symptoms on only the right or left side of the body, the ischemic model did not characterize cold or chemical hypersensitivity or allodynia. Further, in the MCAO model, not only the thalamus but multiple brain regions including the cerebral cortex and amygdala were damaged. Overall, these studies suggest that these models do not completely recapitulate the clinical characteristics of humans with thalamic pain.

In comparison, the uniqueness of the present model is the combination of chronic ipsilateral facial pain and motor dysfunction in contralateral forelimbs. Indeed, chronic facial pain in this rat model of thalamic pain has never been reported according to our literature research, and previous attempts were only focused on the limbs. It was reported that the prevalence of chronic facial pain in patients with CPSP was between 51.61% and 68.75% (24,25), with or without involvement of the limbs. A previous study showed that the

distribution of pain symptoms was probably related to the location of pathology in patients with CPSP. The distribution of CPSP in infratentorial and brainstem lesions has been reported to involve the face and VPL lesion hemi-body, including or excluding the face (26). However, there were no pathological descriptions in patients with thalamic pain.

Although the mechanism of thalamic pain has not been fully elucidated, several theories have been proposed such as disinhibition theory (27), central imbalance theory (28), and neuronal excitability theory (29). In the present study, after cobra venom microinjection, we found that the ultrastructure of neurons was destroyed in the cell body, endoplasmic reticulum, and mitochondria of the ipsilateral thalamus. And a previous study revealed that the neurons injured directly in the central nervous system could result in neuronal hyperexcitability (30). Therefore, it is hypothesized that the signs of pain and motor dysfunction are likely associated with neurons damaged but not destroyed in the thalamus. Indeed, the cobra venom may exert adverse effects including those leading to the changes in cell cycle regulators, limiting cell proliferation and migration and inducing cellular senescence when used at relatively subtoxic concentrations (11). However, we did not observe mechanical hypersensitivity in the limbs in our model, the reason of which might be associated with the location of the injured territory, but one animal experiment cannot reveal the exact relationship between the distribution of pain symptoms and the location of damaged areas in rats. Further study is needed to clarify the factors contributing to the thalamic pain induced by cobra venom.

In this study, electroacupuncture treatment was able to ameliorate neuropathic pain and motor dysfunction, and this effect of electroacupuncture on CPSP has been reported in humans and rodents. Electroacupuncture, which is different from traditional acupuncture, is a new type of therapy in which a long needle inserted into a specific acupoint is attached to a pulse current with the purpose of combining acupuncture with electric current stimulation. Electroacupuncture was reported to effectively relieve CPSP by inhibiting the apoptosis of brain neurons and the activation of aberrant astrocytes in the brain (31). Clinically, both electroacupuncture and transcutaneous electrical nerve stimulation (TENS) belong to acupuncture-like stimulation devices (ASDs). They are similar in both mechanism and effectiveness. However, electroacupuncture treatment has been gradually replaced by TENS, because TENS is a therapy with noninvasive pain compared with electroacupuncture treatment (32). TENS has also been suggested to have temporary effects on CPSP patients, but its efficacy has not been clearly demonstrated (33). However, treatment with PGB did not provide the relief of pain and motor dysfunction in this study. Previous studies demonstrated that anticonvulsants such as gabapentin and PGB have no effects in the long-term treatment of thalamic pain (18,19,34). Hanada et al showed that PGB failed to affect mechanical allodynia and thermal hyperalgesia in a mouse model of CPSP (18). Yang and his colleagues also found that gabapentin insensitivity can occur following long-term treatment in rats with CPSP (19). This phenomenon has also been noted in clinical trials (34). Moreover, we also found that electroacupuncture but not PGB treatment reversed the ultrastructural alterations associated with the thalamic pain rat model induced by cobra venom. This phenomenon is consistent with our previous studies (13,35).

Limitations

Our model had some limitations as well. Firstly, we observed the behavioral outcomes of mechanical hypersensitivity only on the ipsilateral facial skin, which are not congruent with some other thalamic pain models. The reasons for the difference in behavioral outcomes are likely due to the different lesion site in the thalamus (26) and to the fact that detailed examination of lesion localization was not performed. This represents a deviation from other models and warrants further discussion. Moreover, thermal and cold hyperalgesia were not assessed among the 4 groups. Secondly, it was reported that thalamic pain can be triggered by not only ischemic but hemorrhagic infarct in the spinothalamic pathway (2,36). The pathogenic factors were different from the present model and the patients in clinical practice. Thirdly, in this study, we hypothesized that the sign of pain in ipsilateral facial skin resulted from neuronal hyperexcitability in the VPL induced by cobra venom; however, as a pilot study, we did not perform corresponding confirmatory experiments. Further studies, specifically electrophysiological experiments in the thalamic area of the brain, are warranted.

Conclusion

In conclusion, this is the first report of the application of cobra venom to establish an animal model of thalamic pain that is characterized by ipsilateral facial

mechanical hypersensitivity as well as motor dysfunction in the contralateral forelimbs. This cobra venom model may be useful for investigating the pathology and mechanisms of thalamic pain.

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