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Original Article

Differential modulation of electrical stimulation of periaqueductal gray and thalamus on nociceptive behaviors of rats

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Abstract: Deep brain stimulation (DBS) is a surgical treatment which has shown remarkable therapeutic benefits for patients with a variety of neurologic conditions. As an important application, DBS has been used to treat intractable pain for over 60 years. Clinical studies have revealed that the selection of the stimulation sites depended on the types of pain. In this study, we selected ventrolateral periaqueductal gray (vlPAG) and ventral posterior lateral nucleus (VPL) as the target brain areas, which were widely used in clinical treatment of refractory pain, to clarify and compare the effects of vlPAG and VPL stimulation on different models of pain. Acute pain was evoked by thermal stimulation. The chronic inflammatory pain was produced by complete Freund's adjuvant (CFA) injection, while neuropathic pain was induced by spinal nerve ligation (SNL) surgery. Some important results emerged from this study: (1) in the experiment of normal rats, we found that unilateral vlPAG stimulation could lead to a significant increase of the thermal withdrawal threshold in bilateral hindpaws of rats, which means a significant bilateral analgesic action; (2) in the CFA test, both contralateral vlPAG and VPL stimulation significantly alleviated the thermal hyperalgesia, which exhibited analgesic effects to the chronic inflammatory pain; (3) in the SNL experiment, the results revealed that contralateral VPL stimulation could significantly abolish the mechanical allodynia induced by SNL, indicating remarkable analgesic effect to neuropathic pain. But the vlPAG stimulation did not have any effect on the mechanical allodynia. These results suggest that the electrical stimulation of the PAG works more effectively on nociceptive pain, including acute pain and chronic inflammatory pain. Besides, the VPL stimulation is much more sensitive for chronic pain, including chronic inflammatory pain and neuropathic pain.

Key words: deep brain stimulation; chronic inflammatory pain; neuropathic pain; analgesia

中脑导水管周围灰质电刺激与丘脑电刺激对大鼠伤害感知行为的差异调节

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摘 要：深部脑刺激是一种广泛用于治疗中枢神经及精神疾病的功能型手术疗法。深部脑刺激在临床应用于疼痛治疗起源于半个多世纪以前，能够有效治疗多种类型的顽固疼痛，然而其作用机制尚不清楚。为了进一步探索其神经机制，首先需要建立合适的深部脑刺激治疗疼痛的动物模型。本研究在大鼠的中脑导水管周围灰质腹外侧区(ventrolateral periaqueductal gray, vlPAG)或丘脑腹后外侧核(ventral posterior lateral nucleus, VPL)埋置刺激电极，研究深部脑刺激对正常大鼠急性痛、完全弗式佐剂(complete Freund's adjuvant, CFA)注射引起的慢性炎症痛大鼠模型以及脊神经结扎 (spinal nerve ligation, SNL)手术引起神经病理痛大鼠模型的镇痛效果。主要结果如下：(1)在正常大鼠中，单侧vlPAG刺激能够显著提高双侧足底的热辐射痛阈，即产生显著的双侧镇痛作用；(2)在CFA建立的慢性炎症痛模型中，对侧vlPAG刺激和VPL刺激都能够显著提高CFA侧足底的热

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辐射痛阈，即产生显著的镇痛作用；(3)在SNL手术引发的慢性神经源性痛模型中，对侧VPL刺激能够显著提高SNL侧足底的机械痛阈，而vlPAG刺激对SNL引发的触诱发痛没有影响。以上结果提示，PAG刺激对于急性痛以及慢性炎症痛有着较好的镇痛效果，而VPL刺激更适合慢性炎症痛和慢性神经病理痛的镇痛研究。

关键词：深部脑刺激；慢性炎症痛；神经病理痛；镇痛
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Deep brain stimulation (DBS) is a widely used functional surgery therapy for the treatment of central neuronal and mental diseases. As an important application, it has been used to treat chronic intractable pain, such as neuropathic pain, phantom limb pain, facial pain and brachial plexus avulsion, for over 60 years [1, 2]. Many brain structures have been proved as the targets of DBS analgesia, including the septal area, caudate nucleus, periaqueductal gray (PAG), periventricular grey (PVG), ventral posterior lateral nucleus (VPL), medial ventral posterior thalamic nucleus (VPM) and other thalamic nuclei, such as the mediodorsal, centromedian, and parafascicular (Pf) nuclei. Although lots of valuable data have been collected for the analgesic mechanisms of DBS since the development of brain imaging techniques, such as fMRI and CT, the human experiments still have many restrictions on technique and feasibility. Therefore, animal studies became much more important to explore the neuronal mechanisms of DBS analgesia.

Studies have argued that the choice of the targeted areas depended on the type of pain and its distribution [3]. Morgan *et al*. have found that the stimulation on PAG could significantly attenuate the formalin pain and increase the pain threshold of complete Freund's adjuvant (CFA)-induced inflammatory pain [4, 5]. Iwata *et al*. have demonstrated that electrical stimulation within the VPL could effectively modulate some nociceptive phenomena associated with peripheral neuropathic pain [6]. Studies suggested that PAG stimulation is suitable for the nociceptive pain and VPL stimulation is better for those with neuropathic pain [7, 8]. However, given the different parameters of DBS in these studies, the results about the comparison of PAG and VPL stimulation may be inaccurate. Therefore, we employed similar conditions in this study to compare the analgesic effects between PAG and VPL on different pain models of rats, in order to determine the suitable and sensitive target regions for these pain models respectively.

1 MATERIALS AND METHODS

1.1 *Animals*

Male Sprague Dawley rats weighing between 230 to 250 g (Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China) served as subjects. All rats were housed individually on a 12:12 h light/dark cycle (lights on at 07:00) and had *ad libitum* access to food and water, with ambient temperature set at (23 ± 1) °C. Animals were allowed to acclimate for one week before experiments, and were handled daily by the experimenter. All experimental procedures were approved by the Institutional Review Board for Animal Care and Use of the Chinese Academy of Sciences.

1.2 *Experimental design*

Three experiments were performed. The first experiment investigated the effects of PAG (n = 8) and VPL (n = 8) stimulation on the acute pain. After one-week surgery recovery, paw withdrawal latencies (PWLs) of both hindpaws were tested before, during and after PAG/VPL stimulation. Stimulation was repeated three times for one rat. The ipsilateral and contralateral referred to the electrode site.

The second one examined the effects of VPL (n = 9) and PAG (n = 7) stimulation on the chronic inflammatory pain in rats with CFA injection. After one-week surgery recovery, CFA (Sigma) was injected into the contralateral paw as opposed to the side of electrode implantation. PWLs were assessed before and after CFA injection, to make sure that the rats developed thermal hypersensitivity. Six days after CFA injection, PWLs were tested before, during and after VPL or PAG stimulation.

The third one investigated the effects of VPL (n = 8) and PAG (n = 8) stimulation on the neuropathic pain in rats with spinal nerve ligation (SNL). Electrode implantation and SNL were operated at the same time. Withdrawal thresholds were assessed before (baseline) and after surgery. One week after the development of allodynia, withdrawal thresholds to mechanical force were assessed three times before, during and after the

stimulation. The sham groups were treated identical to the stimulation group except that no brain stimulation was given.

1.3 *Surgery*

Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus (Stoelting, USA). A twisted bipolar electrode (0.125-mm diameter/wire) with electrode tips was implanted into VPL, −3.0 mm posterior to bregma (A), 3.0 mm lateral to midline (L) and 6.0 mm ventral to the skull surface (V), and ventrolateral PAG (vlPAG, A: −7.4 mm, L: 0.7 mm, V: 5.6 mm) using the atlas of Paxinos and Watson[9].

Animal model of neuropathic pain was established by L5 SNL according to the procedure of Kim and Chung[10]. The skin was incised in the midline over the lumbar spine, and the left transverse process of the L6 vertebra was removed. The left L5 spinal nerve was isolated and tightly ligated with 3-0 silk thread.

1.4 *Pain tests*

Inflammatory pain was induced by intraplantar injection of 100 μL of CFA into the hindpaw contralateral to the site of electrode. The thermal pain thresholds were assessed using radiant heat stimulation. The definition of PWL was the length of time between the light onset and the paw lift. Before brain stimulation, three trials were conducted with 10 min interval to test the PWL. After the baseline test, each rat received 3 times of brain stimulation, during which PWL was tested. Then PWL was assessed at 10, 20, 30 min after 3 times of stimulation.

Mechanical sensitivity was assessed with Von Frey electronic apparatus (Bioseb) using a metallic tip to estimate the allodynia. The mechanical force was applied onto the plantar side of hind paw until paw withdrawal. The minimal force that caused paw withdrawal was considered as mechanical withdrawal threshold. Before stimulation, three trials were conducted with 10 min interval. Each rat received 3 times of brain stimulation, during which withdrawal threshold was measured. Then withdrawal threshold was tested at 10, 20, 30 min after 3 times of brain stimulation.

1.5 *Stimulation procedure*

Charge-balanced pulses were programmed into the DS-8000 digital stimulator and DLS-100 digital linear isolator (World Precision Instrument). The stimulation parameter was as follows: 50 Hz (frequency), 0.4 ms counterbalanced by 20% current, 2 ms opposite current (pulse width, 0.4 ms + 2 ms), 25 μA (intensity), 10 s duration for experiment 1 and experiment 2, 60 s duration for experiment 3. Brain stimulation was initiated 10 s prior to and remained on throughout thermal tests and was given 3 times for each rat with an interval of 10 min.

1.6 *Histology*

After termination of the experiment, the rats were overdosed with chloral hydrate. The stimulation sites were marked by electrophoretically deposited iron (10–20 μA DC current, 10–20 s duration, anode current) at the tips of electrodes. Animals were perfused through the heart with the solution of 5% potassium ferrocyanide and 4% paraformaldehyde. The brain was post-fixed with the same solution and equilibrated in 20% and 30% sucrose for several days. Then the brain was sectioned at 40 μm in the coronal plane. The sections were mounted on glass slides and observed under a light microscope. The markings of recording sites were easily identified as blue dots.

1.7 *Data analysis*

Statistical comparisons and graphs were done by Statistica 10.0 and GraphPad prism 5.0 softwares. Repeated-measures analysis of variance (RM-ANOVA) followed by Newman-Keuls *post hoc* test was used to evaluate the data. For the data did not exhibit equal variation, we used the Greenhouse-Geisser correction to correct them. Data were presented as means ± SEM. A significant level was considered when $P < 0.05$.

2 RESULTS

2.1 Histological localization of stimulation sites

Figure 1 depicts the location of the recording electrodes in this study. As indicated by arrows, in the PAG, iron deposit was found in the ventrolateral area; in the thalamus, the tip was located in the ventroposterior part.

2.2 Effects of vlPAG and VPL stimulation upon thermal-induced acute pain

We found that vlPAG stimulation induced significant analgesic effects in bilateral hindpaws of rats, which maintained for 20 min (treatment effect: $F_{(1,224)} = 77.87$, $P < 0.001$; side effect: $F_{(1,224)} = 0.17$, $P = 0.684$; time effect: $F_{(4.61,129.09)} = 15.19$, $P < 0.001$, Greenhouse-Geisser-corrected). The results are shown in Fig. 2*A*. We also compared the PWLs pre-, during and post-stimulation in vlPAG (side effect: $F_{(1,28)} = 0.406$; $P = 0.534$, time effect: $F_{(2,28)} = 41.9$; $P < 0.001$, interac-

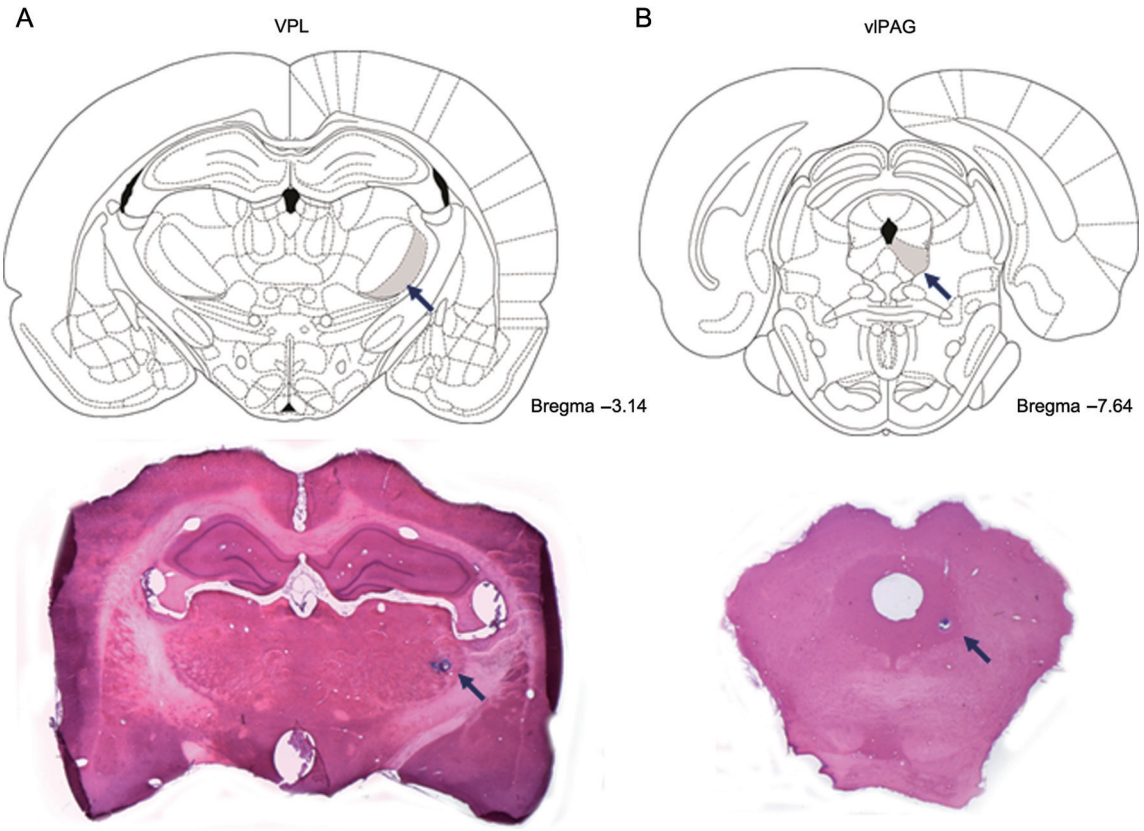

Fig. 1. Histological localization of stimulation sites. *A*: The location of DBS electrodes within the VPL. The gray shaded area marks the VPL for electrodes implantation. The lower panel showed the histochemical staining of the brain section with hematoxylin and eosin. The tip of electrode is indicated by the arrow. *B*: The location of DBS electrodes within the vlPAG. The gray shaded area marks the vlPAG for electrodes implantation. The tip of electrode is indicated by the arrow. DBS: deep brain stimulation; VPL: ventral posterior lateral nucleus; vlPAG: ventrolateral periaqueductal gray.

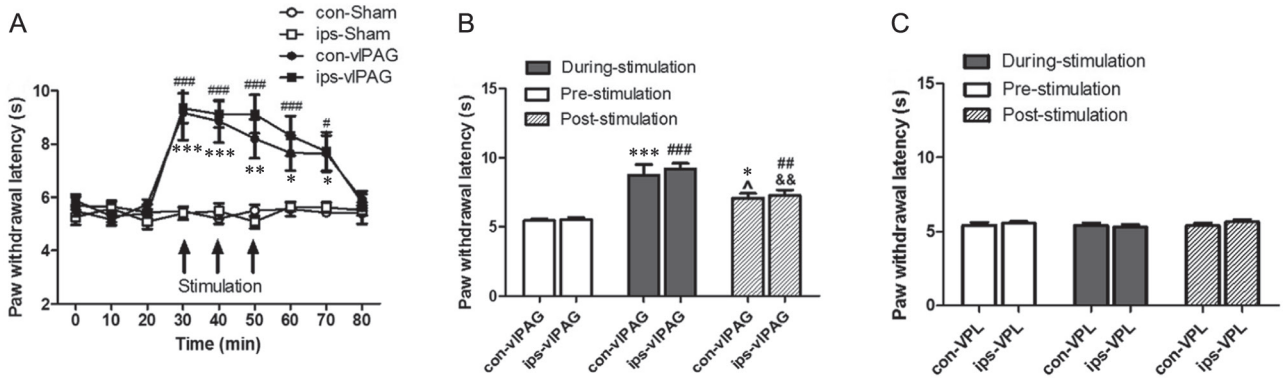

Fig. 2. Effects of vlPAG/VPL stimulation on the PWLs in the thermal acute pain tests. *A*: vlPAG stimulation produced a significant increase in the PWLs of the contralateral and ipsilateral paws compared to sham controls. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with con-sham group; $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ compared with ips-sham group. $n = 8$. *B*: vlPAG stimulation produced a significant increase in the PWLs during and after the stimulation compared with pre-stimulation. $^{***}P < 0.001$, $^{*}P < 0.05$ compared with pre-stimulation of con-vlPAG group; $^{###}P < 0.001$, $^{##}P < 0.01$ compared with pre-stimulation of ips-vlPAG group; $^{\wedge}P < 0.05$ compared with during-stimulation of con-vlPAG group; $^{\&\&}P < 0.01$ compared with during-stimulation of ips-vlPAG group. $n = 8$. *C*: No analgesic effects were observed with DBS of VPL. PWLs: paw withdrawal latencies; DBS: deep brain stimulation; VPL: ventral posterior lateral nucleus; vlPAG: ventrolateral periaqueductal gray; con: contralateral to the electrode implantation; ips: ipsilateral to the electrode implantation.

tion effect: $F_{(2,28)} = 0.138$; $P = 0.871$). As shown in Fig. 2*B*, during and post the stimulation of vlPAG, the PWLs were significantly increased compared with these of pre-stimulation bilaterally (Contralateral of vlPAG: during *vs.* pre-stimulation, $P < 0.001$, post- *vs.* pre-stimulation, $P < 0.05$; Ipsilateral of vlPAG: during *vs.* pre-stimulation, $P < 0.001$; post- *vs.* pre-stimulation, $P < 0.01$). However, the PWLs post-stimulation of vlPAG were still significantly less than these during stimulation bilaterally (Contralateral of vlPAG: $P < 0.05$; Ipsilateral of vlPAG: $P < 0.01$). As shown in Fig. 2*C*, the stimulation in VPL did not induce any significant analgesic effect on either contralateral or ipsilateral hindpaws of rats (all $P > 0.05$). The above results suggested that stimulation in vlPAG could produce significant analgesic effects upon the thermal-induced acute pain in bilateral hindpaws and maintained the effects for about 20 min. But the stimulation in VPL could not evoke any analgesic effect during acute thermal pain.

2.3 Effects of vlPAG and VPL stimulation upon CFA-induced chronic inflammatory hyperalgesia

In this experiment, we firstly evaluated the validity and reliability of the animal model of CFA-induced inflam-

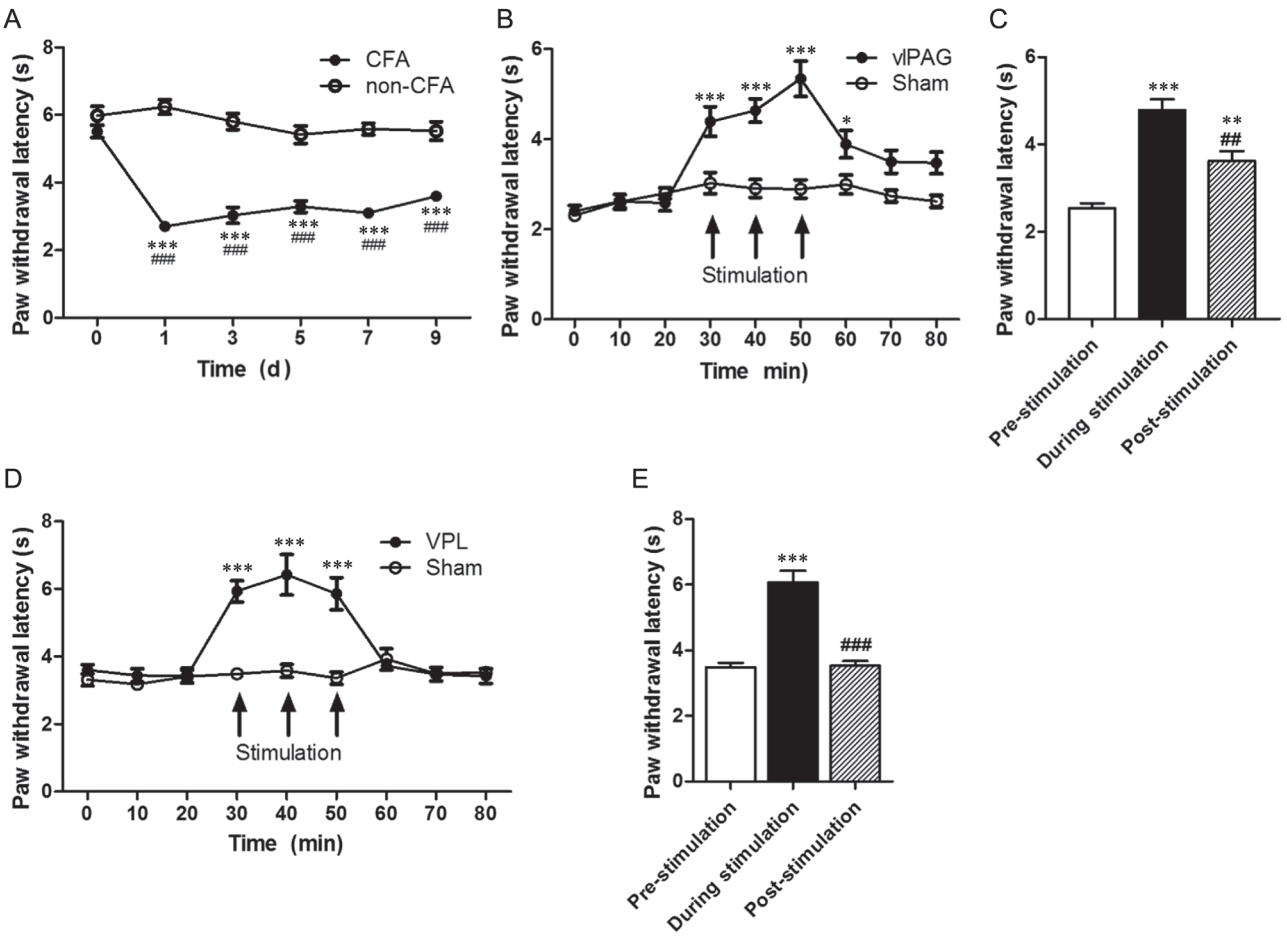

Fig. 3. Effects of vlPAG/VPL stimulation on CFA-induced thermal hyperalgesia. *A*: The time course of CFA-induced thermal hyperalgesia. Noxious radiant heat was delivered to the CFA and non-CFA injected paws. Following the injection of CFA, there was a significant decrease in PWL of the injected paw compared to the pre-CFA baseline and the non-injected paw. $^{***}P < 0.001$, CFA side *vs* non-CFA side; $^{###}P < 0.001$, post- *vs* pre-CFA injection. *B*: Effects of vlPAG DBS on the thermal nociceptive thresholds in rats with CFA-induced inflammatory pain. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with sham group, $n = 7$. *C*: The comparison for PWLs pre-, during and post-vlPAG stimulation. $^{***}P < 0.001$, $^{**}P < 0.01$ compared with pre-stimulation; $^{##}P < 0.01$ compared with during stimulation. $n = 7$. *D*: Effects of VPL DBS on the thermal nociceptive thresholds in rats with CFA-induced inflammatory pain. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with sham group. $n = 9$. *E*: The comparison for PWLs pre-, during and post-VPL stimulation. $^{***}P < 0.001$ compared with pre-stimulation; $^{###}P < 0.001$ compared with during stimulation. $n = 9$. PWLs: paw withdrawal latencies; DBS: deep brain stimulation; VPL: ventral posterior lateral nucleus; vlPAG: ventrolateral periaqueductal gray.

matory pain. Fig. 3*A* shows the time course of thermal hyperalgesia related to CFA injection. Before the CFA injection, there was no significant difference in the PWL among all groups of rats and between left and right hindpaws. Following the injection of CFA, there was a significant decrease in PWL of the injected paw compared to the pre-CFA baseline and the non-injected paw (lateral effect: $F_{(1,150)} = 147.7$, $P < 0.001$; time effect: $F_{(5,150)} = 18.6$, $P < 0.001$; lateral × time interaction effect: $F_{(5,150)} = 16.6$, $P < 0.001$). The thermal hyperalgesia started at day 1 post-CFA and persisted through day 9.

The vlPAG stimulation produced significant analgesic effect on the inflamed hindpaws, which persisted for about 10 min after the stimulation (treatment effect: $F_{(1,96)} = 45.0$, $P < 0.0001$; time effect: $F_{(4.62,55.41)} = 15.5$, $P < 0.001$, Greenhouse-Geisser-corrected; interaction effect: $F_{(4.62,55.41)} = 8.24$, $P < 0.001$, Greenhouse-Geisser-corrected). *Post-hoc* comparison between stimulation and sham groups showed that the PWLs were significantly increased during and after the stimulation time (at 30, 40 and 50 min, all $P < 0.001$; 60 min, $P < 0.05$). These results were shown in Fig. 3*B*. As shown in Fig. 3*C*, we compared the mean PWLs pre-, during and post-stimulation. Stimulation of vlPAG produced strong analgesic effect (one-way ANOVA, $F_{(2, 12)} = 27.6$; $P < 0.001$). Newman-Keuls *post-hoc* analysis indicated that during the stimulation, the PWLs were significantly longer than these of pre-stimulation (during- *vs* pre-stimulation: $P < 0.001$). Besides, PWLs after stimulation were significantly decreased but still longer than these of pre-stimulation [post- *vs.* during stimulation: (3.6 ± 0.2) s *vs.* (4.8 ± 0.3) s, $P < 0.01$; post- *vs*. pre-stimulation: (3.6 ± 0.2) s *vs.* (2.5 ± 0.1) s, $P < 0.01$].

Similarly to the PAG DBS, the VPL stimulation also

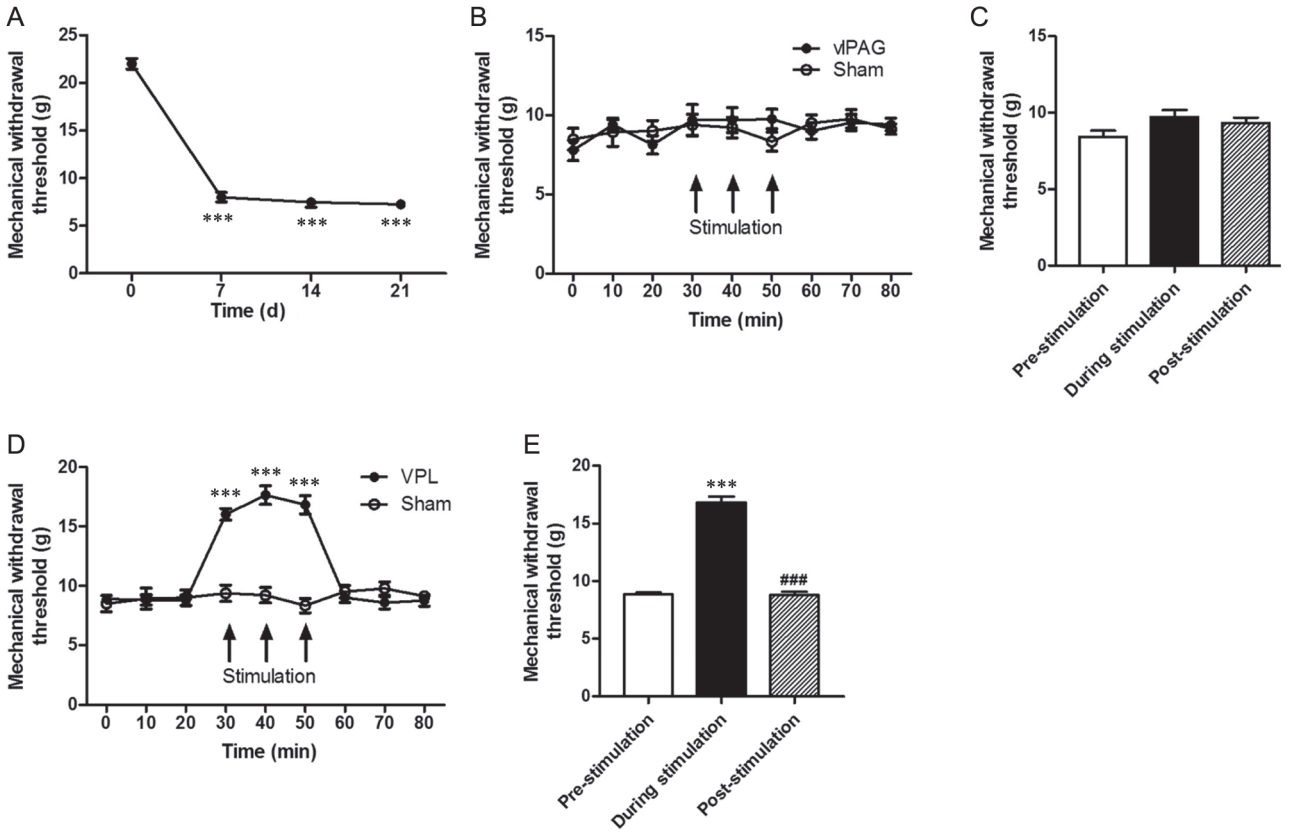

Fig. 4. Effects of vlPAG/VPL stimulation on spinal nerve ligation (SNL)-induced allodynia. *A*: Time course of SNL-induced allodynia. The mechanical withdrawal thresholds (MWTs) of the post-operation period was significantly lower than those of the pre-operation baseline, which started from day 7 post-operation and persisted until day 21. $^{***}P < 0.001$ *vs* 0 day. *B*: vlPAG DBS had no effects on the MWTs in rats with neuropathic pain. *C*: The comparison for MWTs pre-, during and post-vlPAG stimulation. *D*: Effects of VPL DBS on the MWTs in rats with SNL. $^{***}P < 0.001$ compared with sham group. $n = 8$. *E*: The comparison for MWTs pre-, during and post-VPL stimulation. $^{***}P < 0.001$ compared with pre-stimulation; $^{###}P < 0.001$ compared with during stimulation, $n = 8$. DBS: deep brain stimulation; VPL: ventral posterior lateral nucleus; vlPAG: ventrolateral periaqueductal gray.

produced analgesic effect on the inflammatory pain (treatment effect: $F_{(1,128)} = 22.8$, $P < 0.001$; time effect: $F_{(4.58,73.28)} = 15.5$, $P < 0.001$, Greenhouse-Geisser-corrected; interaction effect: $F_{(4.58,73.28)} = 14.7$, $P < 0.001$, Greenhouse-Geisser-corrected). *Post-hoc* comparison between stimulation and sham groups showed that the PWLs were significantly increased during the stimulation period (at 30, 40 and 50 min, all $P < 0.001$). It should be noted that the analgesic effect did not persist and disappeared immediately after the stimulation, as shown in Fig. 3*D*. Figure 3*E* showed the comparison of the mean PWLs pre-, during and post-stimulation using the stimulation in VPL. VPL DBS produced significant analgesic effect but did not exhibit post-effect (one-way ANOVA, $F_{(2, 16)} = 50.12$; $P < 0.001$).

2.4 Effects of vlPAG and VPL stimulation upon SNL-induced allodynia

The analysis on von Frey pain test revealed a strong effect of SNL surgery (one-way ANOVA, $F_{(3,21)} = 233.6$; $P < 0.001$). A strong drop in the mechanical withdrawal thresholds of SNL rats was found in comparison to the baseline level and the low-level thresholds maintained throughout the observation period (all $P < 0.001$), indicating the development of chronic neuropathic pain. The results were shown in Fig. 4*A*.

As shown in Fig. 4*B*, stimulation in vlPAG could not influence the mechanical allodynia (all $P > 0.05$). Figure 4*C* compared the mean mechanical withdrawal thresholds pre-, during and post-stimulation in vlPAG (one-way ANOVA, $P > 0.05$).

It was found that VPL stimulation could significantly influence analgesic effects on SNL-induced mechanical allodynia (treatment effect: $F_{(1, 112)} = 27.71$, $P < 0.0001$; time effect: $F_{(5.33, 74.67)} = 29.53$, $P < 0.001$, Greenhouse-Geisser-corrected; interaction effect: $F_{(5.33, 74.67)} = 32.17$, $P < 0.001$, Greenhouse-Geisser-corrected). Newman-Keuls *post-hoc* comparison between stimulation and sham groups showed that the mechanical withdrawal thresholds were significantly increased during the stimulation period (30, 40 and 50 min from the test beginning, all $P < 0.001$), as shown in Fig. 4*D*. We also compared the mean mechanical withdrawal thresholds pre-, during and post-stimulation of VPL and found strong analgesic effects during the VPL stimulation (one-way ANOVA, $F_{(2, 14)} = 244.6$; $P < 0.001$). Newman-Keuls *post-hoc* analysis indicated that during the stimulation, the mechanical withdrawal thresholds were significantly longer than that of pre-stimulation (pre-stimulation *vs.* during stimulation: $P < 0.001$, increased by nearly 90%). However, there was no significant difference in the mechanical withdrawal thresholds between post- and pre-stimulation. The results demonstrated that VPL stimulation could inhibit the allodynia induced by SNL, but without persistent effect.

3 DISCUSSION

In present study, we investigated the effects of DBS in vlPAG and VPL on thermal nociception, CFA-induced hyperalgesia and SNL-induced allodynia. Results revealed that stimulation of PAG exhibited decreased pain sensitivity to thermal stimulation under both normal and chronic inflammatory pain conditions. We also found that VPL stimulation could attenuate the thermal hyperalgesia induced by CFA and mechanical allodynia induced by SNL. These findings confirmed previous reports that stimulation of PAG is much more useful in cases of nociceptive pain[4, 5, 7]. More importantly, we suggested that VPL stimulation may not only suitable for treatment of chronic neuropathic pain but also could be used in chronic inflammatory pain.

3.1 The different roles of PAG stimulation on nociceptive and neuropathic pain

In 1969, Reynolds found that PAG stimulation could induce significant analgesic effect in rats, which was a very important finding in the modern research of pain [11] and led to the definition of brainstem pain modulatory network. Soon after, Mayer *et al*. proved that stimulation of the mesencephalic central gray matter and periventricular gray matter greatly reduced or totally abolished responsiveness to noxious stimuli [12]. Besides, Fardin *et al*. have clearly distinguished stimulation-produced-analgesia (SPA) from ventral PAG *versus* dorsal PAG and found that the ventral PAG seems to be more preferentially involved in pain modulation [13], which was the reason why we chose vlPAG as a target area.

It is well known that PAG is an important region of the descending inhibitory system for nociceptive inputs. The PAG projects to rostral ventromedial medulla (RVM) and locus coeruleus (LC), which could modulate spinal pain transmission. Therefore, researchers considered that activation of PAG could mobilize the endogenous inhibitory system to induce analgesic effect[14]. Human imaging studies have proved the activation of PAG during the application of noxious

stimuli [15–17]. Animal studies also found similar results. Silva *et al*. have proved that injection of formalin into hindpaw rats could increase glutamate, arginine and aspartate concentration in PAG, which suggested a rapid excitation of the PAG during noxious stimulation [18]. Studies have also found that PAG contains a high density of micro-opioid receptor [19]. It has been revealed that microinjection of morphine into the vlPAG could produce antinociception of rats, in both normal and chronic inflammatory pain [20, 21]. And animal experiments also proved that naloxone could reverse the stimulation produced analgesia [22, 23].

The above theories could explain the inhibitory effect of PAG stimulation on nociceptive pain, including normal and CFA-induced inflammatory pain conditions. In addition, we have found that PAG stimulation could bilaterally decrease the pain sensitivity in the experiment of acute thermal pain. This result was similar with clinical phenomenon that electrical stimulation of PAG could bilaterally treat intractable pain [24, 25] and also proved that the midbrain descending inhibitory system was transmitted bilaterally. Besides, in this study, PAG stimulation did not produce any influence on SNL-induced allodynia. SNL is a classic animal model for chronic neuropathic pain[10]. Studies have suggested that the efficacy of PAG stimulation on neuropathic pain was not consistent. Levy *et al*. have reported that only 23% patients could achieve long-term success when PAG was stimulated for neuropathic pain [26, 27]. But Owen *et al*. have found better pain alleviation when the PVG/PAG stimulation was used on patients with neuropathic pain[28]. These inconsistent results may due to the controversial effect of opioid on neuropathic pain. Eisenberg *et al*. have found that short-term opioids treatments provided equivocal effects on neuropathic pain and intermediate-term studies demonstrated significant efficacy [29]. Smith *et al*. have suggested that central neuropathic pain appeared to respond less well to opioids than peripheral neuropathic pain[30]. To clarify the influence of PAG stimulation on neuropathic pain, we still need further studies to conduct more parameters and employ more animal models.

3.2 The different roles of VPL stimulation on acute and chronic pain

The insights of DBS in VPL came from ablative surgery[31]. A large number of studies have reported that sensory thalamic stimulation had varying effectiveness in chronic pain syndromes [32, 33]. In the present study, we have found that VPL could attenuate CFA-induced hyperalgesia and SNL-induced allodynia. Studies have supported that VPL DBS was much more effective on deafferentation pain. Kupers *et al*., have found that stimulation of the sensory thalamus may alleviate pain of neuropathic origin [34]. Kim *et al*. have indicated that VPL stimulation could attenuate allodynia but not hyperalgesia [35]. There are some theories which could explain analgesic effect of VPL DBS on deafferentation pain. Mazars has hypothesized that as the deafferentation pain was caused by lack of proprioceptive stimuli reaching the thalamus, the stimulation on thalamus could produce signal compensation, which may induce analgesic effect [36]. Employing fMRI, Rezai *et al*. found that thalamus stimulation could activate the primary somatosensory cortex (SI) of chronic pain patients [37]. Besides, a positron emission tomography study reported that the thalamus stimulation was associated with activation of the anterior cingulate cortex (ACC) [38]. Through a study of local field potential (LFP) of rats, Kung and Shyu found that the stimulation of medial thalamic nuclei could evoke LFP in ACC [39]. These studies support the hypothesis that the stimulation on thalamus could compensate the lacking sensory information of cortex, which may change the abnormal activities induced by lacking of proprioceptive stimuli into normal activities and therefore produce analgesic effect on deafferentation pain.

An important result in the present study was the analgesic effect of VPL stimulation on CFA-induced hyperalgesia. This result supports other analgesic mechanisms of thalamus stimulation. Researchers suggested that VPL could retrogradely regulate the descending pain inhibitory pathway through non-opioid pathway. Hosobuchi *et al*. proved that the analgesic effect of VPL stimulation could not be blocked by naloxone[40]. Benabid *et al*. also observed that VPL stimulation could inhibit parafascicularis nociception through non-opioid pathway[41]. A primate study indicated the inhibition of spinothalamic tract neurons by stimulation in VPL[42]. Yamamoto *et al*. also found the inhibition of spinothalamic tract neurons of thalamic nucleus ventralis caudalis (Vc)-DBS in patients with peripheral deafferentation pain[43]. They suggested Vc-DBS could recover the original distribution of receptive field and modulate the rhythm of thalamo-

cortical oscillations, which may play important roles for treatment of deafferentation pain. Some researchers considered that the dopamine and 5-HT were involved in the analgesic effect of VPL DBS [44, 45]. Recently, Yang *et al*. have suggested that VPL-lesion could lead to bilateral mechanical hypersensitivity, which may due to hemorrhagic stroke [46], and this study also suggested that VPL may mediate some descending inhibitory pathways.

In this study, VPL stimulation did not influence the acute pain. This result was similar with previous study that no effect on the withdrawal thresholds at the control side was observed after VPL stimulation [34]. These results suggested that the thalamus condition in chronic pain may be different from that of acute pain. Studies have proved that the receptive field, excitability and firing patterns of neurons in thalamus could be changed by persistent pain stimulations [47–49]. Electrophysiological studies also elucidated that inflammatory pain and neuropathic pain could increase the responsiveness of thalamus neurons [50–52]. Imaging studies showed decreased [53, 54] or abnormally enhanced [55, 56] contralateral thalamus activities of limbs with chronic pain, which could be back to normal when the pain was relieved after clinical treatments. Combined with these studies and our results, we considered that the abnormal condition of VPL under chronic pain could be changed by DBS, which may induce the analgesic effects in CFA and SNL rats. During the acute pain, DBS could not change the normal condition of VPL and therefore caused no effect on acute pain.

3.3 Limitations

The stimulus used in this study was less than 1 min and we detected the analgesic effect immediately after the stimulus. This design could avoid the damage of target brain regions induced by long-term stimulus and could sensitively detect the most effective brain region. However, the analgesic effect may be attenuated by this acute and brief stimulus and could not observe the time summation effect. This may be the reason why we did not find the persistent post-effect induced by VPL stimulation on neuropathic pain. Kupers *et al*. have observed 15 min post-effect after the end of stimulus. They used persistent stimulation in VPL for 30 min and found the alleviation of neuropathic pain[34].

4 Conclusion

Although many studies have examined the analgesic effect of vlPAG and VPL stimulation on pain, the results were still controversial and most of the comparisons were depending on different parameters and different studies. To clarify and compare the effects of vlPAG and VPL stimulation, we employed same stimulation parameters for the two brain regions in each pain model and proved that: (1) the DBS of vlPAG is much more suitable for nociceptive pain, including acute thermal nociception and CFA-induced hyperalgesia; (2) VPL stimulation may produce sensitive analgesic effect on chronic pain, including chronic inflammatory pain and neuropathic pain. These findings may provide evidence for further studies to elucidate the modulation mechanisms of DBS on different pain models and help to build better strategies for treatment of intractable pain.

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