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Inhibition of HCN channel activity in the thalamus attenuates chronic pain in rats

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Abstract

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels regulate neuronal excitability in both peripheral and central nerve systems. Emerging evidence indicates that HCN channels are involved in the development and maintenance of chronic pain. However, the impact of HCN channel activity in the thalamus on chronic pain has not been examined. In this report, we evaluated the effect on nociceptive behaviors after infusion of a HCN channel blocker ZD7288 into the ventral posterolateral (VPL) nucleus of the thalamus in rats with neuropathic pain or monoarthritis. We show that ZD7288 dose-dependently attenuated mechanical allodynia and thermal hyperalgesia in rats with chronic pain. In the thalamus, immunoreactivity of both HCN1 and HCN2 subunits was increased in both rat models. These results suggest that the increased HCN channel activity in the thalamus of the ascending nociceptive pathway contributes to both chronic neuropathic and inflammatory pain conditions.

Keywords

HCN channel; ZD7288; Thalamus; Neuropathic pain; Monoarthritis

1. Introduction

Chronic pain resulting from nerve injury or inflammation often exhibits mechanical allodynia and thermal hyperalgesia, which can persist for a prolonged period of time after the original injury [1]. Hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels are widely expressed in both peripheral sensory neurons and neurons in the central

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⁵Conflict of interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neulet.2016.08.021>.

nervous system [2,3] besides cardiac tissues [4–8]. HCN channels generate inward current (I_h), a mixed Na^+/K^+ current when the membrane potential is hyperpolarized. HCN channel activity plays important roles in behavior and physiological process such as sleep and arousal [9], learning and memory [10,11], and anesthesia [12,13]. Misregulation of HCN channel activity has been shown to contribute to neurological and psychological disorders including pain [14,15], epilepsy [16], addiction [17] and anxiety [18].

Considerable evidence suggests that dysfunction of HCN channel activity is associated with the development and maintenance of chronic pain and inhibition of HCN channel activity produces the anti-nociceptive effect [19–21]. HCN protein accumulation as well as up-regulation of I_h current have been observed in neurons of dorsal root ganglion (DRG) [22] and the spinal cord [23] of rodents with peripheral nerve injury. Local or systemic administration of ZD7288, a HCN channel blocker, reduced nociceptive behavior in animals [23]. At the supraspinal level, increased HCN activity appears to be related to chronic pain and comorbidity. For example, HCN1 expression level was increased in the amygdala of rats with chronic constriction of sciatic nerve (CCI) and inhibition of HCN channels was anti-nociceptive [21]. Increased HCN protein expression level and enhanced I_h current were also observed in the periaqueductal gray of CCI rats, whereas infusion of ZD7288 into this brain region attenuated neuropathic pain [14,24]. Moreover, microinfusion of ZD7288 into the medial prefrontal cortex or the anterior cingulate cortex also produced the antinociceptive effect in mice with spared nerve injury [25] or CCI [15]. These data suggest that HCN activities in ascending nociceptive pathways may be an important contributor of chronic pain conditions.

The thalamus is the brain structure that receives the input from multiple ascending pain pathways and connects to the cortex and the limbic system. Human imaging study suggests changes in the thalamus in response to neuropathic pain [26]. The thalamic neuronal firing pattern also became irregular in patients with intractable pain [27,28]. To date, it remains unclear whether the HCN activity in the thalamus has a direct impact on nociceptive behavior and whether HCN protein expression in the thalamus would change under neuropathic and/or inflammatory pain conditions. Both possibilities were examined in this study and we found that HCN immuno-reactivity was increased in the thalamus of rats with chronic pain and inhibition of HCN activity attenuated mechanical allodynia and thermal hyperalgesia in rats.

2. Materials and methods

2.1. Experimental animals

Adult male Sprague–Dawley rats weighing 250–270 g were purchased from Charles River Laboratories (Wilmington, MA). The experimental protocols were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

2.2. Surgical procedures

2.2.1. Chronic constriction injury (CCI) and sham operation—The surgical procedure was performed aseptically on rats anesthetized with pentobarbital (50 mg/kg, i.p.).

CCI was performed according to the method of Bennett and Xie [29]. Briefly, one side the rat's sciatic nerve was exposed in the mid-thigh, four ligatures using 4.0 mm chronic gut sutures were loosely placed around the sciatic nerve with a 1.0–1.5 mm interval between each ligature. Skin incision was closed with wound clips. Rats in sham groups underwent the same procedure but without nerve ligation.

2.2.2. Complete Freund's adjuvant (CFA)-induced hindpaw monoarthritis—

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). CFA (50 μ L) (Sigma F4258) or saline was injected into one side of the ankle articular cavity using a 28 gauge needle [30].

2.2.3. Implantation of a guide cannula for intra-thalamus microinjection—

Rats were intraperitoneally anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a Stoelting stereotaxic instrument. A guide cannula (C315G with an infusion cannula C315I, Plastics One, Roanoke, VA) was implanted in the ventral posterolateral (VPL) nucleus of the thalamus contralateral to the injury or CFA injection side. The implantation of guide cannula was performed according to the following coordinates from the rat brain atlas [31]: 3.4 mm posterior to the Bregma, 3.5 mm lateral to the midline, and 6.5 mm ventral to the skull surface. The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. A dummy cannula (33 gauge stainless steel wire) was inserted into the guide cannula to reduce the incidence of occlusion. Saline and drug solutions were infused into the thalamus using a microinjection unit (33 gauge cannula) that extended 0.5 mm beyond the tip of the guide cannula. The microinjection unit was attached to a Hamilton microsyringe via polyethylene tubing (PE-10), and an infusion pump was programmed to deliver a volume of 0.5 μ L over a period of 1 min into the VPL nucleus of the thalamus. The needle was held for 1 min before retraction, and the injection site was confirmed by visual examination of thalamus sections upon the completion of each experiment. ZD7288 was purchased from Sigma (St Louis, MO, USA) and dissolved in sterile saline.

2.3. Behavioral tests

All behavioral experiments were carried out with the investigators being blinded to treatment conditions. Animals were habituated to the test environment for two consecutive days (30 min per day) before baseline testing.

2.3.1. Mechanical allodynia—A von Frey filament was perpendicularly applied to the plantar surface of each hindpaw. A threshold force of response (in grams) was defined as the first filament that evoked at least two withdrawals out of five applications [32].

2.3.2. Thermal hyperalgesia—Response to radiant heat was assessed using the foot-withdrawal test [33]. Each animal underwent three trials and their results were averaged to yield mean withdrawal latencies. The cutoff was set at 20 s to avoid tissue damage.

2.4. Immunohistochemistry and western blot

Immunostaining and Western blot were carried out as previously reported [21] using the following primary and secondary antibodies: mouse anti-HCN1 antibody (1:800; ab84816, Abcam), mouse anti-HCN2 antibody (1:1000; ab84817, Abcam), Cy3- or FITC conjugated

goat anti-mouse antibody (1:300; Jackson ImmunoResearch Laboratories Inc.), and HRP-conjugated donkey anti-mouse antibody (1:10,000; Santa Cruz) Primary antibody omission was used as a control. Sections were examined and captured with an Olympus fluorescence microscope. Western blot was analyzed using ImageJ.

2.5. Statistical analysis of behavioral data

Behavioral data were analyzed using two-way analysis of variance (ANOVA) [21]. *Post-hoc* Waller-Duncan K-ratio *t* test was performed to determine the source(s) of differences. GraphPad Prism5 software was used for the statistical analyses. All data were expressed as mean \pm SEM and the statistically significant level was set at $P < 0.05$.

3. Results

3.1. Intra-thalamus infusion of HCN channel blocker ZD7288 attenuated nociceptive behavior of CCI rats

Rats subjected to unilateral CCI exhibited mechanical allodynia and thermal hyperalgesia in the ipsilateral hindpaw as compared to sham controls (Fig. 1A and B). To determine the impact of I_h inhibition in the thalamus on neuropathic pain, nociceptive behavior was assessed in CCI rats after acute infusion of saline or ZD7288 into the VPL nucleus of the thalamus contralateral to the CCI side at fourteen days after injury. Both 1 μ g and 5 μ g of ZD7288 infusion attenuated mechanical allodynia and the effect of 5 μ g of ZD7288 lasted for 90 min (Fig. 1C and E). Measured at 30 min after 5 μ g of ZD7288 infusion, the threshold of CCI rats to mechanical stimulus nearly returned to its pre-injury (baseline) level. Thermal hyperalgesia was also reduced after the ZD7288 infusion in CCI rats and the higher ZD7288 dose (5 μ g) did not further enhance the effect (Fig. 1D and F). In contrast, the nociceptive behavior in CCI or CFA rats was not affected by saline infusion (Suppl Fig. S1). ZD7288 (5 μ g) infusion into the VPL nucleus (Fig. 2C) of the thalamus did not alter either mechanical or thermal sensitivity of sham rats (Fig. 2A and B).

3.2. Protein expression of HCN channels was increased in the thalamus of CCI rats

HCN1 and HCN2 proteins are expressed in the thalamus in rodents [5,6,8], which is considered to be relevant to pain signaling in both peripheral and central nerve systems [15,21,22]. Accordingly, we analyzed the HCN1 and HCN2 expression in the thalamus of CCI rats. By fourteen days after CCI, protein expression and immuno-reactivity of both HCN1 and HCN2 were increased in the contralateral thalamus as compared with that of sham rats analyzed by Western blot (Fig. 3A) and immunohistochemistry (Fig. 3B). Since changes in HCN protein expression affects HCN channel activity [34], the increased HCN1 and HCN2 expression in the thalamus may contribute to persistent nociception after CCI.

3.3. Intra-thalamus infusion of ZD 7288 attenuated nociceptive behavior in rats with inflammatory monoarthritis

The VPL nucleus of the thalamus is the final relay of the spinothalamic tract. HCN channel activity is increased in both peripheral and central nerve system in chronic pain induced by persistent inflammation [15,35]. To determine whether increased HCN channel activity in the thalamus contributes to the maintenance of inflammatory pain, we tested the effect of

ZD7288 in rats with chronic inflammatory monoarthritis. Rats subjected to CFA injection into the ankle joint developed persistent mechanical allodynia and thermal hyperalgesia in the hindpaw compared with sham controls (Fig. 4A and B). Ten days after CFA or saline injection, nociceptive behavior was assessed in the rats after infusion of saline or ZD7288 in the VPL nucleus of the thalamus contralateral to the CFA injection side. Saline infusion did not alter the hindpaw mechanical or thermal withdrawal threshold in CFA rats. However, ZD7288 (1 μ g) infusion produced a prolonged anti-nociceptive effect on the hindpaw ipsilateral to CFA injection, which lasted over 120 min (Fig. 4C–F). Mechanical allodynia was significantly attenuated when assessed at 15 min after the ZD7288 infusion and the protective effect peaked at 30 min (Fig. 4C and E), whereas thermal withdrawal threshold returned to the pre-injury level at 15 min after 1 μ g of ZD7288 infusion (Fig. 4D and F).

4. Discussion

We report that infusion of a HCN blocker ZD7288 into the VPL nucleus of the thalamus attenuated nociceptive behavior in rats with chronic pain induced by peripheral nerve injury or inflammation. We also observed that HCN1 and HCN2 protein expression was increased in the thalamus of the same rats after nerve injury.

Accumulating evidence suggests that abnormal HCN channel activity contributes to the development and maintenance of chronic pain. In neurons, I_h influences resting membrane potential and input resistance, thereby altering neuronal excitability [36]. Altered I_h induced by nerve injury has been linked to the increased firing of sensory neurons [35,37,38]. Deletion of HCN2 in sensory neurons reduced mechanical hyperalgesia in mice with CFA-induced inflammatory pain [35]. Despite the known presence of HCN channels in the thalamus, the role of the thalamic HCN channel activity in chronic pain condition has not been studied. Our data suggest that HCN channel activity in the thalamus contributes to the behavioral manifestation of chronic pain in two rodent models without changing thermal or mechanical nociceptive threshold in sham rats. These results are in line with a recent finding that HCN channel activity following periphery nerve injury enhanced synaptic transmission between the thalamocortical projection and anterior cingulate cortex [15].

The VPL nucleus is a major relay site of the spinothalamic track for pain and temperature sensation [39]. In rats with neuropathic pain, neurons in the VPL nucleus exhibited higher spontaneous firing as well as evoked response [40]. Moreover, increased HCN channel activity may contribute to ectopic firing under chronic pain [20,37]. Our data show that inhibition of HCN channel activity in the VPL nucleus alleviated chronic pain. It has been shown that intrathecal or intra-periaqueductal gray application of HCN channel inhibitor reduced neuropathic pain behaviors [23,24]. These data lend strong support for a critical role of the brain HCN channel activity in chronic pain.

Among four HCN channel subunits (HCN1–4), HCN1 and HCN2 subunits are expressed in neurons of both peripheral and central nerve systems and are highly relevant to pain processing and modulation [41–45]. A recent study using inducible HCN2 gene knockout mice, which showed a dramatic reduction in HCN2 protein expression in the brain including thalamus and cortex, were protected from mechanical allodynia and thermal hyperalgesia

after CFA-induced inflammation [35]. In addition, HCN1 gene knockout mice were partially protected from developing cold allodynia [3]. On the other hand, increased HCN1 and HCN2 protein expression has been observed in the PAG and amygdala in rodents with chronic pain [21,24]. In this study, we also found that HCN1 and HCN2 immunoreactivity was increased in the thalamus of rats with chronic neuropathic pain.

While the exact mechanisms by which HCN channel activity in the thalamus regulates chronic pain remains to be examined, the current data suggest that inhibition of HCN subtype channel activity, such as HCN1 and HCN2, may be a method to alleviate chronic pain. Due to bradycardia resulting from blocking the sinoatrial HCN4 with all known I_h blockers, broad HCN channel blockers may not be useful when applied systemically. The present results suggest that searching for HCN subtype blockers relevant for pain would be beneficial to patients with chronic pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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HIGHLIGHTS

- Inhibition of HCN channel activity in the VPL nucleus of the thalamus attenuated chronic pain.
- HCN1 and HCN2 protein expression levels were increased in the thalamus under chronic pain condition.
- Increased HCN channel activity in the thalamus contributed to the maintenance of chronic pain.

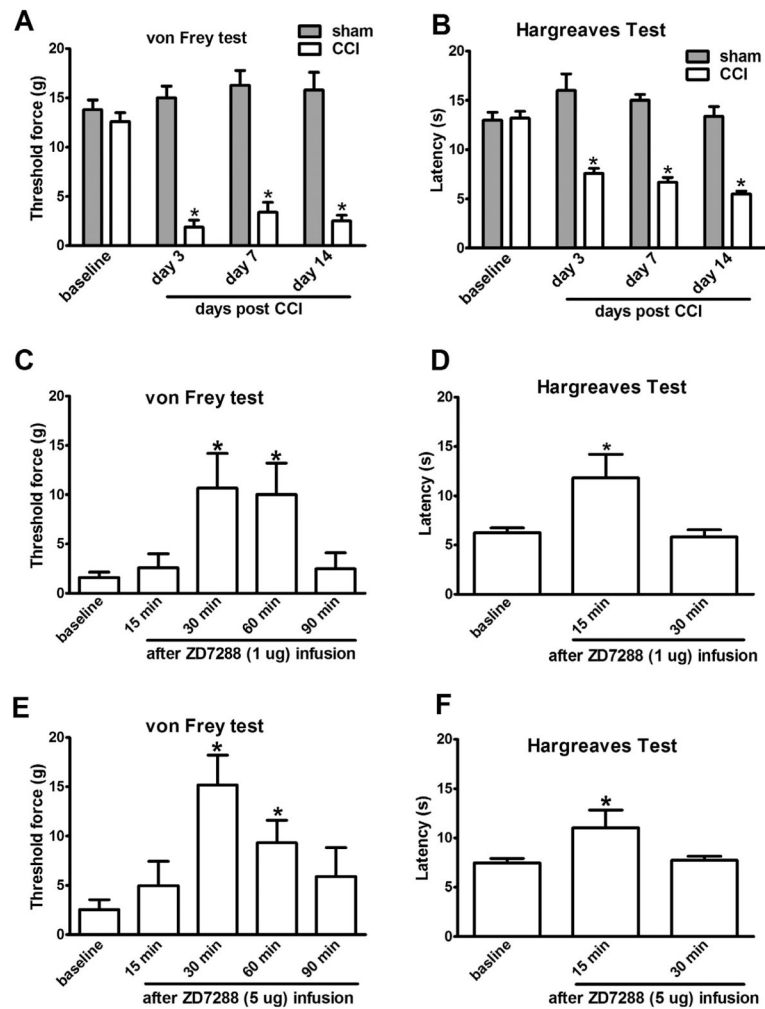


Fig. 1. Intra-thalamus infusion of ZD7288 reduced nociceptive behavior in CCI rats. (A, B) CCI-induced mechanical allodynia and thermal hyperalgesia lasted for weeks in rats (sham vs CCI). * $P < 0.05$, $n = 6$). (C,E) Infusion of ZD7288 into the VPL nucleus contralateral to the CCI side attenuated mechanical allodynia for 90 min. (D,F) Infusion of ZD7288 into the VPL nucleus contralateral to the CCI side transiently reduced thermal hyperalgesia for at least 30 min. (baseline vs after ZD7288 injection * $p < 0.05$, $n = 6-8$).

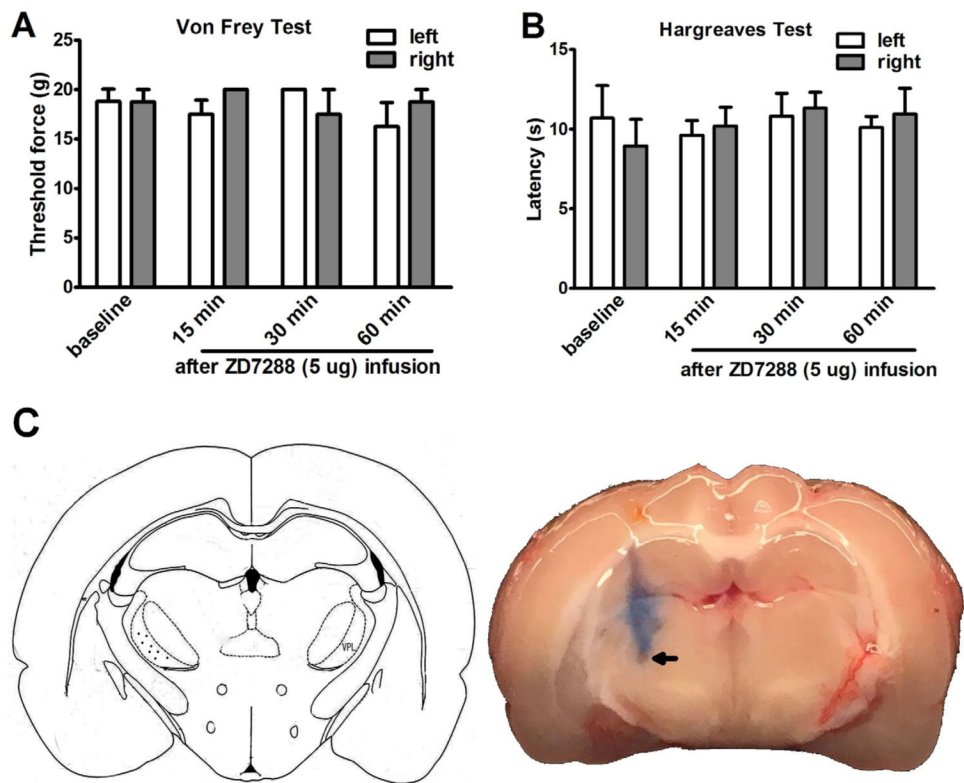


Fig. 2. Intra-thalamus infusion of ZD7288 did not alter nociceptive threshold in sham rats. (A, B) Infusion of ZD7288 (5 μ g) into the right side of the VPL nucleus did not change the hindpaw mechanical and thermal threshold of sham rats. (C,D) Schematic drawing of the injection site in the thalamus and a representative brain section showing ZD7288 or saline injection site.

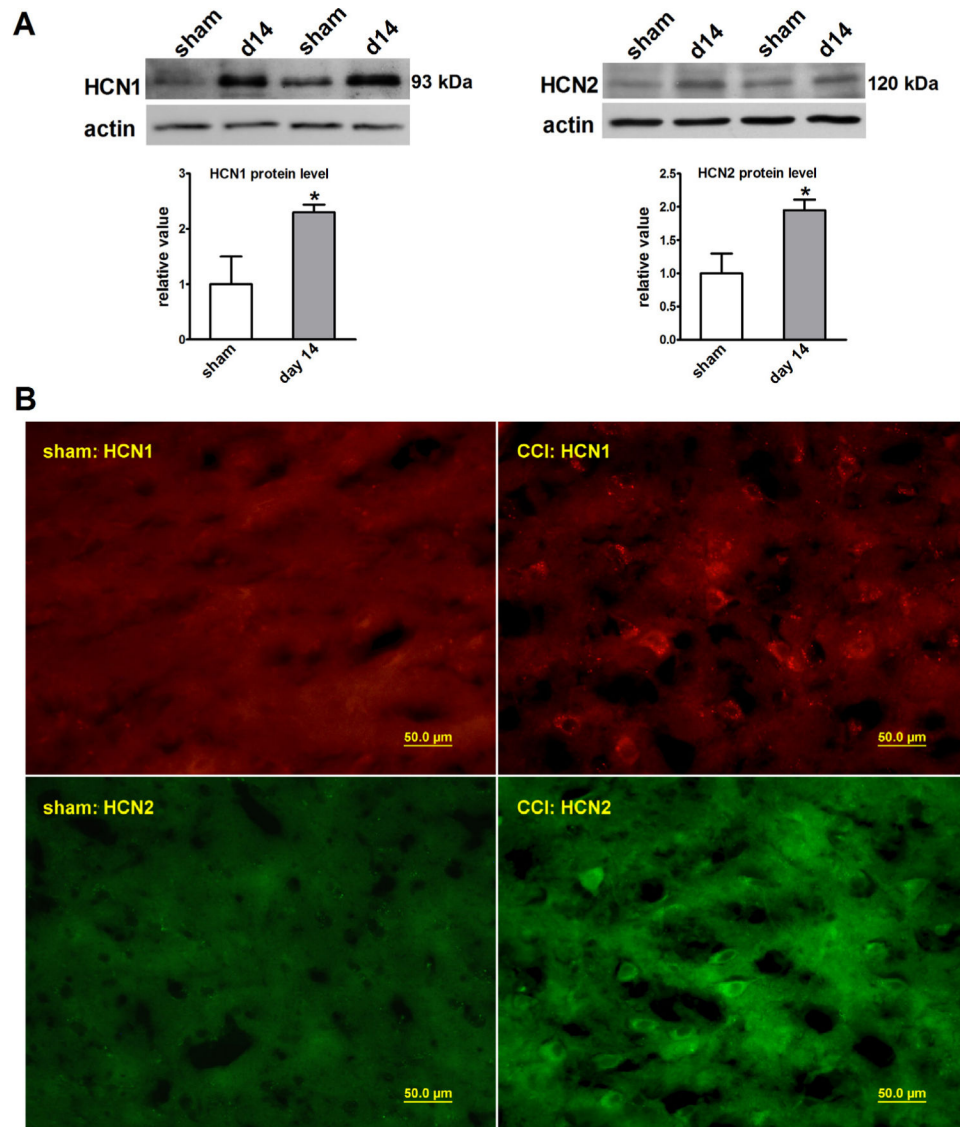


Fig. 3. HCN1 and HCN2 protein expression and immunoreactivity were increased in the thalamus of CCI rats. (A) The HCN1 (93 kDa) and HCN2 (120 kDa) protein levels were increased when analyzed at 14 days after CCI (sham vs CCI, $n = 5-6$, $*P < 0.05$). (B) HCN1 and HCN2 immunostaining showed the increased number of HCN1 and HCN2 positive cells in the thalamus of CCI rats (40 \times magnifications).

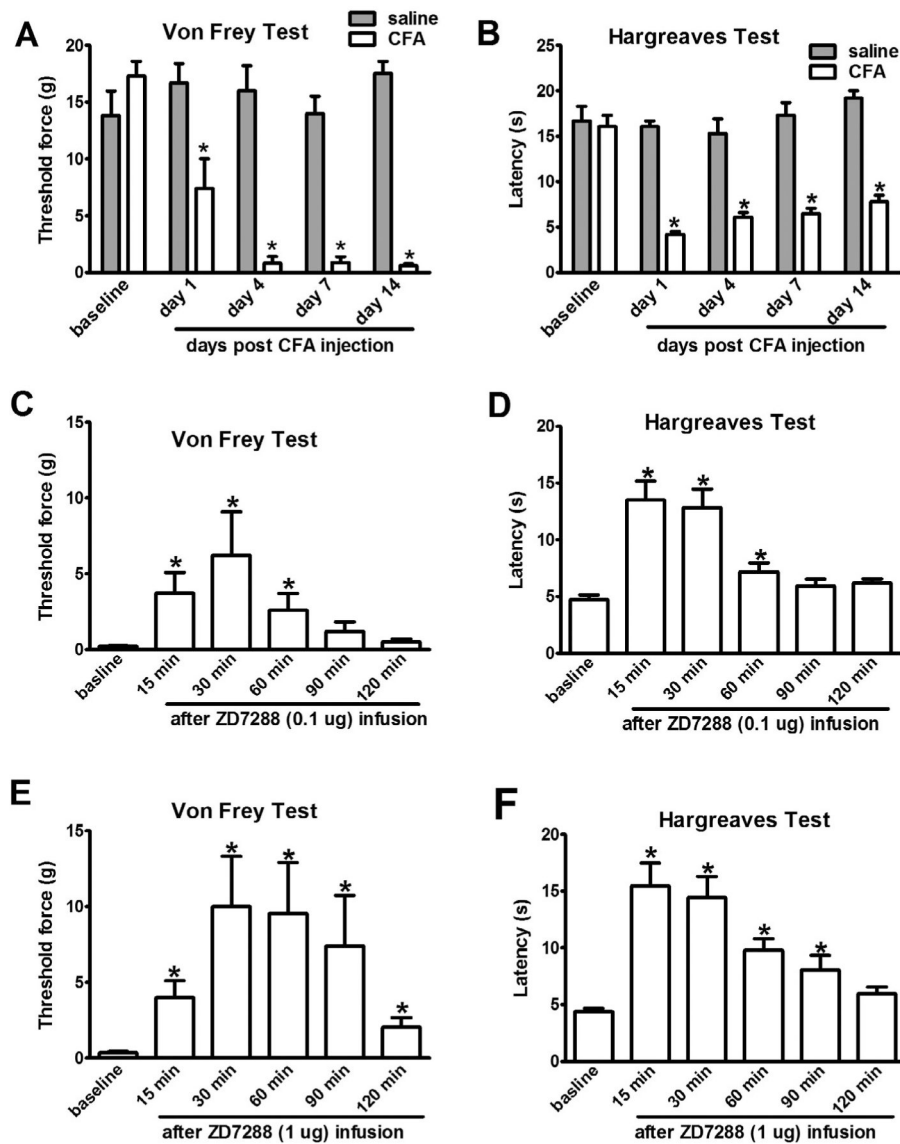


Fig. 4. Intra-thalamus infusion of ZD7288 reduced nociceptive behavior in rats with CFA-induced inflammatory monoarthritis. (A, B) CFA injection into the ankle joint induced mechanical allodynia and thermal hyperalgesia lasting for weeks in rats (saline vs CFA. * $P < 0.05$, $n = 6$). (C,E) Infusion of ZD7288 into the VPL nucleus contralateral to the CFA injection side attenuated mechanical allodynia for up to 120 min. (D,F) At 15 min after infusion of ZD7288 into the VPL nucleus contralateral to the CFA side, thermal hyperalgesia was significantly attenuated (baseline vs after ZD7288 injection * $p < 0.05$, $n = 6-8$).