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Facial pain induces the alteration of transient receptor potential vanilloid receptor 1 expression in rat trigeminal ganglion

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Abstract: Objective To investigate the involvement of transient receptor potential vanilloid receptor 1 (TRPV1) in the facial inflammatory pain in relation to thermal hyperalgesia and cold pain sensation. **Methods** Facial inflammatory pain model was developed by subcutaneous injection of turpentine oil (TO) into rat facial area. Head withdrawal thermal latency (HWTL) and head withdrawal cold latency (HWCL) were measured once a day for 21 d after TO treatment using thermal and cold measurement apparatus. The immunohistochemical staining, cell-size frequency analysis and the survey of average optical density (OD) value were used to observe the changes of TRPV1 expression in the neurons of the trigeminal ganglion (TG), peripheral nerve fibers in the vibrissal pad, and central projection processes in the trigeminal sensory nuclei caudalis (Vc) on day 3, 5, 7, 14, and 21 after TO injection. **Results** HWTL and HWCL decreased significantly from day 1 to day 14 after TO injection with the lowest value on day 5 and day 3, respectively, and both recovered on day 21. The number of TRPV1-labeled neurons increased remarkably from day 1 to day 14 with a peak on day 7, and returned back to the normal level on day 21. In control rats, only small and medium-sized TG neurons were immunoreactive (IR) to TRPV1, and the TRPV1-IR terminals were abundant in both the vibrissal pad and the Vc. Within 2 weeks of inflammation, the expression of TRPV1 in small and medium-sized TG neurons increased obviously. Also the TRPV1 stained terminals and fibers appeared more frequent and denser in both the vibrissal pad skin and throughout laminae I and the outer zone of laminae II (IIo) of Vc. **Conclusion** Facial inflammatory pain could induce hyperalgesia to noxious heat and cold stimuli, and result in increase of the numbers of TRPV1 positive TG neurons and the peripheral and central terminals of TG. These results suggest that the phenotypic changes of TRPV1 expression in small and medium-sized TG neurons and terminals might play an important role in the development and maintenance of TO-induced inflammatory thermal hyperalgesia and cold pain sensation.

Keywords: vanilloid receptors; facial pain; hyperalgesia; trigeminal ganglion

1 Introduction

Transient receptor potential vanilloid receptor 1 (TRPV1), also called vanilloid receptor 1 (VR1), is a non-selective cation channel gated by noxious heat, proton and capsaicin[1,2]. Under normal state, regarding primary sensory neurons, TRPV1 is expressed in small (TG) neurons, which dominate the skin and cornea with their unmyelinated axons, and project to the brain stem with their central processes[3]. Under peripheral tissue injury and inflammation, TRPV1 expression is largely increased in peripheral terminals[4]. It is thus believed that the level of peripheral TRPV1 is likely an index of peripheral sensitization of thermal nociceptors and is probably responsible for the production and maintenance of hyperalgesia.

TRPV1 is essential for the development of thermal hyperalgesia, as mice lacking this receptor lack thermal hyperalgesia after inflammation[5]. In addition, the upregulation of TRPV1 expression contributes to the development of inflammatory heat hyperalgesia[6-8]. It has been found that the proportion of unmyelinated axons expressing TRPV1 increases in the inflamed tissues, and that inflammation increases the number of TRPV1-expression primary sensory neurons in the dorsal root ganglia (DRG)[6,7]. Studies *in vitro* have shown that substances produced by local inflammation such as ATP[9], bradykinin (BK)[10] and

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nerve growth factor (NGF)[11] could increase the capsaicin-evoked current in TRPV1 expressed cells. These functional modulations of TRPV1 channels, however, cannot explain why the number of active neurons increases after inflammation. Therefore novel recruitment of TRPV1 expression would be necessary. Other studies have also demonstrated the changes in TRPV1 protein expression in rat hind paw skin, sciatic nerve and DRG on day 2 and day 7 after complete Freund's adjuvant (CFA) injected[4,8].

Previous reports were focused on the role of TRPV1 during relatively short period (less than or equal to 1 week) after peripheral inflammation. Only a few studies reported the expression of TRPV1 in TG, vibrissal pad and the trigeminal sensory nuclei caudalis (Vc) at different time points during a relative long period of facial inflammatory pain. Therefore, in the present study, by using behavioral survey and immunohistochemical analyses, we attempted to determine whether the hyperlagesia and cold pain sensation induced by inflammation have correlations with the change of TRPV1 expression in the TG neurons with different size as well as in the TG peripheral and central terminals during the full time course of inflammatory nociception.

2 Materials and methods

2.1 Animals The experiments were carried out on the male adult Sprague-Dawley (SD) rats (150–200 g) purchased from the Experimental Animals Center of Tongji Medical College of Huazhong University of Science and Technology. All animal experiments were approved by the Animal Care Committee of Huazhong University of Science and Technology and complied with the ethical guidelines of the International Association for the Study of Pain (IASP)[12].

SD rats were individually housed in cages with a natural light/dark cycle and had accessed water and food *ad lib*. Total 24 rats were used for the present experiment, 20 rats were treated with turpentine oil (TO) and persisted experimental observations until day 3, 5, 7, 14, and 21 ($n = 4$ rats for each time point), respectively, and the left 4 rats were used as control without treatment. Animals were habituated to the pain testing paradigms for 3–5 d before data collection.

2.2 Establishment of facial inflammatory pain model Three days after acclimation to the laboratory environment and measurement of basic pain threshold, 50 μL turpentine oil (TO, Shanghai chemical industry, China) solution (TO: paraffinum = 1:1) was subcutaneously injected into the left supraorbital, infraobital and vibrissae skin of the rat under light anesthesia with 10% chloral hydrate by abdominal injection. Typical signs of acute inflammation including edema, redness and heat were most obvious on days 1 to day 3 after injection and lasted for more than 2 weeks.

2.3 Behavioral tests

2.3.1 Thermal hyperalgesia The latency to radiant heat (s) was measured by a radiant heat apparatus (Department of Physiology of the Fourth Military Medical University, China), prior to 0 d and lasted for 3 weeks after TO administration. Rats were allowed to acclimatize them within a (8×10×18) cm^3 transparent plastic membrane sheath. A radiant heat source (*i.e.* high-intensity projector lamp bulb) was controlled with a timer to switch on and focused on the beard area. Both lamp and timer were halted by a photocell when the head withdrew, and head withdrawal thermal latency (HWTL) was measured at the same time. Five trials with a 10-min intertrial interval were conducted on each rat's beard area. The voltage was adjusted to derive an average baseline thermal threshold of approximately 20 s, and a maximal cut-off of 30 s was employed to prevent tissue damage. Results were expressed as mean ± SEM.

2.3.2 Cold pain sensation According to the principles of cold plate test[13] and the animal models of cold pain sensation[14], ice cool water [(0±1) ºC] was taken in a hermetical test-tube. Use the tip of this cold test-tube to lightly touch the facial area of the rats and the head withdrawal cold latency (HWCL) (s) was measured just prior to 0 d and lasted for 3 weeks after TO injection. Five trials with a 10-min intertrial interval were conducted on each rat's beard area. The cut off time was 30 s. Results were expressed as mean ± SEM.

2.4 Immunohistochemical staining On day 3, 5, 7, 14, and 21 after TO injection and pain measurements, rats were deeply anesthetized with 10% chloral hydrate (350 mg/kg, i.p.) and transcardialy perfused with 300 mL of saline followed by 500 mL of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.6). After the perfusion, the TG and vibrissal pad of the injecting side and the brainstem were removed, kept in the same fixative for 6 h, and then cryoprotected in 30% sucrose in 0.1 mol/L PB (4 ºC) overnight. The sections (20 μm) were cut on a cryostat, collected sequentially in 4 vials (with a minimum separation of 80 μm between the sections), and immersed in 0.05 mol/L Tris Buffer Saline (TBS, pH 7.4). Immunohistochemical staining for TRPV1 was performed with avidin-biotin-horse-

radish peroxidase complex (ABC) detection method (Vector Laboratories, Burlingame, CA, USA). Sections were incubated with rabbit anti-TRPV1 serum (1:20 000, Sigma) for 24 h at 4 ºC in a humid atmosphere. After rinsed with 0.05 mol/L TBS, the sections were incubated with biotinylated goat anti-rabbit IgG (1:200) for 1 h in a humid atmosphere at room temperature and followed by ABC complex (1:800) for 1 h. All antisera were diluted in 0.05 mol/L TBS containing 0.3% Triton X-100. Then the sections were stained for 5 min in a solution containing 0.05% DAB and 0.2% nickel-amoniumaulphate activated with 0.01% H_2O_2. Finally, the sections were mounted on gelatin coated glass slides, air-dried, dehydrated in a graded series of alcohols, cleared in xylene and cover-slipped with resin (Shanghai, China). Control sections were incubated without the primary antibody. In this condition, no staining was observed.

2.5 Image analysis DAB-stained sections (see above) were selected from the TG of 4 animals at each time point to analyze the proportion of TRPV1-labeled neuronal profiles and the size frequency distribution. In addition, the immunoreactive alteration of nerve terminal fibers in vibrissal pad and medulla oblongata was observed. Signals on TG neurons, TG peripheral terminals in the vibrissae skin, and TG central terminals in the medulla dorsal horn were analyzed under × 10, × 20 and × 4 microscopic visual fields (Olympus BH-2), respectively. Photographs were taken with a digital camera (Panasonic, DMC-F1, Japan), and digitally processed and printed. Digital image was obtained by a microscopy-digital camera system (TK-C1381EG; Victor Company of Japan Limited, Japan). Signal intensity and area of each neuron were calculated by using the High Vivid Color Pathological Photo Analyze System (HPIAS-1000; Huazhong University of Science and Technology) on a SΛMSUNG computer. The cross-sectional area of those cell bodies containing the nucleolus was recorded. Only neurons with visible nuclei were processed for calculation. The ratio of TRPV1-positive cells to the total neuronal profiles was calculated to identify any relative changes in TRPV1 expression in TG neurons. Absolute value of the TRPV1-positive neuronal profiles per each TG that belong to corresponding cell size was compared for size frequency distribution. In addition, the average optical density (OD) value of the immunoreactive terminals in medulla oblongata was measured to determinate the variance of TRPV1 expression in the Vc.

2.6 Statistical analysis Changes in HWTL and HWCL of TO treated side (ipsilateral) were compared with that of control groups and the non-injected side (contralateral) at the corresponding time. Differences among time points were analyzed with one-way ANOVA for repeated measures by SPSS11.5, followed by Dunnett's *post-hoc* test. $P < 0.05$ was considered statistically significant.

3 Results

3.1 General behavioral changes Rats did not show marked ongoing behavioral abnormalities and/or stress reaction after TO injection, and they gained weight normally. In about 50% of rats, increased asymmetric facial grooming/scratching could be seen as evidenced by hair loss; and in few cases, superficial injury of the vibrissal pad region ipsilateral to the injection was detected.

3.2 Thermal hyperalgesia The baseline of HWTL in facial area was stable, ranging from 6.97 s to 8.96 s. There was no significant difference between the two sides of facial area ($n = 4$, $P > 0.05$, Fig. 1A). Subcutaneous injection of 50 μL TO into the left supraorbital, infraobital and vibrissae skin of the rat produced marked inflammation (edema and erythema) and thermal hyperalgesia, which peaked on day 5 and showed little change in magnitude in the following 3 d. Mean withdrawal latency in the inflamed facial area decreased to (3.7±0.70) s on day 5 and showed significant difference compared with that of control [(7.85±0.09) s, $n = 4$, $P < 0.01$, Fig. 1A]. The decrease lasted for 14 d and completely recovered to the control level on day 21 after treatment. The HWTL of both contralateral and non-injected facial area ($n = 4$) showed no obvious changes at all post-injection time points during the entire observation period compared with baseline values ($n = 4$, $P > 0.05$, ANOVA, Fig. 1A).

3.3 Cold pain sensation We examined the development of cold pain sensation at (0±1) ºC in TO model rats over time and found a reduction in HWCL from day 1 to approximate 2 weeks after TO injection. The reduction was peaked on day 3 after TO injection. Mean withdrawal latency in the inflamed facial area decreased to (2.48±0.90) s on day 3 and showed significant difference compared with that of control [(12.09±2.16) s, $n = 4$, $P < 0.05$, Fig. 1B]. The decrease lasted for 2 weeks and totally recovered on day 21, which was well in accordance with that of thermal hyperalgesia. There was no significant change in the enhanced response to cold stimulation in the controls ($n = 4$, $P > 0.05$, ANOVA, Fig. 1B).

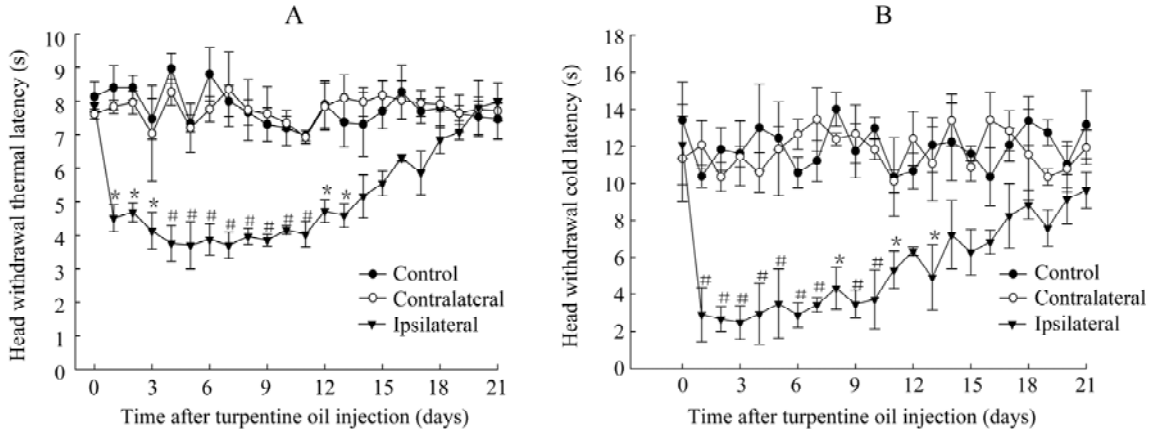

Fig. 1 Changes in head withdrawal thermal latency (HWTL) and head withdrawal cold latency (HWCL) to stimulation with heat irradiant (A) and cold light touch (B) of ipsilateral and contralateral facial areas after TO injection (n = 4). Data were collected from the day before TO injection (day 0) to day 21 after TO injection. Head withdrawal latency from day 1 to day 14 after TO injection decreased significantly compared with the control group. Data are shown as mean ± SEM. * $P < 0.05$, # $P < 0.01$ *vs* control with Dunnett's test.

3.4 TRPV1 expression in the TG

3.4.1 The distribution and cell size of TRPV1 expression neurons in the TG of untreated rats Based on the intensity of staining, two types of neurons were observed in TG: the strongly-stained neurons with the immunohistochemical reactive product located in the cytoplasm and the light stained neurons with weak staining restricted to the plasma membrane and cytoplasm (Fig. 2). Since the strong staining throughout the cytoplasm disappeared in the control sections, whereas the weak staining in the membrane and cytoplasm remained unchanged (suggesting that the light staining was unlikely to be specific for TRPV1), only strongly-stained neurons were analyzed in this study. The TG contained abundant TRPV1-immunoreactive (-IR) neurons. In total, 23.73% (206/871) of TG neurons presented positive staining for TRPV1. As shown in Fig. 3A, TRPV1-IR neurons were mostly the small with some in medium size [(353±145) μm^2, ranging 98 μm^2 to 798 μm^2; mean±SEM]; 30.8% (134/435) of TG neurons were smaller than 400 μm^2 and 19.8 % (72/363) were in the range of 400–800 μm^2. Virtually no TG neuron larger than 800 μm^2 was seen TRPV1-IR positive (0% or 0/73).

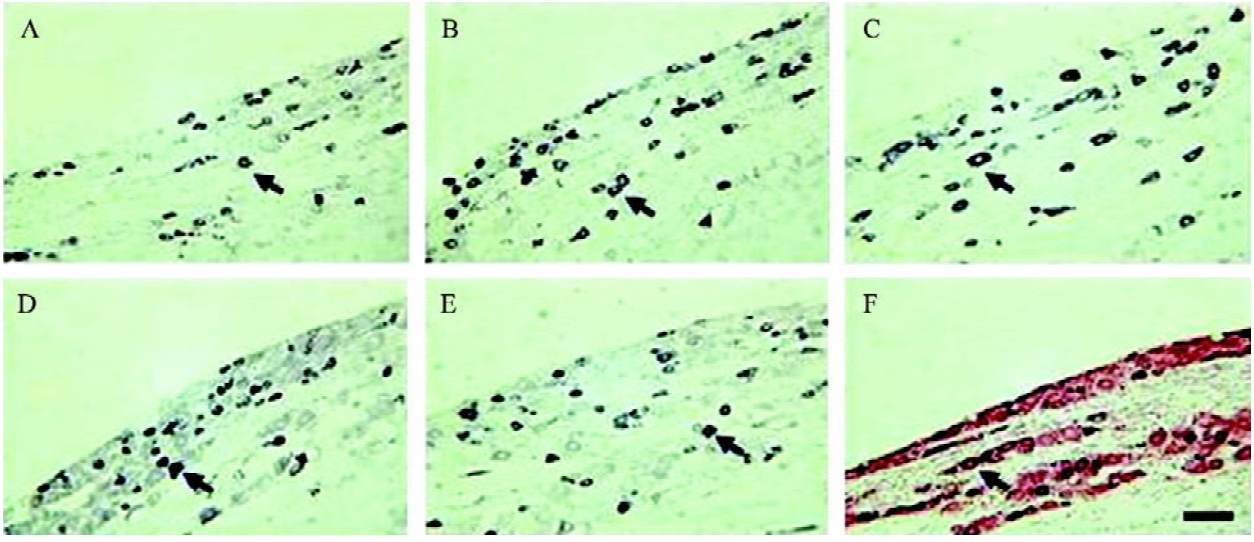

Fig. 2 Immunohistochemical expression of TRPV1 in the TG of the control and inflamed animals. A: TRPV1 expression is restricted to most of small and some of medium-sized neurons (arrowhead) in control animal. B, C: Day 5 and day 7 after inflammation, TRPV1 staining appeared more frequently in small and medium sized neurons (arrowhead). D, E: The staining pattern of TRPV1-positive neurons returned back to the normal level at 14 d and 21 d after inflammation. F: The section was counterstained with neutral red in order to calculate the number of TRPV1-positive neurons (deep colored positive cells compared with the red negative cells). Scale bar, 100 μm.

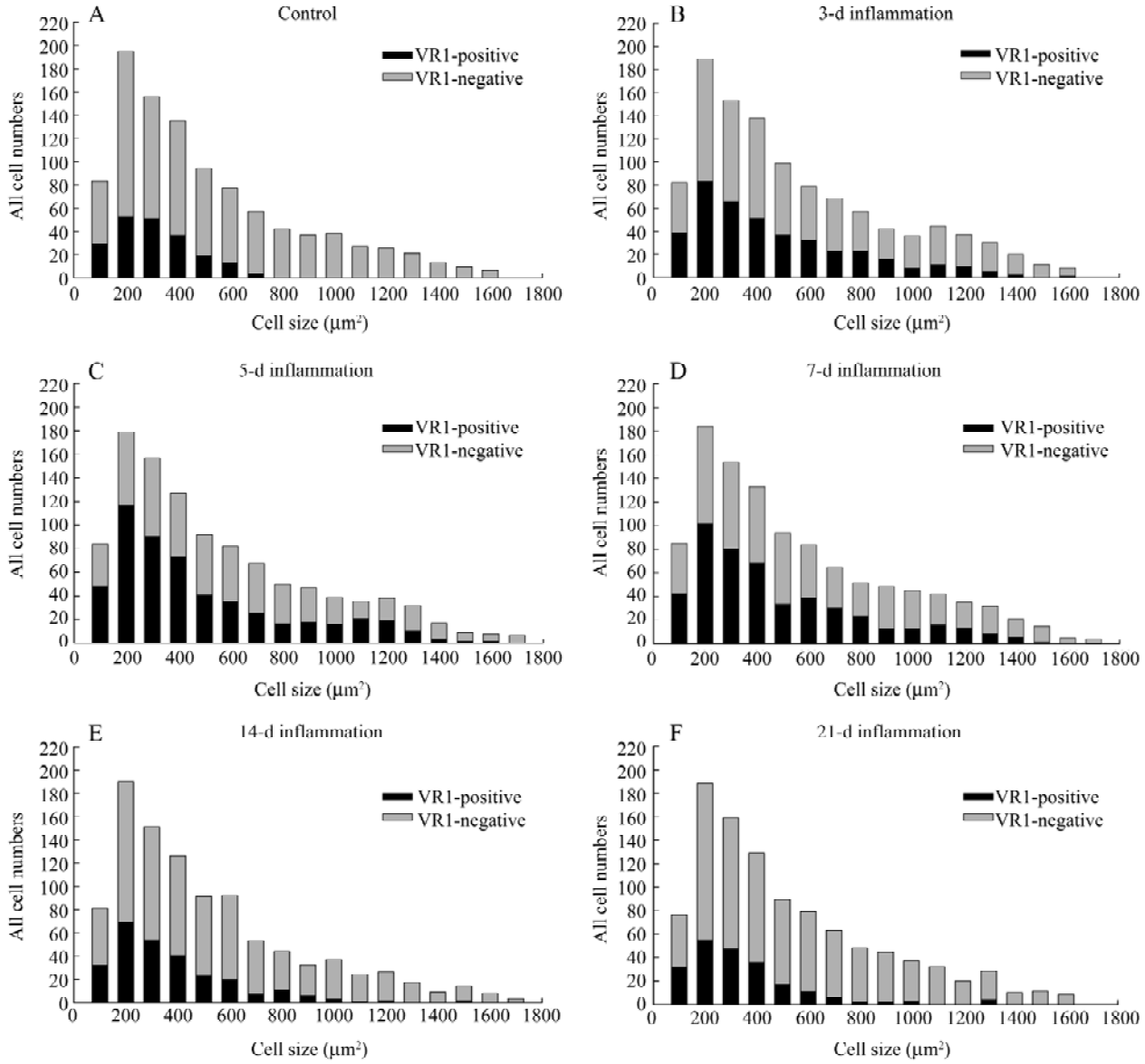

Fig. 3 Area–frequency distribution of TRPV1-positive neurons in the ipsilateral TG in rats at the pre-injection day (A) and day 3 (B), 5 (C), 7 (D), 14 (E) and 21 (F) after TO injection. The absolute value of numbers of TRPV1-expressing neurons from each TG that belonged to corresponding cell size was indicated. C, D: TRPV1-positive staining appeared more frequently in medium-sized (600–1200 μm²) TG neurons; E, F: the distribution pattern was similar to the control after 14 d.

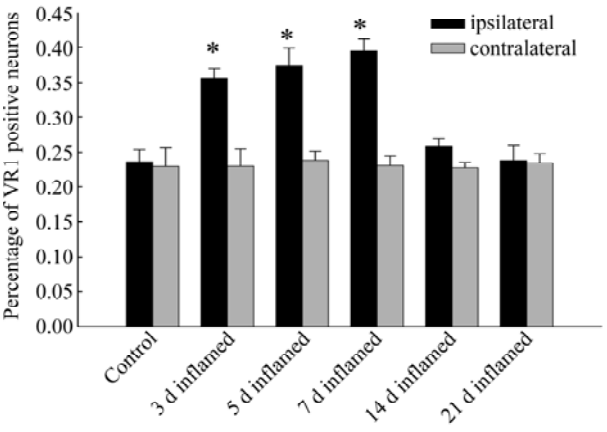

Fig. 4 Ratio of TRPV1-expression neurons to the total number of ipsilateral (n = 4) and contralateral (n = 4) TG in each group at different time points. The ratio increased significantly on day 3, 5 and 7 and returned to the normal level after 14 d in inflamed animals. * P < 0.05.

3.4.2 Change of TRPV1 expression in the ipsilateral trigeminal ganglion after TO injection The number of TRPV1-IR neurons in TG was measured on the pre-injection day (day 0) and on day 3, 5, 7, 14 and 21 after TO injection. Results from day 0 and day 5, 7 and 14 are shown in Fig. 2. The percentage of TRPV1 positive neurons at different time points mentioned in Fig. 3 are shown in Fig. 4. The ratio of the number of positive neurons to the number of total neurons in inflamed TG on day 3, 5 and 7 after TO injection increased significantly compared with that on day 0 ($n=4, P<0.05$). Area frequency histograms (Fig. 3) showed that TRPV1 expression on day 0 was almost entirely in small

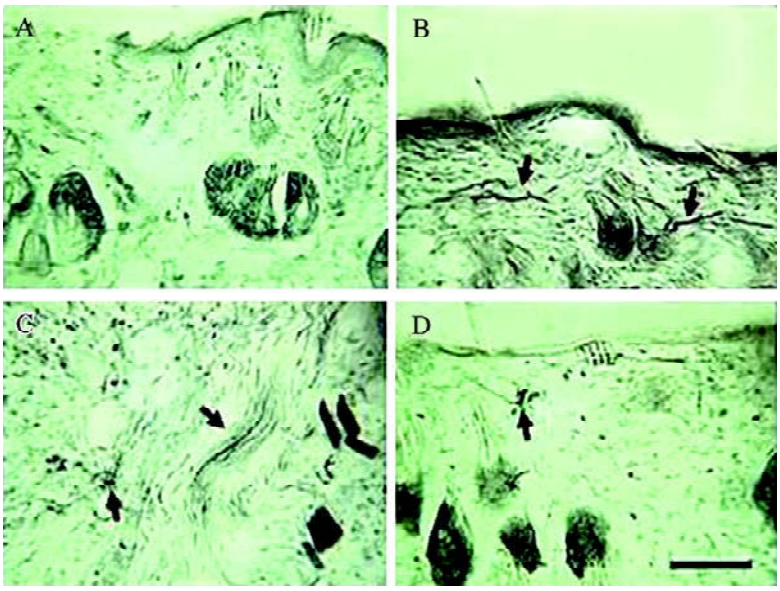

Fig. 5 Immunohistochemical staining for TRPV1-positive nerve fibers in the superficial dermal tier and deep dermal tier of the rats' vibrissal pad. A: TRPV1-IR nerve fibers occasionally penetrate the cutaneous of vibrissal pad in normal rats. B, C: On day 3 and 7 after TO induced inflammation, the TRPV1 positive fibers appeared more frequent and denser than the control. D: On day 14, TRPV1-positive fibers began to retrieve to the control level. Scale bar, 100 μm.

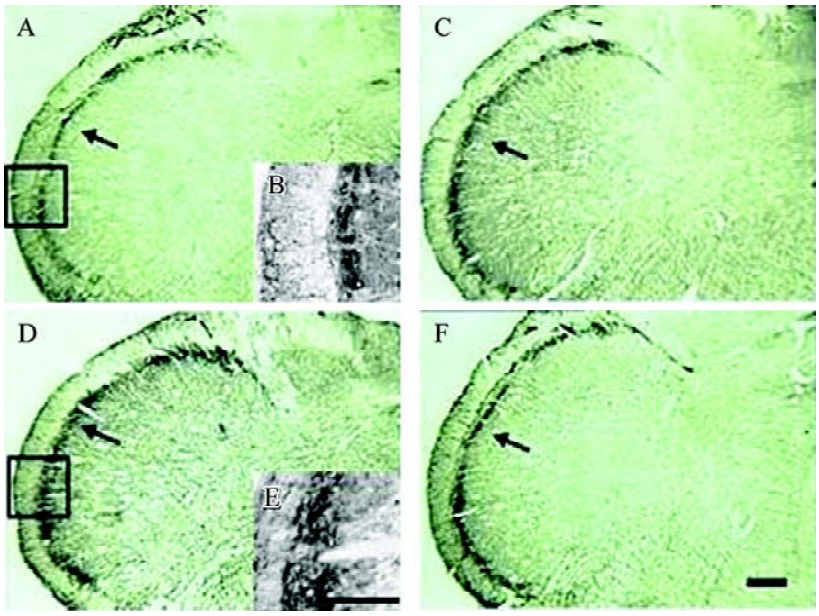

Fig. 6 Immunohistochemical staining for TRPV1 at the trigeminal caudal nucleus (Vc). A: The TRPV1-positive fibers and terminals are dense in laminae I and IIo throughout the dorsoventral and mediolateral aspects of Vc in normal rats. C, D: TRPV1 positive staining is stronger and denser on day 3 and 7 after inflammation, respectively. B and E: The high magnification of the boxed area in A and D, TRPV1 positive terminals appear denser in E than those in B. F: TRPV1-positive staining returned back to the control level on day 14 after TO injection. Scale bar, 100 μm in B, E; 200 μm in A, C, D, F.

and medium-sized neurons (less than 700 μm^2). On day 3, 5 and 7, the number of TRPV1-positive neurons conspicuously increased within small to medium sized (600–1200 μm^2) neurons compared with that on day 0 (Fig. 2B, C; Fig. 3B–D).

3.5 TRPV1-positive nerve fibers in vibrissal pad One of the main noxious sensory fields of TG is the vibrissal pad. Figure 5A shows a cross-section through the pad skin in the normal rats, and TRPV1-IR nerve fibers occasionally penetrated the cutaneous and deep dermal tier. On day 3 and day 7 after inflammation, it can be clearly seen that TRPV1 positive staining nerve fibers appeared more frequent and denser both in the superficial and deep dermal tier of the vibrissal pad skin (Fig. 5B, C). However, it was not obvious on day 14 after TO injection (Fig. 5D).

3.6 TRPV1 expression in Vc TRPV1-positive terminals in Vc were dense in laminae I and the outer zone of laminae II (IIo) in normal rats, this pattern was seen throughout the dorsoventral, mediolateral, and rostrocaudal extensions of these two layers (Fig. 6A). TRPV1-positive fibers were also dense in the external part of the spinal trigeminal tract, where they gave off axon collaterals terminating in laminae I and IIo (Fig. 6B). On day 3 and day 7 after inflammation, TRPV1-positive staining was denser (Fig. 6C, D) than that in controls. The average OD values on day 3 (0.54±0.05) and day 7 (0.56±0.04) were both significantly lower than the control group (0.98±0.13, n = 4; P = 0.013 and 0.019, respectively, ANOVA, Fig. 7). On day 14 after inflammation, TRPV1-positive staining began to return to the control level (Fig. 6E), and the average OD value was 0.91±0.03. There was no significant change compared with the control group (n = 4, P = 0.89, ANOVA, Fig. 7).

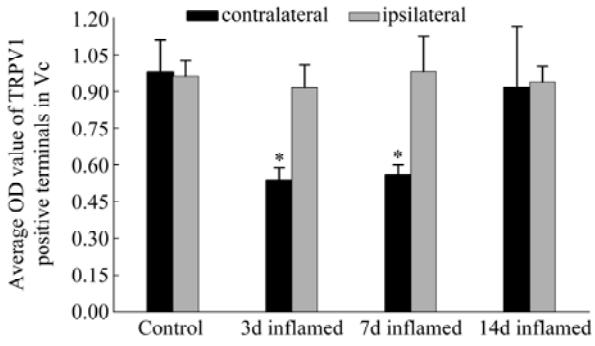

Fig. 7 Average optical density (OD) value of TRPV1-positive fibers and terminals in the trigeminal caudal nucleus (Vc) in each group at different time points. The average OD value decreased significantly on day 3 and 7 and returned back to the control level on day 14 after facial inflammation. * P < 0.05.

4 Discussion

The results of present study demonstrated that subcutaneous injection of TO in rat face could result in sterile inflammation and hypersensitivity lasting for at least 2 weeks. Immunohistochemical study on normal animals indicated that TRPV1 expression was restricted to small and medium sized TG sensory neurons (less than 700 μm^2), and the TRPV1-positive TG terminal fibers which project to both the periphery and the central area were abundant. However, after local inflammation, the number of TRPV1-positive neurons was significantly increased mainly in medium sized neurons (600–1200 μm^2). The ratio of neurons containing TRPV1-positive staining reached 1.5-fold to the basal level, suggesting that the up-regulation of TRPV1 expression in TG neurons occurred after inflammation and that the neurons, which were negative for TRPV1, could express this receptor when inflammatory hyperalgesia persisted. In addition, TRPV1-positive staining terminal fibers, either in the vibrissal pad or in the Vc, appeared more frequent and denser.

In the present study, we found that the number of TRPV1-positive neurons in the ipsilateral TG increased most significantly on day 3, 5 and 7 after facial inflammation. The rats simultaneously showed the lowest value of HWTL, which represented the rats' most significant hyperalgesia. However, two weeks later after inflammation, the expressions of both TRPV1 and HWTL began to return. These results demonstrated that there is an intimate correlation between the alteration of TRPV1 expression and the changes in thermal sensitivity in facial inflamed rats.

It is confirmed that TRPV1 is an important molecule for the development of thermal hyperalgesia under the inflammatory pain state. Selective TRPV1 antagonists could alleviate thermal hyperalgesia in the formalin and the carrageenan models of pain in rats[15,16]. Mice lacking this receptor do not develop thermal hyperalgesia after local inflammation[5,17]. Previous electrophysiological studies suggested that several chemical mediators produced within inflamed tissue could lower the TRPV1 threshold for capsaicin, pH and heat[10]. Induction of hyperalgesia immediately after inflammation seems to be, therefore, mainly due to activation of TRPV1 channel activity. These studies and our results indicate that increased expression of TRPV1 in the TG enhances the transport of this receptor, which subsequently up-regulates TRPV1 density and the behavioral sensitivities in the nerve terminals of inflamed tissue.

This variation of structural plasticity of sensory neurons could be an important mechanism that causes hyperalgesia following inflammation.

We successfully extended the method of cold pain sensation[14] to the facial area and performed it successfully. Interestingly, the results were almost consistent with the thermal hyperalgesia test, that is, rats showed enhanced response to cold stimulus for about 2 weeks, with a peak on day 3. Immunohistochemical observation showed that the striking difference in TRPV1 distribution between the control and inflamed animals was that more medium sized TG neurons expressed TRPV1 after inflammation. Generally, small TG neurons that are considered to be polymodal nociceptors with unmyelinated C-fibers[18], and medium sized neurons are likely with myelinated Aδ fibers[14]. Moreover, it is certainly believed that cold stimulation is conducted by myelinated Aδ fibers and unmyelinated C-fibers, but thermal stimulation is mainly conducted through unmyelinated C-fibers[19]. Early research works have demonstrated that the sensory fibers innervating cold receptors in rats had predominantly unmyelinated axons[20], and all mechanosensitive Aδ fibers were excited by noxious cold stimuli[14]. In addition, by using a heavy chain marker NF200 (also as a marker of myelinated neurons) to double-label TRPV1-IR DRG neurons, Amaya *et al*. found that the proportion of TRPV1-expressing neurons in the NF200-positive neurons was significantly increased in the inflamed animals, but the large NF200-positive neurons (more than 1200 μm^2) did not express TRPV1. This confirmed that a considerable number of thin myelinated Aδ neurons began to express TRPV1 after local inflammation[6]. These results together with ours suggest that the response to noxious cold stimulation might be mediated partly through capsaicin sensitive Aδ fibers. This structural plasticity of Aδ neurons and TRPV1 probably play an important role in cold pain sensation following inflammation.

In the normal rats, TRPV1-IR fiber terminals are existed in the vibrissal pad and Vc. Abundant TRPV1-IR terminals can be seen in the laminae I and IIo of Vc. Less TRPV1-IR fiber terminals is evidence in the epithelium of the vibrissal pad. Our immunohistochemical analysis of inflamed rats demonstrated that local persistent inflammation within 14 d dramatically increased the number of TRPV1-IR fiber terimals in both the vibrissal pad and Vc, which almost parallels with the increase of small and medium-sized TG neurons after inflammation. Previous report demonstrated that inflammation induced in rat hindpaw could produce an increased expression of TRPV1 in DRG neurons as well as the fibers in the sciatic nerve and the peripheral fiber terminals in the epidermis and dermis[8]. They also observed a very small increase in the spinal dorsal horn, but not very significant. In the present study, we found that TRPV1-positive staining in Vc was denser in laminae I and IIo, which is a little different from the previous observation. Based on an early electrophysiological study on TG in monkeys, researchers inferred that unmyelinated (C) heat sensitive fibers project to laminae I and IIo of Vc[21], and Vc contains much less dense labeling (TRPV1-positive fibers) in laminae IIi than in IIo. Such a distribution pattern is just opposite for the spinal dorsal horn[3,22]. The difference may be due to the fact that the orofacial afferents, consisting of unmyelinated C and thin myelinated Aδ fibers from small to medium-sized TG neurons, terminate in laminae I and IIo of Vc, whereas thick myelinated fibers from big DRG neurons project to the spinal dorsal horn and its deep laminae. Thus, in our recent study, the increase of TRPV1-positive fiber terminals both in the vibrissal pad and Vc during local inflammation could be due to the increased expression of TRPV1 in small and medium-sized TG neurons, which may contribute to the development of hyperalgesia.

In conclusion, the present study showed that subcutaneous injection of TO into the facial skin of rats could induce the increase of TRPV1 expression, suggesting that the changes of plasticity on TRPV1 expression in the TG, vibrissal pad and Vc may be involved in and play an important role in the thermal hyperalgesia and cold pain sensation.

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面部炎症痛诱发大鼠三叉神经节神经元中辣椒素受体表达的改变

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摘要 目的 探讨辣椒素受体(transient receptor potential vanilloid receptor 1，TRPV1)参与和面部炎症痛相关的热痛觉过敏与冷痛觉感受的可能机制。**方法** 于大鼠面部皮下注射松节油造成面部炎症痛模型，分别应用热测痛和冷测痛装置测量热缩头潜伏期(head withdrawal thermal latency, HWTL）和冷缩头潜伏期(head withdrawal cold lentency, HWCL)的变化，每天测量一次，连续21天。应用免疫组织化学染色，细胞大小频率分析和平均光密度值分析来研究面部炎症痛后第3、5、7、14、21天支配大鼠面部表皮区三叉神经节(trigeminal ganglion，TG)初级感觉神经元、触须部皮肤末梢神经纤维和投射至三叉神经感觉尾侧亚核(trigeminal sensory nuclei caudalis, Vc)中枢突TRPV1表达的改变。**结果** 注射松节油后第1至14天，热退缩反应潜伏期与冷退缩反应潜伏期均明显下降，分别于注射后第5天和第3天达到最低，第21天恢复到正常水平；注射松节油后第1至14天，TRPV1表达的细胞数量增加，并于第7天达到最大，第21天恢复到正常水平。正常大鼠TRPV1主要表达于TG的中小神经元，触须部皮肤以及三叉神经尾侧亚核含丰富的TRPV1阳性末梢；面部炎症痛后2周内，TG的中小神经元，触须部皮肤末梢以及Vc的Ⅰ和Ⅱ外层均可见明显的TRPV1表达增加。**结论** 面部炎症痛可以引起大鼠对伤害性热刺激和冷刺激的痛觉过敏，并导致三叉神经节中TRPV1阳性神经元和外周与中枢阳性神经纤维末梢数目增加，表明TRPV1在三叉神经节的中小神经元和末梢轴突表型的改变可能对松节油引起面部炎症痛时热痛觉过敏和冷痛觉感受的形成与维持起重要作用。

关键词：辣椒素受体；面部痛；痛觉过敏；三叉神经节