Bilateral mechanical and thermal hyperalgesia and tactile allodynia after chronic compression of dorsal root ganglion in mice

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Abstract: Objective Low back pain is one of the most inextricable problems encountered in clinics. Animal models that imitate symptoms in humans are valuable tools for investigating low back pain mechanisms and the possible therapeutic applications. With the development of genetic technology in pain field, the possibility of mutating specific genes in mice has provided a potent tool for investigating the specific mechanisms of pain. The aim of the present study was to develop a mouse model of chronic compression of dorsal root ganglion (CCD), in which gene mutation can be applied to facilitate the studies of chronic pain. **Methods** Chronic compression of L4 and L5 dorsal root ganglia was conducted in mice by inserting fine stainless steel rods into the intervertebral foramina, one at L4 and the other at L5. Mechanical allodynia and thermal hyperalgesia were examined with von Frey filaments and radiating heat stimulator, respectively. **Results** The CCD mice displayed dramatic mechanical and thermal hyperalgesia as well as tactile allodynia in the hindpaw ipsilateral to CCD. In addition, this mechanical and thermal hyperalgesia as well as tactile allodynia was also found to spread to the contralateral hindpaw. **Conclusion** This model, combined with the possible genetic modification, will strengthen our knowledge of the underlying mechanisms of low back pain. It also favors the development of new treatment strategies for pain and hyperalgesia after spinal injury and other disorders which affect the dorsal root ganglion in humans.

Keywords: chronic compression of dorsal root ganglion; mice; hyperalgesia; allodynia

1 Introduction

Chronic pain is a major challenge to clinical practice and basic science. Low back pain is one of the most common chronic pain syndromes, which is often caused by trauma, degenerative disorders as well as other diseases of the lumbar spine^[1]. Since the past few decades, our

understanding of the mechanisms of low back pain has progressed with the establishment of animal models which mimic its clinical syndromes. Among these pain models, chronic compression of dorsal root ganglion (CCD), which involves the implantation of a metal rod into the intervertebral foramen of the rat, is considered to closely resemble dorsal root ganglion (DRG) compression in humans as a consequence of an acutely herniated lumbar disc, foraminal stenosis, tumors, or other diseases of the spine^[2,3]. In this model, compression of one or 2 lumbar ganglia in rat induces abnormal spontaneous activity of primary sensory neurons and elicits chronic pain behaviors including hy-

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peralgesia and allodynia^[4–6]. Although traditional pharmacological, electrophysiological and behavioral studies in rats have provided valuable insights into pain mechanisms, the development of genetic technology in mice has opened unprecedented new perspectives in the understanding as well as in the treatment of chronic pain disorders. Molecular techniques and genetic engineering are regarded as the prime methods for addressing the cellular and the molecular mechanisms underlying chronic pain^[7]. Therefore, developing a mouse model of CCD will help elucidate the cellular and the molecular basis for development and maintenance of low back pain. The present study aimed to present a method of producing chronic compression of L4 and L5 DRGs in mice by inserting fine stainless steel rods into intervertebral foramina, one at L4 and the other at L5, thereby producing stenosis of the intervertebral foramina.

2 Materials and methods

2.1 Animals and surgery Adult C57BL/6 mice (12–16 weeks old, $n = 24$) were provided by the Laboratory Animal Center of the Fourth Military Medical University, and divided into CCD and sham operation groups ($n = 12$ in each group). The mice were housed in plastic boxes at 22–26 °C with access to food and water *ad libitum* in the colony room. A 12:12 h light/dark cycle with lights on at 08:00 was maintained and testing was done between 09:00 and 18:30. The mice were habituated to the test boxes for at least 30 min each day for 5 d before the testing. All the experimental procedures were approved by the Institutional Animal Use and Care Committee.

The surgical procedures were similar to those described previously^[2]. Briefly, mice were anesthetized with sodium pentobarbital (40 mg/kg, i.p., supplemented if necessary). The skin was incised on the left side of lumbar vertebrae between L3 and L6 and the paraspinal muscles were separated from vertebrae at the L3–L6 level to expose the L4 and the L5 intervertebral foramina clearly. A fine, L-shaped needle was inserted into each foramen at a 30° angle with respect to the dorsal middle line and a 20° angle with respect to the vertebral horizontal line. When the needle tip reached DRG, the ipsilateral leg exhibited a slight and transient twitch. Then, the needles were with-

drawn from the L4 and the L5 intervertebral foramina and stainless L-shaped steel rods (2.2 mm in length and 0.25 mm in diameter) were inserted into the intervertebral foramina, one at L4 and the other at L5, along the path of the needle. This was intended to produce steady and persistent compression of L4 and L5 DRGs. Then the muscular layer and skin were sutured and antibiotics were administered. Mice in sham control group received the same surgical procedure except the insertions of stainless steel rods into the intervertebral foramina.

2.2 Behavioral testing

2.2.1 Mechanical hyperalgesia and allodynia The mouse was placed in a Plexiglas box with a metal mesh floor and von Frey filaments were applied to the plantar surface of the bilateral hindpaws through the metal mesh floor. A series of von Frey filaments with increasing bending forces (0.07, 0.16, 0.40, 0.60, 1.00, 1.40, and 2.00 g) were applied to the testing site of the hindpaw. For each force of filament, 10 trials were conducted and paw withdrawal response frequency was calculated as the percentage of the number of positive responses to the number of stimuli. The occurrence of a 50% frequency of paw withdrawal was expressed as the mechanical threshold. This test was performed before operation and on days 1, 2, 3, 4, 7, 10, 14, 21, and 28 after CCD operation.

2.2.2 Thermal hyperalgesia To examine thermal hyperalgesia, the mouse was placed on the surface of a 2-mm-thick glass covered with a Plexiglas box. The sensitivity to heat stimuli was measured with a radiant heat stimulator. The heat source produced a light spot with a diameter of 2.5 mm, which was directed alternatively onto the plantar surfaces of the bilateral hindpaws, with 5-min inter-stimulus intervals between different paws and 10-min intervals in the same paw. Six stimuli were administered at the same testing site. The latency of paw withdrawal was recorded. The test was conducted before operation and on days 1, 2, 3, 4, 7, 10, 14, 21, and 28 after CCD operation. To avoid excessive tissue injury, the maximal duration of heat stimulation was 30 s.

2.3 Statistical analysis Data were presented as mean $\pm$ SEM, and analyzed by analysis of variance (ANOVA) for

random measures, followed by *post hoc* Fisher's test or Dunnett's test to determine statistically significant differences for all data. $P < 0.05$ was considered to be statistically significant.

3 Results

All the animals were in good health and did not show any signs of autotomy following CCD. Compared to the control mice, CCD mice were often seen to lift the affected hindpaw from the floor and hold it in a protected position next to flank while standing or sitting. Sometimes when the affected hindpaw touched the floor, mice appeared to

reduce the weight placed on it by sitting on the other side of the haunch.

3.1 Mechanical hyperalgesia and allodynia induced by CCD Sensitivity of the hindpaw ipsilateral to DRG compression to mechanical stimuli was tested on days 1, 2, 3, 4, 7, 10, 14, 21, and 28 after operation. Sensitivity of the contralateral hindpaw was also investigated at the same time points. As shown in Fig. 1A, in the basal state, the mean incidence of paw withdrawal response increased monotonically with the increase in von Frey filament bending force. Following CCD, mice showed the characteristic leftward and upward shift in the stimulus-response curve

Fig. 1 Sensitivity of ipsilateral hindpaw to mechanical stimuli in CCD and sham control groups. **A:** The response frequency of CCD mice to different forces of von Frey filaments prior to and on days 3 and 14 following unilateral CCD. CCD induced a characteristic leftward and upward shift in the stimulus-response curves over the basal curve. **B:** Sham control mice did not show any significant deviation in stimulus-response curves. **C:** The response thresholds of CCD mice to plantar von Frey hair application prior to and on days 1, 2, 3, 4, 7, 10, 14, 21 and 28 following unilateral CCD. **D:** The response thresholds of sham control mice to von Frey filament application prior to and at the corresponding time points after sham operation. All data were represented as mean $\pm$ SEM. * $P < 0.05$ vs the basal level.

over basal curve, reflecting allodynia (measured here as responses to 0.07–0.4 g forces, which were non-noxious in basal state) as well as mechanical hyperalgesia (measured here as responses to 0.6–2.0 g forces, which elicited withdrawal behavioral responses in basal state) in the ipsilateral hindpaw (Fig. 1A). Ipsilateral mechanical hyperalgesia and allodynia in CCD mice began on day 1 post operation, reaching the peak in week 2 and persisting over the whole testing time period. In comparison with the CCD mice, mice receiving sham operation did not show deviation in stimulus-response curve from basal state, as shown in Fig. 1B. The time courses of changes in mechanical thresholds

(defined here as the minimum force required to elicit the 50% response frequency) of the ipsilateral hindpaw in CCD and sham control mice were analyzed. As shown in Fig. 1C, mice showed a dramatic drop in mechanical threshold following CCD operation over the whole testing period ($P < 0.05$ at all time points tested). The mean threshold decreased from a basal level of (0.71 ± 0.09) g to (0.26 ± 0.06) g on day 3 post operation and to (0.11 ± 0.02) g on day 14 post operation. In contrast, mechanical threshold in sham control mice was significantly lowered only on day 1 post operation and returned to the basal level from day 2 (Fig. 1D).

Fig. 2 Sensitivity of contralateral hindpaw to mechanical stimuli in CCD and sham control groups. **A:** Comparison of response frequency to different forces of von Frey filaments in mice prior to and on days 3 and 14 following unilateral CCD. CCD induced a characteristic leftward and upward shift in the stimulus-response curves over the basal curve. **B:** Sham control mice did not show any significant deviation in stimulus-response curves. **C:** Response thresholds of CCD mice to plantar von Frey hair application prior to and on days 1, 2, 3, 4, 7, 10, 14, 21 or 28 following unilateral CCD. **D:** Response thresholds of control mice to von Frey filament application prior to and at the corresponding time points after sham operation. All data were represented as mean $\pm$ SEM. * $P < 0.05$ vs the basal level.

Similar to the ipsilateral hindpaw, the contralateral hindpaw in CCD mice also exhibited hypersensitivity to the mechanical stimuli. As shown in Fig. 2A, a leftward and upward shift of stimulus-response curve from basal curve was observed in contralateral hindpaw of mice following CCD operation, reflecting the contralateral mechanical hyperalgesia and allodynia. Analysis of mechanical threshold showed that the contralateral mechanical threshold dropped significantly from day 2 post operation and persisted through the whole time period tested (Fig. 2C, $P < 0.05$). In contrast, no significant mechanical hyperalgesia or allodynia was found in sham control mice (Fig. 2B, D).

3.2 Thermal hyperalgesia induced by CCD Sensitivities of the hindpaws ipsilateral and contralateral to DRG compression to heat stimuli were tested at the time points of days 1, 2, 3, 4, 7, 10, 14, 21, and 28 after operation. In the basal state, the mean thermal latency of ipsilateral paw withdrawal reflex to radiant heat stimuli was (10.50 ± 0.45) s. Following CCD operation, the thermal latency was significantly decreased (Fig. 3A, $P < 0.05$). The decrease in thermal latency began on day 1 post operation and persisted through the whole testing period. For example, the thermal latency dropped to (7.22 ± 0.63) s on day 3 post operation and to (5.63 ± 0.36) s on day 14 post operation. This reduction in thermal latency of ipsilateral hindpaw

Fig. 3 Sensitivities of ipsilateral and contralateral hindpaws to heat stimuli in CCD and sham control mice. A and B: The thermal latency of ipsilateral hindpaw to heat stimuli prior to and on days 1, 2, 3, 4, 7, 10, 14, 21 and 28 following unilateral CCD (A) and sham operation (B) in mice. C and D: The thermal latency of contralateral hindpaw to heat stimuli prior to and at the corresponding time points following unilateral CCD (C) and sham operation (D) in mice. All data were represented as mean $\pm$ SEM. * $P < 0.05$ vs the basal level.

indicated the occurrence of ipsilateral thermal hyperalgesia after chronic DRG compression. In contrast, thermal latency was not altered in sham control mice (Fig. 3B, $P > 0.05$).

Similarly, thermal latency in the contralateral hindpaw was reduced significantly from day 1 post operation and this reduction persisted for over 4 weeks (Fig. 3C, $P < 0.05$). In contrast, contralateral thermal hyperalgesia was not found in sham control mice (Fig. 3D, $P > 0.05$).

4 Discussion

Animal models in pain research offer great promise for investigating pain mechanisms and the possible therapeutic applications^[8,9]. With a high incidence in patients, low back pain has been imitated in various animal models. The rat model of CCD, which involves the placement of a stainless metal rod into the intervertebral foramen, is considered to closely resemble low back pain symptoms in humans resulting from herniated lumbar disc, foraminal stenosis, tumor, or other diseases of the spine^[2]. Traditional pharmacological and electrophysiological studies have provided much evidence on the mechanisms of low back pain^[4-6,10]. However, in recent years, with the development of genetic technology in pain field, our understanding of pain mechanisms was greatly strengthened. The possibility of mutating specific genes provides a potent tool for investigating the specific mechanisms of pain. So far, many mice models with specific pain phenotypes through gene mutation have been developed, and they, combined with other traditional techniques, can help us elucidate the specific cellular and molecular mechanisms of pain, particularly the chronic pain, which is a multifactorial phenomenon. Therefore, here CCD model was extended to mice and the pain behaviors induced by CCD were characterized.

The present study presented a method for imitating clinical low back pain and sciatica in mice by inserting 2 fine stainless-steel rods into the special locations of L4 and L5 ganglia (one for each) through the intervertebral foramina to maintain steady compression of DRG. Then the time course of pain behavior occurrence induced by DRG compression was observed. We found that chronic L4/L5 DRG compression produced strong mechanical

and thermal hyperalgesia as well as mechanical allodynia in the affected hindlimb. The hyperalgesia and allodynia started immediately on day 1 post compression and persisted for over 4 weeks. The rapid onset and persistence of pain hypersensitivity are similar to those observed in rat CCD model^[4-6,11]. Most interestingly, we observed that mechanical and thermal hyperalgesia as well as mechanical allodynia in CCD mice was not limited to the ipsilateral hindlimb, but spread to the contralateral hindlimb. The time course of contralateral hyperalgesia and allodynia corresponded to that observed in the ipsilateral side. However, previous findings on contralateral hyperalgesia and allodynia in rat CCD model were not consistent, with some revealing no hyperalgesia or allodynia in the contralateral side after CCD operation^[2,6], while others reporting remarkable contralateral hyperalgesia and tactile allodynia in CCD rats^[12,13]. Although this discrepancy has not been well explained, it is likely that the development of contralateral hyperalgesia and allodynia is related to the diameter of the rod for compression. Zhang *et al.* observed that the rod with a smaller diameter (*e.g.* 0.5–0.6 mm) tended to produce less contralateral mechanical hyperalgesia and failed to induce contralateral allodynia^[12]. Those studies finding contralateral hyperalgesia and allodynia after operation usually employed the compression rod with a diameter being 0.7 mm or larger. In the present study, different diameters of steel rods to compress mice ganglion were tested, and the diameter of 0.25 mm was finally selected, since with this diameter, rod compression induced marked hyperalgesia and allodynia with no motor deficits.

It is possible that the pain behaviors induced by DRG compression result from both direct, persistent compression and the secondary local inflammation of the DRG. The intervertebral foramen has a limited size, and it becomes further narrowed with the insertion of a metal rod, as described in CCD surgery. This narrowing would be exacerbated by any swelling of the tissue surrounding the DRG, thus establishing chronic, steady compression of the DRG. It has been shown that local compression of the DRG can induce an intraneural edema and reduce the blood flow to the sensory nerve cell somata^[14,15], which

may result in a change in neuronal excitability and thus cutaneous hyperalgesia and allodynia. In addition, the ischemia caused by DRG compression may initiate an inflammatory reaction and release a variety of inflammatory mediators derived from the circulation and the nervous system, which may intensify the effects of DRG compression^[5]. For example, administration of tumor necrosis factor (TNF) to DRG in rats induces cutaneous hyperalgesia to mechanical stimuli. Conversely, blocking the activity of certain inflammatory cytokines in DRG partially relieves the CCD-induced hyperalgesia^[16,17]. In addition, increased activity of satellite glial cells in the DRG has been recently shown to play an important role in CCD-induced neuronal hypersensitivity^[18]. However, the mechanisms underlying the spread of mechanical and thermal hyperalgesia as well as mechanical allodynia observed in CCD mice remain still unclear and require further investigation.

In summary, unilateral chronic compression of L4 and L5 DRGs in mice may result in rapid-onset, long-lasting mechanical and thermal hyperalgesia as well as mechanical allodynia on both ipsilateral and contralateral hindlimbs. Combined with the genetic modification technique, this experimental DRG compression model will strengthen the knowledge of the underlying mechanisms and facilitate the development of treatment strategies for pain and hyperalgesia induced by spinal injury and other disorders that affect the DRG in humans.

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References:

- [1] Devor M. Pain arising from the nerve root and the dorsal root ganglion. In: Weinstein JN, Gordon SL (eds). *Low back pain: a scientific and clinical overview*. Rosemont, IL: American Academy of Orthopaedic Surgeons, 1995: 187–208.
- [2] Hu SJ, Xing JL. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain* 1998, 77: 15–23.
- [3] Carette S, Fehlings MG. Cervical radiculopathy. *N Engl J Med* 2005, 353: 392–399.
- [4] Zhang JM, Song XJ, LaMotte RH. Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. *J Neurophysiol* 1999, 82: 3359–3366.
- [5] Ma C, LaMotte RH. Enhanced excitability of dissociated primary sensory neurons after chronic compression of the dorsal root ganglion in the rat. *Pain* 2005, 113: 106–112.
- [6] Song XJ, Hu SJ, Greenquist KW, Zhang JM, LaMotte RH. Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. *J Neurophysiol* 1999, 82: 3347–3358.
- [7] Kuner R. Genetic approaches for the study of pain. In: Zhuo M, Gebhart GF (eds). *Molecular pain*. China: Higher Education Press, 2006.
- [8] Mogil JS. Animal models of pain: progress and challenges. *Nat Rev Neurosci* 2009, 10: 283–294.
- [9] Decosterd I, Berta T. Animal models and neuropathic pain. In: Basbaum AL, Bushnell MC (eds). *Science of pain*. San Diego: Academic Press, 2009: 857–864.
- [10] Ma C, Greenquist KW, LaMotte RH. Inflammatory mediators enhance the excitability of chronically compressed dorsal root ganglion neurons. *J Neurophysiol* 2006, 95: 2098–2107.
- [11] LaMotte RH, Chao MA. Hyperexcitable neurons and altered non-neuronal cells in the compressed spinal ganglion. *Acta Pharmacologica Sinica* 2008, 60: 597–602.
- [12] Zhang JM, Li HQ, Brull SJ. Perfusion of the mechanically compressed lumbar ganglion with lidocaine reduces mechanical hyperalgesia and allodynia in the rat. *J Neurophysiol* 2000, 84: 798–805.
- [13] Chen XW, Zhang XJ, Xie YB, Zhang TW, Wang T, Xie RG, *et al.* Behaviors of mechanical and cold allodynia in chronically compressed multiple DRG rats. *Chin J Pain Med* 2009, 15: 352–362.
- [14] Igarashi T, Yabuki S, Kikuchi S, Myers RR. Effect of acute nerve root compression on endoneurial fluid pressure and blood flow in rat dorsal root ganglia. *J Orthopaedic Res* 2005, 23: 420–424.
- [15] Rydevik BL, Myers RR, Powell HC. Pressure increase in the dorsal root ganglion following mechanical compression: closed compartment syndrome in nerve roots. *Spine* 1989, 14: 574–576.
- [16] Homma Y, Brull SJ, Zhang JM. A comparison of chronic pain behavior following local application of tumor necrosis factor alpha to the normal and mechanically compressed lumbar ganglia in the rat. *Pain* 2002, 95: 235–246.
- [17] Zhang JM, Li H, Liu B, Brull SJ. Acute topical application of tumor necrosis factor α evokes protein kinase A-dependent responses in rat sensory neurons. *J Neurophysiol* 2002, 88: 1387–1392.
- [18] Zhang HJ, Mei XF, Zhang P, Ma C, White FA, Donnelly DF, *et al.* Altered functional properties of satellite glial cells in compressed spinal ganglia. *Glia* 2009, 57: 1588–1599.

小鼠背根节慢性压迫诱致的双侧机械性痛敏和热痛敏行为

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摘要: **目的** 腰背痛是一个临床常见的棘手问题。动物模型可以很好地模拟人类的临床症状, 并为研究腰背痛的机制及其药物治疗带来希望。随着转基因技术的发展, 特定的转基因小鼠模型为研究疼痛机制提供了一个强有力的工具。本研究旨在建立慢性背根节压迫小鼠模型, 为应用转基因动物深入研究慢性痛的发生机制提供重要工具。**方法** 向小鼠腰4和腰5椎间孔内插入细钢柱造成腰4和腰5背根节慢性压迫模型, 采用von Frey细丝和辐射热刺激仪检测机械性痛敏和热痛敏。**结果** 小鼠一侧腰4和腰5背根节持续压迫后导致同侧肢体出现显著的机械性痛敏和热痛敏。另外, 压迫的对侧肢体也出现了明显的机械性痛敏和热痛敏。**结论** 此模型的建立可以使转基因动物发挥优势, 并为治疗人类脊髓损伤和背根节异常后的疼痛和痛觉过敏提供新的对策。

关键词: 背根节慢性压迫; 小鼠; 痛敏; 触诱发痛