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Effects of traditional "*Juci*" (contralateral acupuncture) on orofacial nociceptive behavior in the rat

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Abstract

Purpose. "*Juci*", one of the traditional acupuncture techniques, means contralateral acupuncture; i.e., implanting a needle into an acupoint to treat a given disease or disorder, but on the side of the body opposite to the diseased side. The aim of this study was: (1) to assess acupuncture effects on formalin-induced nociceptive behavior in the orofacial region in the rat, and (2) to evaluate the efficacy of *Juci* in the orofacial formalin test.

Methods. Forty-four adult male Wistar rats were used in the present study. A 1.0% formalin solution (25 μl s.c., diluted in saline) was injected into the right upper lip. The rats were randomly assigned to five groups. (1) The control group ($n = 9$), which received formalin injection without acupuncture pretreatment; (2) the ipsilateral Ho-ku (see note below) acupuncture group ($n = 10$); (3) the contralateral Ho-ku acupuncture group ($n = 11$); (4) the acupuncture plus naloxone group ($n = 9$), where intraperitoneal naloxone ($1.0 mg \cdot kg^{-1}$) was injected immediately before acupuncture pretreatment; and (5) the sham acupuncture group ($n = 5$). "Ho-ku" is the term used for the "Large Intestine 4" acupoint, located between the first and second metacarpal bones.

Results. The injection of formalin produced the characteristic biphasic behavioral response. Acupuncture significantly inhibited the response in the early and late phases. Naloxone significantly reversed these effects. There were no statistically significant differences between the ipsilateral and *Juci* acupuncture groups. Sham acupuncture did not exert any significant effect on the formalin-induced behavior.

Conclusion. Our results showed that the degree of effectiveness of *Juci* was similar to that of the ipsilateral acupuncture technique. Therefore, the *Juci* technique is also useful for the treatment of orofacial pain.

Key words Acupuncture · *Juci* · Orofacial formalin test · Nociception · Rat

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Introduction

Acupuncture, an ancient therapeutic procedure in traditional Chinese medicine, has been practiced for over 2000 years [1–4]. There has been growing interest in the West in the application of acupuncture to control pain since the visit of United States President Nixon to China in 1971 [1,3,4]. More recently, the NIH Consensus Development Panel on Acupuncture has agreed that it is useful for the treatment of postoperative and chemotherapy-induced nausea and vomiting, and postoperative dental pain, based on over 2000 scientific articles [3].

A wide variety of acupuncture techniques exists [2], one of which is *Juci*. "*Juci*" is a technique advocated in the oldest text on traditional Chinese medicine that includes acupuncture, the "*Huang Di Nei Jing*" or the *Yellow Emperor's Classic of Internal Medicine* [5]. Whereas conventional acupuncture treatment is performed on acupoints ipsilateral or bilateral to the diseased side [6], *Juci* means contralateral acupuncture; i.e., implanting a needle into the indicated acupoint to treat a given disease or disorder, but on the side of the body opposite to the disease side. This traditional technique contributes to the successful treatment of various diseases, such as hemihidrosis, postherpetic neuralgia, acute traumatic pain, spinogenic dizziness, and apoplectic hemiplegia [7–9]. The contralateral technique is useful when ipsilateral acupuncture is contraindicated, e.g., in the presence of local infection, trauma, anomaly, and post-amputation. Although several clinical reports support the efficacy of the intriguing *Juci* technique, no studies so far have compared the efficacy of conventional ipsilateral acupuncture and that of *Juci* acupuncture, especially in the trigeminal region.

There are relatively few behavioral models in laboratory animals dedicated to the study of nociception in the trigeminal region. One of the important models used to assess nociceptive processes in the orofacial region is the orofacial formalin test [10]. The formalin test in the paw was originally described by Dubuisson and Dennis [11], and it has been used widely to test various analgesic effects, but not for trigeminal areas [12]. Clavelou et al. [13] developed an orofacial formalin test to assess pain in the trigeminal region. In both models, the time course of the response is biphasic, with an early and short-lasting first phase, followed, after a quiescent period, by a late prolonged (tonic) phase that mimics some features of post-injury pain in humans [10]. In spite of the usefulness of the assessment of trigeminal pain by the orofacial formalin test, there are no available data to assess the antinociceptive effect of acupuncture using this test. Therefore, in the present study, using the orofacial formalin test, we evaluated the analgesic effects of acupuncture and, in addition, we assessed whether the effects of traditional *Juci* were significant or not.

Materials and methods

Animals

Forty-four adult male Wistar albino rats, weighing 200–250 g, were used in the present study. They were housed in plastic cages with soft bedding (4 per cage) with free access to food and water. The animals were maintained in a climate- and light-controlled room (23 ± 1°C; 12-h: 12-h dark-light cycle with lights on at 08: 00 a.m.) for at least 1 week prior to the experiment. Testing was carried out between 11: 00 a. m. and 5: 00 p. m. in a glass box (dimensions 30 × 30 × 30 cm, with three mirrored sides). The rats did not have any access to food or water during the test. Each rat was used only once in this study. All experiments conformed to the ethical guidelines recommended by the International Association for the Study of Pain, for experimental pain in conscious animals [14], and were approved by the Nagasaki University Animal Care Committee.

Testing procedure

The acupoint used was the Ho-ku (Large Intestine 4) located between the first and second metacarpal bones. Ho-ku is one of the acupoints commonly used for the treatment of orofacial pain [15]. A stainless steel needle (0.2 mm in diameter and 3.0 mm in length; KN-107 J No. 3; Seirin, Shizuoka, Japan) was inserted at the Ho-ku to a depth of 3.0 mm. In the sham acupuncture group, the same type of needle was inserted to a non-acupoint between the third and fourth metacarpal bones as deeply as in the Ho-ku acupuncture group. The animal was placed in the test box for a 30-min habituation period. Following this habituation period, 25 µl of 1.0%

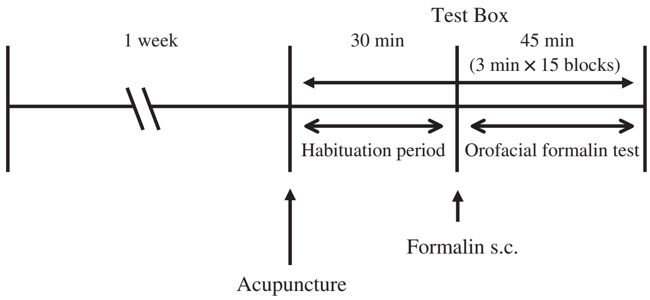

Fig. 1. Diagrammatic summary of the experimental procedure

formalin (diluted in sterile saline from stock formaldehyde 37% solution) was subcutaneously injected into the upper lip, just lateral to the nose, through a 30-gauge needle, and the animal was placed back in the test box for a 45-min observation period. The acupuncture needle was not withdrawn until the end of the experiment. Leaving the needle in place for long periods of time, even for days, is one of the variations of the needling technique, using an intradermal needle or perpendicular intradermal needle, in the clinical field [16]. The recording time was divided into 15 blocks of 3 min, and a pain score was determined for each block by measuring the number of seconds during which the animals spent rubbing the injected area with the ipsilateral fore- or hindpaw. Figure 1 shows the time-line of the experimental procedures.

The rats were randomly assigned to five groups: (1) a control group ($n = 9$), which received formalin injection without acupuncture pretreatment; (2) an ipsilateral acupuncture group ($n = 10$); (3) a contralateral acupuncture group ($n = 11$); (4) an acupuncture plus naloxone group ($n = 9$), where intraperitoneal naloxone (1.0 mg·kg^{-1}) was injected immediately before acupuncture pretreatment; and (5) a sham acupuncture group ($n = 5$).

A digital video camera (GR-DVX35K; Victor Company of Japan, Yokohama, Japan) was used to record the behavioral nociceptive response during the orofacial test. Analysis of the behavior was made by an investigator who was blinded to the animals' group assignments.

Statistical analysis

The reaction to the formalin injection was analyzed in terms of early and late phases; the early phase being the first 3 min after the formalin injection, and the late phase the period between 12 and 45 min after the formalin injection.

Data were analyzed using one-way analysis of variance (ANOVA), followed by Fisher's protected least

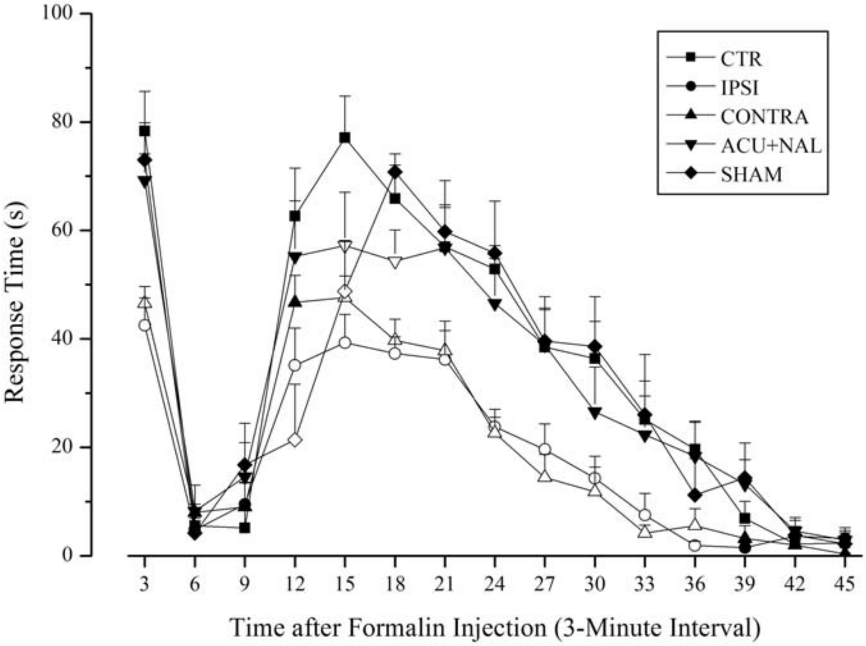

Fig. 2. Time course of the orofacial formalin test. Face-rubbing responses after the injection of 25 µl of 1.0% formalin. In all groups, typical biphasic response was observed. Namely, an early-phase response began almost immediately and generally disappeared within the first 3-min interval. A late-phase response generally began during the fourth interval (9 to 12 min), peaked during the fifth interval (12 to 15 min), and usually dissipated by the fifteenth interval (42 to 45 min). *CTR*, control group, which received formalin injection without acupuncture pretreatment ($n = 9$); *IPSI*, ipsilateral acupuncture group ($n = 10$); *CONTRA*, contralateral acupuncture group ($n = 11$); *ACU+NAL*, acupuncture plus naloxone group ($n = 9$); *SHAM*, sham acupuncture group ($n = 5$). *Open symbols* indicate $P < 0.05$ compared to the control group. *Error bars* indicate SEM

significant difference (PLSD) test for multiple comparisons between groups. For all tests, the level of significance was set at $P < 0.05$. Data are presented as mean ± SEM.

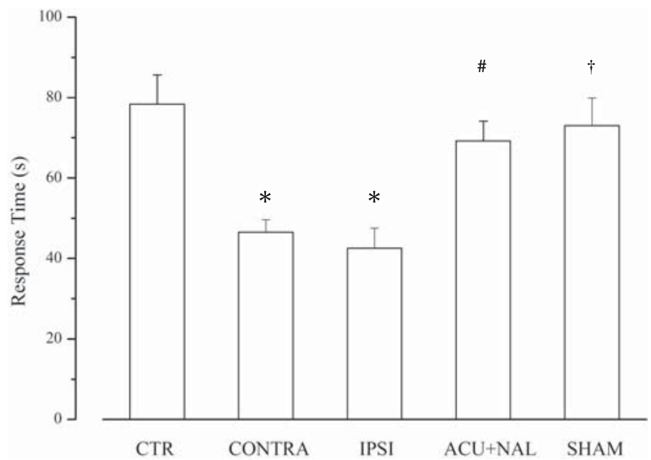

Fig. 3. Comparison of face-rubbing responses in the early phase. Contra- and ipsilateral acupuncture pretreatment significantly inhibited the face-rubbing response. Intraperitoneally administered naloxone ($1\,mg \cdot kg^{-1}$) significantly reversed the reduced behavior. *$P < 0.05$ compared to the control group. $^{\#}P < 0.05$ compared to the acupuncture group. $^{\dagger}P < 0.05$ compared to the ipsilateral acupuncture group. *Error bars* indicate SEM. *CTR*, control group; *CONTRA*, contralateral acupuncture group; *IPSI*, ipsilateral acupuncture group; *ACU+NAL*, acupuncture plus naloxone group; *SHAM*, sham acupuncture group

Results

Effect of formalin on orofacial nociception

The injection of formalin produced the characteristic biphasic behavioral response (Fig. 2). The early-phase responses began almost immediately on the rat's return to the test box and generally disappeared within the first 3-min interval. The mean (± SEM) response time of the early phase was 78.3 (± 7.3) s. The late-phase responses generally began during the fourth interval (9 to 12 min), peaked during the fifth interval (12 to 15 min), and had usually dissipated by the fifteenth interval (42 to 45 min). The mean response time of the late phase was 409.2 (± 44.1) s.

Effect of acupuncture on the early phase

The mean response time of the early phase in the ipsilateral acupuncture group and contralateral acupuncture group were 42.5 (± 5.0) and 46.5 (± 3.1) s, respectively. In both acupuncture pretreatment groups, the early-phase response was significantly inhibited by acupuncture stimulation. However, there was no significant difference between the ipsilateral acupuncture group and the contralateral one. Naloxone significantly reversed the reduced nociceptive behavior in the early phase (Fig. 3), indicating that endogenous opioids were involved in the suppression of the orofacial nociceptive behavior evoked by the formalin injection.

Effect of acupuncture on the late phase

The mean response durations of the late phase in the ipsilateral acupuncture group and the contralateral one

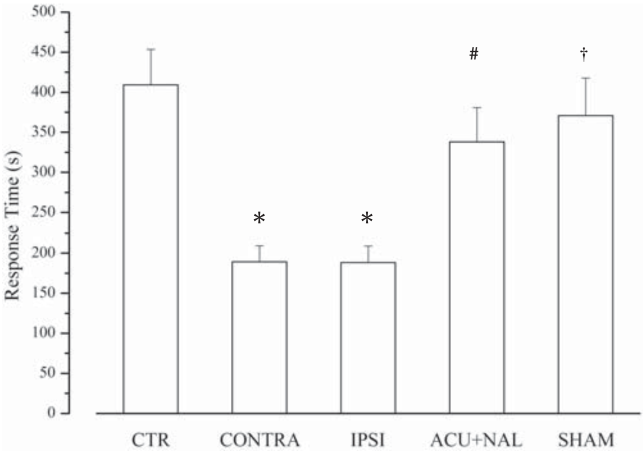

Fig. 4. Comparison of face-rubbing responses in the late phase. Contra- and ipsilateral acupuncture pretreatment significantly inhibited the face-rubbing response. Intraperitoneally administered naloxone ($1\,mg \cdot kg^{-1}$) significantly reversed the reduced behavior. *$P < 0.05$ compared to the control group. $^{\#}P < 0.05$ compared to the acupuncture group. $^{\dagger}P < 0.05$ compared to the ipsilateral acupuncture group. *Error bars* indicate SEM. *Abbreviations* are the same as those in Fig. 3

were 188.2 (± 20.6) and 189.4 (± 19.8) s, respectively. As in the early phase of the orofacial formalin test, the late-phase response was significantly inhibited in both acupuncture pretreatment groups. There was no significant difference between the ipsilateral and contralateral acupuncture groups. Naloxone significantly reversed the effect of acupuncture in the late phase (Fig. 4).

Effect of sham acupuncture on the formalin-induced behavior

The characteristic biphasic behavioral response was observed in the sham acupuncture group, as was in all the other groups (Fig. 2). The mean response time of the early phase was 73.0 (± 6.9) s. This was significantly different from that in the ipsilateral acupuncture group, and there was no significant difference between the control group and the sham acupuncture group (Fig. 3). The mean response time of the late phase was 371.0 (± 46.8) s. Similar to the early phase, this was significantly different from that in the ipsilateral acupuncture group, and there was no significant difference between the control group and the sham acupuncture group (Fig. 4).

Discussion

In the present study, we demonstrated that the orofacial formalin test is useful for assessing the analgesic effects induced by acupuncture in the trigeminal system and, furthermore, we showed that contralateral acupuncture is as effective as the ipsilateral one. This is in agreement with several studies which have suggested that unilateral acupuncture exerts its effects bilaterally or contralaterally in non-trigeminal regions [17,18]. A significant contralateral effect of acupuncture has been reported in some studies [17–19]. However, the mechanism underlying it is not fully understood.

Contralateral effects mediated by spinal interneurons, in particular, are a putative mechanism of the *Juci* effect, i.e., interaction between both sides of the spinal cord have been demonstrated in recent studies [20–22]. Electrophysiological studies have shown that a significant proportion of neurons in the dorsal horn are inhibited by noxious stimulation of the homologous contralateral territory [20,22]. With regard to acupuncture, Bing et al. [23] have investigated the effects of manual acupuncture on the activities of medullary neurons located in the subnucleus reticularis dorsalis of the rat. Although the responses of the wide dynamic range neurons to contralateral stimuli were three to five times greater than those to the corresponding ipsilateral stimuli, the responses of nociceptive specific neurons were only slightly weaker from ipsilateral parts of the body than from their contralateral counterparts. Actually, it has been reported that unilateral interventions, including acupuncture, produce a bilateral or contralateral effect [7–9,24–26]. Bileviciute-Ljungar and Lundeberg [25,26] have reported that a contralateral injection with a local anesthetic reduced nociceptive behavior and inflammatory edema formation in rats following acute carrageenan-induced inflammation and mononeuropathy. Further, an electrophysiological study by Bileviciute-Ljungar et al. [24] was in agreement with these findings. These observations suggested that spinal interneurons have an important role in producing the *Juci* effect. Besides interactions between the two sides, projections of trigeminal afferents to the contralateral medullary horn contribute to the *Juci* effect. In several studies, it has been demonstrated that neurons within the medullary dorsal horn receive input from both the ipsi- and contralateral trigeminal areas [27,28]. In addition, the trigeminal nuclei also receive afferent input from the upper extremities [15]. Therefore, the neuronal activities elicited by contralateral hoku acupuncture may exert their effects by modulating neuronal input from the pathological region with which the Ho-ku region share a pathway.

Other mechanisms underlying the *Juci* effect are analgesia via endogenous opioids [29] and/or diffuse noxious inhibitory controls (DNIC) [30]. Acupuncture is known to stimulate the release of endogenous opioids, and the analgesic effect of acupuncture has been reported to be reversible by the opioid receptor antagonist naloxone [29,31]. This effectively explains why acupuncture in one part of the body can affect pain in another region. Le Bars et al. [32,33] have shown that the activities of neurons in the spinal cord and trigeminal dorsal horn are inhibited by various noxious stimuli applied to areas of the body surface remote from the excitatory receptive fields of the neurons, and they dubbed the phenomenon DNIC. A close relationship between DNIC and acupuncture analgesia has been suggested [30,34]. DNIC-induced analgesia is also reversed by naloxone [30,35]. In the present study, the antinociceptive effect of acupuncture on the formalin-induced behavior was confirmed to be reversed by the pretreatment with naloxone.

Recently, Cho et al. [36] demonstrated that a specific acupoint (Bladder 67) appeared to be associated with the vision-related occipital cortex, using functional magnetic resonance imaging (fMRI), providing evidence to support the hypothesis that acupoints are related to specific internal organs. Hui et al. [37] have reported that unilateral acupuncture stimulation showed bilateral neuronal modulation of cortical and subcortical structures. Another study also found that unilateral acupuncture exerted an effect on the central nervous system bilaterally [38]. Although the mechanisms underlying these acupuncture-induced central changes have not been clarified, these findings offer some rationale for the contralateral *Juci* effect of unilateral acupuncture.

In the orofacial formalin test, intervention should not affect motor performance, because rubbing is the only parameter of the nociception [10]. In the present study, we investigated the effect of needling on a non-acupoint and proved that mere needling beside Ho-ku, did not affect the rubbing activities. Therefore, the reduced nociceptive behavior in the "*Juci*" and ipsilateral acupuncture groups is attributed to the acupuncture effect.

In summary, we investigated the efficacy of the '*Juci*' technique using the orofacial formalin test. Ipsi- and contralateral acupuncture techniques produced similar anti-nociceptive effects. Our results provide supporting data for the use of the "*Juci*" technique, because it was as effective as the ipsilateral technique.

References

1. Hansson P, Lundeberg T (2004) Transcutaneous electrical nerve stimulation, vibration and acupuncture as pain-relieving measures. In: Wall PD, Melzack R (eds) Textbook of pain, 4th edn. Churchill Livingstone, Edinburgh, pp 1341–1351
2. Uom ES, Min BI, Kim JH, Cho YW (2001) Analgesic effect of the acupuncture using the method of quick insertion and withdrawal of the needle in rats. Neurosci Lett 298:21–24
3. Ramsay D, Bowman M, Greenman P, Jiang S (1998) Acupuncture. JAMA 280:1518–1524
4. Audette JF (2004) Acupuncture. In: Warfield CA, Bajawa ZH (eds) Principles and practice of pain medicine, 2nd edn. McGraw-Hill, New York, pp 785–791
5. Unschuld PU (2003) Invasive therapies. In: Huan Di nei jing su wen. University of California Press, Berkeley, pp 265–283
6. Beijing College of Traditional Chinese Medicine, Shanghai College of Traditional Chinese Medicine, Nanjing College of Traditional Chinese Medicine, The Acupuncture Institute of the Academy of Traditional Chinese Medicine (1980) A brief introduction to acupuncture analgesia. In: Essentials of Chinese acupuncture. Foreign Language Press, Beijing, pp 416–425
7. Lu F (1997) Clinical application of contralateral acupuncture technique. J Tradit Chin Med 17:124–126
8. Cheng B (1996) 206 cases of spinogenic dizziness treated by contralateral acupuncture. J Tradit Chin Med 16:35–36
9. Sui S, Huang X (2004) Acupuncture methods for treatment of hemiplegia. J Tradit Chin Med 24:46–48
10. Raboisson P, Dallel R (2004) The orofacial formalin test. Neurosci Biobehav Rev 28:219–226
11. Dubuisson D, Dennis SG (1977) The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4:161–174
12. Sawynok J, Reid A (2001) Antinociception by tricyclic antidepressants in the rat formalin test: differential effects on different behaviours following systemic and spinal administration. Pain 93:51–59
13. Clavelou P, Pajot J, Dallel R, Raboisson P (1989) Application of the formalin test to the study of orofacial pain in the rat. Neurosci Lett 103:349–353
14. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110
15. Ekblom A, Hansson P (1985) Extrasegmental transcutaneous electrical nerve stimulation and mechanical vibratory stimulation as compared to placebo for the relief of acute oro-facial pain. Pain 23:223–229
16. Bischko J (1978) The technique of acupuncture and the reactions it causes. In: An introduction to acupuncture, Haug Verlag, Heidelberg, pp 37–47
17. Farber PL, Tachibana A, Campiglia HM (1997) Increased pain threshold following electroacupuncture: analgesia is induced mainly in meridian acupuncture points. Acupunct Electrother Res 22:109–117
18. Milne RJ, Dawson NJ, Butler MJ, Lippold OC (1985) Intramuscular acupuncture-like electrical stimulation inhibits stretch reflexes in contralateral finger extensor muscles. Exp Neurol 90:96–107
19. Toda K (1981) Peripheral afferent nerve impulses for conveying electroacupuncture effects on the jaw opening reflex evoked by tooth pulp stimulation in rat. Am J Chin Med 9:319–325
20. Fitzgerald M (1982) The contralateral input to the dorsal horn of the spinal cord in the decerebrate spinal rat. Brain Res 236:275–287
21. Koltzenburg M, Wall PD, McMahon SB (1999) Does the right side know what the left is doing? Trends Neurosci 22:122–127
22. Sotgiu ML, Biella G (1998) Contralateral inhibitory control of spinal nociceptive transmission in rats with chronic peripheral nerve injury. Neurosci Lett 253:21–24
23. Bing Z, Villanueva L, Le Bars D (1991) Acupuncture-evoked responses of subnucleus reticularis dorsalis neurons in the rat medulla. Neuroscience 44:693–703
24. Bileviciute-Ljungar I, Biella G, Bellomi P, Sotgiu ML (2001) Contralateral treatment with lidocaine reduces spinal neuronal activity in mononeuropathic rats. Neurosci Lett 311:157–160
25. Bileviciute-Ljungar I, Lundeberg T (2000) Contralateral but not systemic administration of bupivacaine reduces acute inflammation in the rat hindpaw. Somatosens Mot Res 17:285–293
26. Bileviciute-Ljungar I, Lundeberg T (2000) Contralateral treatment with xylocaine reduces nociceptive behaviour in mononeuropathic rats. Neuroreport 11:291–295

27. Jacquin MF, Chiaia NL, Rhoades RW (1990) Trigeminal projections to contralateral dorsal horn: central extent, peripheral origins, and plasticity. Somatosens Mot Res 7:153–183
28. Marfurt CF (1981) The central projections of trigeminal primary afferent neurons in the cat as determined by the tranganglionic transport of horseradish peroxidase. J Comp Neurol 203:785–798
29. He LF (1987) Involvement of endogenous opioid peptides in acupuncture analgesia. Pain 31:99–121
30. Bing Z, Villanueva L, Le Bars D (1990) Acupuncture and diffuse noxious inhibitory controls: naloxone-reversible depression of activities of trigeminal convergent neurons. Neuroscience 37:809–818
31. Pomeranz B, Chiu D (1976) Naloxone blockade of acupuncture analgesia: endorphin implicated. Life Sci 19:1757–1762
32. Le Bars D, Dickenson AH, Besson JM (1979) Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain 6:283–304
33. Le Bars D, Dickenson AH, Besson JM (1979) Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. Pain 6:305–327
34. Murase K, Kawakita K (2000) Diffuse noxious inhibitory controls in anti-nociception produced by acupuncture and moxibustion on trigeminal caudalis neurons in rats. Jpn J Physiol 50:133–140
35. Le Bars D, Chitour D, Kraus E, Dickenson AH, Besson JM (1981) Effect of naloxone upon diffuse noxious inhibitory controls (DNIC) in the rat. Brain Res 204:387–402
36. Cho ZH, Chung SC, Jones JP, Park JB, Park HJ, Lee HJ, Wong EK, Min BI (1998) New findings of the correlation between acupoints and corresponding brain cortices using functional MRI. Proc Natl Acad Sci USA 95:2670–2673
37. Hui KK, Liu J, Makris N, Gollub RL, Chen AJ, Moore CI, Kennedy DN, Rosen BR, Kwong KK (2000) Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. Hum Brain Mapp 9:13–25
38. Lee JD, Chon JS, Jeong HK, Kim HJ, Yun M, Kim DY, Kim DI, Park CI, Yoo HS (2003) The cerebrovascular response to traditional acupuncture after stroke. Neuroradiology 45:780–784