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Acupuncture Research

Low-Frequency Electroacupuncture Alleviates Chronic Constrictive Injury-Induced Mechanical Allodynia by Inhibiting NR2B Upregulation in Ipsilateral Spinal Dorsal Horn in Rats*

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ABSTRACT **Objective**: To study the effects of electroacupuncture (EA) in chronic constrictive injury (CCI) rat model and the expression of N-methyl-D-aspartate receptor type 2B (NR2B) in ipsilateral spinal dorsal horn in rats to explore the analgesic mechanisms of EA. **Methods**: According to the random number table, totally 180 rats were evenly divided into a sham group, a CCI group, and an EA group. CCI model was conducted with four 4–0 chromic gut ligatures loosely ligated around the left sciatic nerve 1 cm above the trifurcation. Rats in the EA group received 2 Hz EA therapy bilaterally at acupoints of Zusanli (ST 36) and Sanyinjiao (SP 6) once daily (30 min/d) for 30 days after surgery. Paw withdrawal thresholds (PWTs) were measured on 0 (baseline), 1, 3, 7, 15, 30 days after surgery. Rats were sacrificed on 0, 1, 3, 7, 15 and 30 days after surgery, and the L4–5 segments of spinal cord were removed to detect the expression of NR2B by immunohistochemistry and quantitative polymerase chain reaction. **Results**: PWTs in the CCI group were significantly lower than the sham group at Day 1–30 after surgery, and reached its lowest at Day 1 (P<0.01). After EA treatment, the PWTs recovered rapidly and were significantly higher than those in the CCI group on 3, 7, 15 and 30 days after surgery (P<0.01). The numbers of NR2B-immunoreactive cells of the CCI group significantly increased after CCI surgery compared with the sham group (P<0.01). Compared with the CCI group, stimulation of EA markedly decreased the numbers of NR2B-immunoreactive cells at Day 3, 7, 15 and 30 (P<0.05). In the sham group, NR2B mRNA was expressed at a low level. It increased after CCI surgery, which increased rapidly at Day 7 (P<0.01) and reached its peak value at Day 15 (P<0.01). After EA stimulation, relative quantity of NR2B mRNA expression was less than that in the CCI group at Day 15 and 30 (P<0.05). **Conclusions**: Low frequency of EA had antinociceptive effect in CCI rat model. The analgesic effects of EA might be through the inhibition of NR2B.

KEYWORDS sciatica, electroacupuncture, chronic constrictive injury, spinal dorsal horn, N-methyl-D-aspartate receptor type 2B, Chinese medicine

Sciatica is one of the most common chronic pain, which affects approximately 20% of the population.[1] Typically the patients suffer from spontaneous or evoked of the lower limb pain, usually accompanied by numbness and weakness. International guidelines recommend pharmacological interventions for sciatica pain, including non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, opioids, antidepressants, anticonvulsants, skeletal muscle relaxants and vitamin D, among others.[2] However, there is limited information on the safety, efficacy and tolerability for the pharmacological treatment of sciatica. Thus it is very important for us to find an effective and safe treatment.

Acupuncture is one of forms of Chinese medicine (CM). The proposed mechanism of acupuncture analgesia is its effect on the central nervous system (CNS), particular on opioid receptors, and consequent regulation of neurotransmitters and hormones.[3,4]

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Electroacupuncture (EA) is a modified acupuncture method which combined with electrical stimulation and has a good analgesic effect. Recent studies showed that EA at low or high frequencies could reduce acute pain and chronic inflammatory pain.[5,6] However, the mechanism how EA analgesia works is unclear. N-methyl-D-aspartate receptors (NMDARs) in the CNS are composed of N-methyl-D-aspartate receptor 1 (NR1) and NR2 subunits, which form a complex of 2 NR1 and 2 NR2 subunits. NR2 subunit has different subtypes including NR type 2A (NR2A), NR2B, NR2C and NR2D. It is known that presynaptic NMDARs in neuropathic rats are mainly composed of NR2B.[7] It is consistent with and supported by an increase of NR2B subunit protein expression in both the dorsal root ganglion and spinal dorsal horn in neuropathic rats. Our previous studies found that the expression of NR2B increased with paw withdrawal thresholds (PWTs) decline in the chronic constrictive injury (CCI) model, and CM such as Wenjing Tongbi Decoction (温经通痹汤) has an analgesic effect by lowering NR2B expression.[8] In this study, we investigated whether EA stimulation in CCI rat model could have an antinociceptive effect and evaluated the relation of low-frequency EA in CCI rat model and the expression of the NR2B to explore the mechanisms of EA analgesic.

METHODS

Animals and Grouping

Totally 180 male Sprague-Dawley rats [specific pathogen-free grade, certificate No. SCXK (Hu) 2013-0016; Shanghai, China] weighing 220±20 g and aged 6–7 weeks were raised 5 per cage and maintained in a light- and temperature-controlled room (a standard 12-h light/dark cycle, (24±2) ℃, and 45% humidity). All rats were allowed free access to water and food. All interventions on groups were performed between 9:00 and 12:00. All animals were treated in compliance with the regulations of the State Science and Technology Commission for the care and use of experimental animals (State Science and Technology Commission Order No. 2, 1988). According to the random number table, all rats were randomly divided into a sham group, a CCI group, and an EA group after 3-day adaptive feeding (*n*=60 per group).

Establishment of CCI Model

Fasting and water deprivation were conducted for 12 h before surgery. Rats in surgery were anesthetized with 4% (v/v) choral hydrate (30–40 mg/kg) by an intra injection peritoneal. CCI model was conducted with four 4–0 chromic gut ligatures loosely ligated around the left sciatic nerve 1 cm above the trifurcation as described previously.[9] The ligation strength of 4–0 chromic gut first knot unified one Newton. After the surgery, rats were cared for at least 30 min before recovery, and no drugs were used. The same procedure of CCI surgery was performed in the sham group without ligation of the nerves.

EA Treatment

The EA treatment procedure was the same as reported in our previous study.[10] The bilateral acupoints of Zusanli (ST 36) and Sanyinjiao (SP 6) were selected for EA treatment. Four stainless steel acupuncture needles of 0.25 mm in diameter were inserted a depth of 5 mm into bilateral ST 36 and SP 6. The acupoint ST 36 was located between the tibia and fibula, laterally to the distal end of the cranial tuberosity of the tibia, 5 mm lateral to the anterior tubercle of the tibia, in the tibialis anterior muscle, innervated by the deep peroneal nerve. The acupoint SP 6 was situated approximately 3 mm proximal the largest prominence of the medial malleolus at the posterior tibia, and in the space between the achilles tendon and the distal part of the tibia.[11] The ends of the needles were attached to a pair of electrodes from an electrical stimulator (LH-202H, Huawei Co. Ltd., China). EA (2 Hz, 2 mA, 0.4 ms pulse width) was administered for 30 min once daily from Day 1 to Day 30 after surgery. Animals kept awake and calmed by placing the heads in black hoods during EA treatment. The rats in the sham and CCI groups were handled in the same way as the rats in the EA group except the acupuncture stimulation.

Measurement of PWTs

After habituation, all rats were placed on a metal mesh table. The mechanical stimulus was delivered to the plantar surface of left hind paw by an automated testing device (dynamic plantar aesthesiometer 37450, UGO Basile, Italy). A steel rod was pushed against the hind paw with linear ascending force. The force went from 0 to 50 g over a 20-s period. When the rat withdrew its hind paw, the mechanical stimulus automatically stopped, and the force at which the rat withdrew its paw was recorded as PWTs. PWTs were measured on 0 (baseline), 1, 3, 7, 15 and 30 days after surgery and determined as the mean of 3 consecutive tests with intervals of 60 s.

Harvests

Spinal cord was quickly excised. Rats were sacrificed on 0, 1, 3, 7, 15 and 30 days after surgery by an intra injection peritoneal of 10% (v/v) choral hydrate in a volume of 3.5 mL/kg bodyweight and perfused with 150 mL cold sterilized saline followed by 500 mL cold, fresh 4% (v/v) paraformaldehyde in 0.1 mol/L phosphate-buffered saline (PBS, pH 7.4). The left L4–5 segments of spinal cord were removed. Five animals of each group were postfixed in the same fixative for 6 h at 4 ℃ before transfer to 15%, 30% sucrose for cytoprotection. Tissue was cut using a cryostat at a thickness of 30 μm. The other five animals of each group in the same time point were perfused with ice cold saline to clear contaminating blood. The same region of spinal cord used for quantitative polymerase chain reaction (qPCR) was frozen in liquid nitrogen and stored at –80 ℃.

Immunohistochemistry Assay

Antigen retrieval was performed using 10 mmol/L sodium citrate (pH 6.0), microwaved for 15 min. Following antigen retrieval, tissues were blocked in 3% H_2O_2-methanol for 15 min at room temperature, washed with double distilled water (ddH_2O) and PBS, and then incubated overnight at 4 ℃ with rabbit anti-rat NR2B (1:50, Invitrogen, USA) in a humidified chamber. Tissues were washed extensively in phosphate-buffered saline Tween-20 (PBST) and detection was performed using a horseradish peroxidase (HRP)-conjugated secondary antibody followed by colorimetric detection using a diaminobenaidine (DAB) kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene. The NR2B-immunoreactive cells are measured by using Image pro plus 5.0 (Media Cybernetics Inc., Maryland, USA). Briefly, the area of interesting was given using manual operation. Then the threshold of NR2B-immunoreactive cells was given. Finally, the number or optical density of NR2B-immunoreactive cells could be counted by the software.

Quantitative Real-Time PCR

Total RNA was isolated from L4–5 segments of spinal cord using lysis reagent (QIAzol, QIAGEN) following the manufacturer's instructions. One μg DNA-free total RNA was transcribed into cDNA using the first strand cDNA synthesis kit (SuperScript Ⅲ set, Life Technologies). Expression of NR2B genes was analyzed by real-time qPCR used the EXPRESS SYBR GreenER reagent (Life Technologies, USA) in a real-time PCR system (CFX96™, Bio-Rad Laboratories, USA). Cycling conditions used for all the qPCR are 3 min at 95 ℃ followed by 40 cycles of 15 s at 95 ℃ and 45 s at 60 ℃, a common step for annealing and extension at which step data were collected. Rpl32 was used as an internal standard for normalization.[12] All the experiments were performed in triplicate and repeated 3 times. Primers were synthesized (Table 1).

Table 1. Primers of Real-Time qPCR

Gene		Sequence	Product size (bp)
NR2B	Sense	5'-GAGACATTCTTTATCATAGTAGG-3'	103
	Anti-sense	5'-GAACGAATGAAACCAAAAC-3'	
Rpl32	Sense	5'-CACAGCTGGCCATCAGAGTCA-3'	83
	Anti-sense	5'-AAACAGGCACACCAAGCCATCTATTC-3'	

The amount of NR2B mRNA, normalized to the Rpl32 and relative to a calibrator, was given by $2^{-\triangle\triangle Cq}$, with Cq indicating the cycle number at which the fluorescence signal of the PCR product crossed an arbitrary threshold set with the exponential phase of the PCR.

Statistical Analysis

All data were expressed as mean ± standard deviation ($\bar{x} \pm s$). All data were performed using SPSS 17.0 (SPSS Inc., IL, USA). A one-way analysis of variance (ANOVA) followed by least-significant-difference post-hoc tests were applied. The criterion for statistical significance was $P<0.05$.

RESULTS

Sciatic Nerve Ligation Causes Behavioral Changes

The rats survived after CCI surgery and would like to arch their back and curl up. They were much more sensitive to the outside stimulation. When walking, they liked to lift the left foot, and showed in slight valgus shape. After EA treatment, the rats appeared similer as the CCI group.

Change of PWTs Levels with EA Stimulation

No difference in PWTs was observed among the 3 groups at baseline. PWTs in the CCI group were obviously lower than those in the sham group at corresponding time points after surgery ($P<0.01$), and reached its lowest value at Day 1. After EA treatment, the PWTs recovered rapidly and were significantly higher than the CCI group on 3, 7, 15 and 30 days

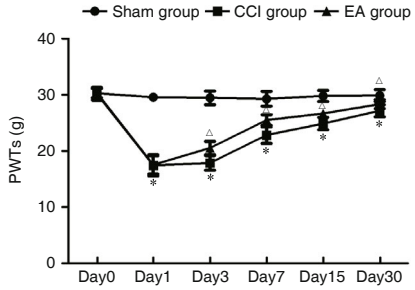

Figure 1. Paw Withdrawal Thresholds of Rats in Each Group at Different Time-Points after Surgery ($\bar{x} \pm s$, n=10)

Notes: *P<0.01 *vs*. the sham group; △P<0.01 *vs*. the CCI group. CCI: chronic constrictive injury; EA: electroacupuncture; PWTs: paw withdrawal thresholds

after surgery (P<0.01, Figure 1).

Change of NR2B Level with EA Stimulation

As shown in Figures 2 and 3, the numbers of NR2B-immunoreactive cells of the CCI group significantly increased in the L4–5 left spinal dorsal horn from 1 day after CCI surgery, compared with those in the sham group (P<0.01). While stimulation of EA markedly decreased the numbers of NR2B-immunoreactive cells, compared with the CCI group at Day 3, 7, 15 and 30 (P<0.01).

In the sham group, NR2B mRNA was expressed at a low level. Before CCI, no significant differences in the level of NR2B mRNA were found in each group (P>0.05). Compared with the sham group, relative quantity of NR2B mRNA expression in the CCI group increased at Day 1 and 3 (P<0.05), increased rapidly at Day 7 (P<0.01) and reached its peak value at Day 15 (P<0.01). After EA treatment, it decreased gradually compared with the CCI group, and showed significant difference at Day 15 and 30 (P<0.05, Figure 4).

DISCUSSION

Sciatica has been characterized by a series of

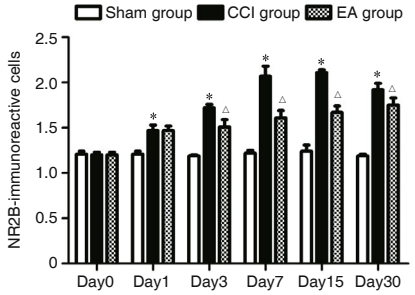

Figure 3. Comparison of NR2B-Immunoreactive Cells on Dorsal Horn in Rats of Each Group at Different Time-Points ($\bar{x} \pm s$, n=5)

Notes: *P<0.01 *vs*. the sham group; △P<0.01 *vs*. the CCI group. CCI: chronic constrictive injury; EA: electroacupuncture; NR2B: N-methyl-D-aspartate receptor type 2B

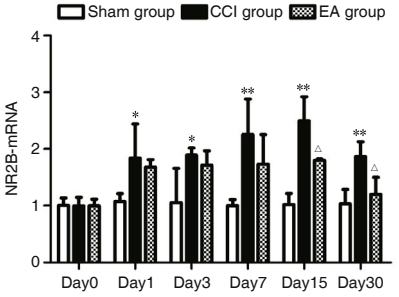

Figure 4. Comparison of NR2B mRNA Levels on Dorsal Horn in Rats at Different Time-Points ($\bar{x} \pm s$, n=5)

Notes: *P<0.05, **P<0.01 *vs*. the sham group; △P<0.05 *vs*. the CCI group. CCI: chronic constrictive injury; EA: electroacupuncture; NR2B: N-methyl-D-aspartate receptor type 2B

clinical symptoms, whose pain is typically felt from the low back to behind the thigh and radiates down below the knee.[13] The sciatic nerve is the largest nerve in the body and begins from nerve roots in the lumbar spinal cord in the low back and extends through the buttock area to send nerve endings down the lower limb. Because of its adverse clinical consequences, sciatica is one of the most important contributors to longer hospital stays and higher costs.[2] A way to promote recovery of sciatica has been desired

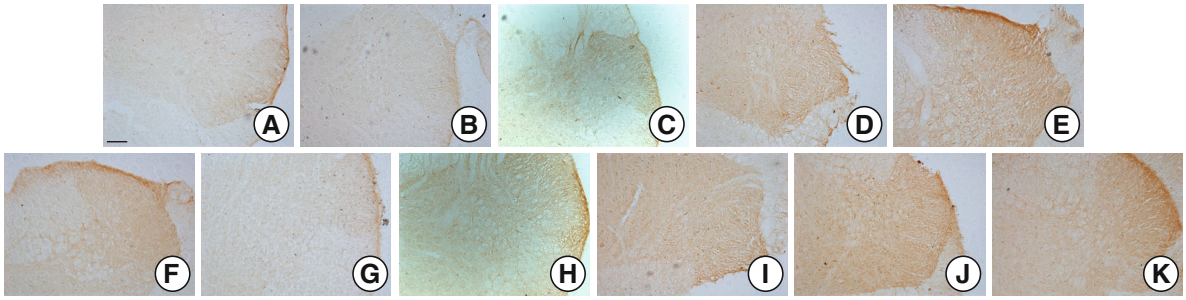

Figure 2. Representative Immunohistochemistry of NR2B Expressions in Ipsilateral L4–5 Spinal Cord Horn in Rats of Each Group (×100)

Notes: A: the sham group, B–F: the EA group, G–K: the CCI group. Scale bar:100 μm. CCI: chronic constrictive injury; EA: electroacupuncture; NR2B: N-methyl-D-aspartate receptor type 2B

for decades, but the results are disappointing.[14,15] Treatments for sciatica depend on the underlying cause and the severity of the pain. In the present study, it is demonstrated that EA stimulating on ST 36 and SP 6 decreased the expression of NR2B in the spinal dorsal horn, and improved the ipsilateral PWTs of rat after CCI operation.

It is well-known that plasticity forming of the dorsal horn in spinal cord is the mechanism of neuropathic pain.[16] Dorsal horn in spinal cord was not only the primary centre of transferring and integrating information, but also the important parts of the central sensitization mechanism.[17] There the nociceptor releases signalling molecules called neurotransmitters that activate neurons in the dorsal horn and prompt them to transmit the alarm message up to the brain. The change of the receptor expression on neurons membrane in the dorsal horn can inhibit upload and descending of pain.

NMDARs, a kind of ionic glutamate receptors, play a role in excitement when combining with glutamate, which belong to an excitatory neurotransmitter.[18] NR2B, a subtype of NR2 subunits, is mainly distributed in Ⅰ, Ⅱ layer in the dorsal horn of the spinal cord and the forebrain, which is closely related to the pain conduction. The latest evidence showed that NMDARs, especially the NR2B subunits in the dorsal horn of the spinal cord, played an important part in the central sensitivity of inducing and maintaining a state of pain.[19]

EA combines traditional acupuncture and modern electrotherapy, whose stimulation can be altered objectively and quantitatively.[20] It has distinct analgesic effects at low or high frequencies that may occur in specific neural pathways. A former study showed that 2 Hz of EA significantly attenuated hyperalgesia, whereas 120 Hz of EA did not exert beneficial effect in diabetic neuropathic pain model.[21] The effectiveness of low-frequency EA is also documented in other neuropathic pain models, such as sciatic nerve ligation model.[22,23] Jiang, et al[24] showed that EA at 2 Hz could effectively relieve spinal nerve ligation-induced neuropathic pain, accompanied by a reduction of calcitionin gene-related peptide in sensory neurons.

In CM, ST 36 of "Wei (Stomach) Meridian of Foot-Yangming" and SP 6 of "Pi (Spleen) Meridian of Foot-Taiyin" are commonly used in human acupuncture to treat a wide range of health conditions.[11] In animal research, they are frequently used to study acupuncture effects on various physiological regulatory mechanisms and control system changes. Consistent with previous findings,[25] this study showed that EA at ST 36 and SP 6 acupoints could effectively elicit analgesia in rats.

In conclusion, the present study evaluated that low frequency of EA had antinociceptive effect in CCI rat model. The analgesic effects of EA might be through the inhibition of NR2B.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Zhao WS and Jiang ZN designed and performed the experimental protocols. Wang YC and Shi H performed the animal experiments, EA treatments and measurements of PWTs. Xu LL and Yang Y detected the NR2B by immunohistochemistry assay and qPCR. Jiang ZN wrote the initial draft of the manuscript, performed statistical analysis and image acquisition. Zhao WS and Jiang ZN supervised the data analyses, manuscript design and revisions. All of the authors have read and approved the final manuscript.

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REFERENCES

1. Goldberg DS, McGee SJ. Pain as a global public health priority. BMC Public Health 2011;11:770-774.
2. Bonakdar RA. Integrative pain management. Med Clin North AM 2017;101:987-1004.
3. Chen S, Wang S, Rong P, Wang J, Qiao L, Feng X, et al. Acupuncture for visceral pain: neural substrates and potential mechanisms. Evid Based Complement Alternat Med 2014;2014:609594.
4. Harris RE, Zubieta JK, Scott DJ, Napadow V, Gracely RH, Clauw DJ. Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on μ-opioid receptors (MORs). Neuroimage 2009;47:1077-1085.
5. Liao HY, Hsieh CL, Huang CP, Lin YW. Electroacupuncture attenuates induction of inflammatory pain by regulating

opioid and adenosine pathways in mice. Sci Rep 2017;7:42531-42539.
6. Li A, Wang Y, Xin J, Lao L, Ren K, Berman BM, et al. Electroacupuncture suppresses hyperalgesia and spinal Fos expression by activating the descending inhibitory system. Brain Res 2007;1186:171-179.
7. Yan X, Jiang E, Gao M, Weng HR. Endogenous activation of presynaptic NMDA receptors enhances glutamate release from the primary afferents in the spinal dorsal horn in a rat model of neuropathic pain. J Physiol 2012;591:2001-2019.
8. Zhao WS, Shi H, Fang JF, Fang JQ. The value of Wenjing Tongbi Decoction on pain threshold of sciatica and expression of NR2B in spinal cord. J Zhejiang Chin Med Univ (Chin) 2014;38:17-21.
9. Chiang CY, Liu SA, Sheu ML, Chen FC, Chen CJ, Su HL, et al. Feasibility of human amniotic fluid derived stem cells in alleviation of neuropathic pain in chronic constrictive injury nerve model. PLoS One 2016;11:e0159482.
10. Fang JQ, Du JY, Liang Y, Fang JF. Intervention of electroacupuncture on spinal p38 MAPK/ATF-2/VR-1 pathway in treating inflammatory pain induced by CFA in rats. Mol Pain 2013;9:13.
11. Senna-Fernandes V, França DL, de Souza D, Santos KC, Sousa RS, Manoel CV, et al. Acupuncture at "Zusanli" (ST.36) and "Sanyinjiao" (SP.6) points on the gastrointestinal tract: a study of the bioavailability of (99m) tc-sodium pertechnetate in rats. Evid Based Complement Alternat Med 2011;2011:823941.
12. Frank D, Kuhn C, Brors B, Hanselmann C, Lüdde M, Katus HA, et al. Gene expression pattern in biomechanically stretched cardiomyocytes: evidence for a stretch-specific gene program. Hypertension 2008;51:309-318.
13. Vulfsons S, Bar N, Eisenberg E. Back pain with leg pain. Curr Pain Headache Rep 2017;21:32-41.
14. Bauer BA, Tilburt JC, Sood A, Li GX, Wang SH. Complementary and alternative therapies for chronic pain. Chin J Integr Med 2016;22:403-411.
15. Konstantinou K, Dunn KM, Ogollah R, Lewis M, van der Windt D, Hay EM. Prognosis of sciatica and back-related leg pain in primary care: the ATLAS cohort. Spine J 2018;18:1030-1040.
16. Ro LS, Chang KH. Neuropathic pain: mechanisms and treatments. Chang Gung Med J 2005;28:597-605.
17. Rohini Kuner. Central mechanisms of pathological pain. Nature Med 2010;16:1258-1266.
18. Carvajal FJ, Mattison HA, Cerpa W. Role of NMDA receptor-mediated glutamatergic signaling in chronic and acute neuropathologies. Neur Plast 2016;2016:2701526.
19. Liu H, Zhang Y, Qi D, Li W. Downregulation of the spinal NMDA receptor NR2B subunit during electro-acupuncture relief of chronic visceral hyperalgesia. J Physiol Sc 2017;67:197-206.
20. Ishiko N, Yamamoto T, Murayama N, Hanamori T. MElectroacupuncture: current strength-duration relationship for initiation of hypesthesia in man. Neurosci Lett 1978;8:273-276.
21. He XF, Wei JJ, Shou SY, Fang JQ, Jiang YL. Effects of electroacupuncture at 2 and 100 Hz on rat type 2 diabetic neuropathic pain and hyperalgesia-related protein expression in the dorsal root ganglion. J Zhejiang Univ Sci B 2017;18:239-248.
22. Liang Y, Du JY, Qiu YJ, Fang JF, Liu J, Fang JQ. Electroacupuncture attenuates spinal nerve ligation-induced microglial activation mediated by p38 mitogen-activated protein kinase. Chin J Integr Med 2016;22:704-713.
23. Shao XM, Shen Z, Sun J, Fang F, Fang JF, Wu YY, et al. Strong manual acupuncture stimulation of "Huantiao" (GB 30) reduces pain-induced anxiety and p-ERK in the anterior cingulate cortex in a rat model of neuropathic pain. Evid Based Complement Alternat Med 2015;2015:235491.
24. Jiang YL, Yin XH, Shen YF, He XF, Fang JQ. Low frequency electroacupuncture alleviated spinal nerve ligation induced mechanical allodynia by inhibiting TRPV1 upregulation in ipsilateral undamaged dorsal root ganglia in rats. Evid Based Complement Alternat Med 2013;2013:170910.
25. Kim JH, Chung JY, Kwon YK, Kim KJ, Yang CH, Hahm DH, et al. Acupuncture reduces alcohol withdrawal syndrome and c-Fos expression in rat brain. Am J Chin Med 2005;33:887-896.

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