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***Basic Study***

**Comparison of the analgesic effects between electro-acupuncture and moxibustion with visceral hypersensitivity rats in irritable bowel syndrome**

Zhao JM *et al*. Comparison of EA and Moxibustion

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**Abstract**

***AIM***

To observe whether there are differences in the effects of electro-acupuncture (EA) and moxibustion (Mox) in rats with visceral hypersensitivity.

***METHODS***

EA at 1 mA and 3 mA and Mox at 43 °C and 46 °C were applied to the Shangjuxu (ST37, bilateral) acupoints in model rats those with visceral hypersensitivity. Responses of wide dynamic range neurons in dorsal horns of the spinal cord were observed through the extracellular recordings. Mast cells (MC) activity in the colons of rats were assessed, and 5-hydroxytryptamine (5-HT), 5-hydroxytryptamine 3 receptor (5-HT3R) and 5-HT4R expressions in the colons were measured.

***RESULTS***

Compared with normal control group, responses of wide dynamic range neurons in the dorsal horn of the spinal cord were increased in the EA at 1 mA and 3 mA groups (1 mA: 0.84 ± 0.74 *vs* 2.73 ± 0.65, *P* < 0.001; 3 mA: 1.91 ± 1.48 *vs* 6.44 ± 1.26, *P* < 0.001) and Mox at 43°C and 46°C groups (43 °C: 1.76 ± 0.81 *vs* 4.14 ± 1.83, *P* = 0.001; 46 °C: 5.19 ± 2.03 *vs* 7.91 ± 2.27, *P* = 0.01). MC degranulation rates and the expression of 5-HT, 5-HT3R and 5-HT4R in the colon of Mox 46 °C group were decreased than model group (MC degranulation rates: 0.47 ± 0.56 *vs* 0.28 ± 0.78, *P* < 0.001; 5-HT: 1.42 ± 0.65 *vs* 7.38 ± 1.12, *P* < 0.001; 5-HT3R: 6.62 ± 0.77 *vs* 2.86 ± 0.88, *P* < 0.001; 5-HT4R: 4.62 ± 0.65 *vs* 2.22 ± 0.97, *P* < 0.001).

***CONCLUSION***

The results indicates that the analgesic effects of Mox at 46 °C are greater than those of Mox at 43 °C, EA 1 mA and EA 3 mA.

**Key words:** Electro-acupuncture; Moxibustion; Visceral hypersensitivity; Analgesic effect; Rats

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**Core tip:** Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, and the visceral hypersensitivity is considered to be one of the most important factors in its pathogenesis. Both acupuncture and moxibustion can regulate visceral hypersensitivity in IBS; however, the underlying mechanisms remain unclear. The present study is designed to observe whether there are differences in the effects of electro-acupuncture with different current intensities and moxibustion at varying temperatures on visceral hypersensitivity and to explore the potential analgesic mechanisms of these two stimulations.

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**INTRODUCTION**

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by abdominal discomfort or pain associated with abnormal bowel movements. The pathogenesis of IBS is complex and has not been fully elucidated. However, current data suggest that it is associated with visceral hypersensitivity, altered gut motility and dysfunction of the brain-gut axis[1-4]. Recently, visceral hypersensitivity was considered to be one of the specific indicators for IBS, and it can, to some extent, induce symptoms, including urgent defecation, flatulence and abdominal pain[5]. Studies have shown that acupuncture can greatly regulate visceral hypersensitivity in IBS[6-8], but the underlying mechanism has not been identified. Other research has indicated that the stimulation from acupuncture and moxibustion can converge with visceral nociceptive afferent impulses the spinal cord[9-11]. To provide experimental evidence for electro-acupuncture (EA) and moxibustion (Mox) in treating IBS with visceral hypersensitivity, this study attempted to observe the convergence of afferent impulse induced by EA with different current intensities and Mox at varying temperatures in wide dynamic range (WDR) neurons in the dorsal horns of the spinal cord, and the subsequent influence on activation of mast cells and changes in expression of 5-HT, 5-HT3R and 5-HT4R in the colon.

**MATERIALS AND METHODS**

***Animals***

Sprague-Dawley (SD) rats (male, Specific Pathogen Free, 250-300 g) were supplied by the Experimental Animal Center of the Chinese Academy of Traditional Chinese Medicine. Animal used in this study were treated according the animal rights and animal welfare, the care of laboratory animal and the animal experimental operation have conforming to *Beijing Administration Rule of Laboratory Animal*. All efforts were made to minimize the number of animals utilized and their suffering. All animal experiments in this study were performed under the guidelines approved by the Animal Ethics Committee of the China Academy of Traditional Chinese Medicine.

***Visceral hypersensitivity model establishment***

An experimental rat model of visceral hypersensitivity was established as previously described[12]. On the second day after the rats were fasted, the experiment was begun. Rats in the normal group were stimulated through manual manipulation around the anus, while rats in the other groups were stimulated by distending the colorectum (CRD). Daily CRD using an inflatable balloon (constructed from a latex glove finger, length 4 cm, inflated with air) attached to an intravenous line connected via a Y connector to a manual pump and a sphygmomanometer. The balloon was inserted into the colon while the animal was sedated, and CRD was induced while animals were fully awake. The distention was repeated twice daily at a 30-minute interval. The rats were reared until they reached adulthood (at least 6 week old), and behavioral responses to visceral pain induced by acute CRD were then examined.

***Groups and treatments***

Fifty D-IBS rats were randomly assigned to five groups as follows: (1) EA 1 mA group (*n =* 10): needles (0.22 mm diameter, 13 mm length, Hwato, Suzhou Medical Appliance Factory, Ltd, Suzhou, China) were inserted 3-5 mm into the skin at the ST37 acupoints (Shangjuxu, bilateral), and each acupuncture needle was connected to a HANS-100 pain relieving apparatus (Nanjing Jisheng Medical Science and Technology, Ltd, Nanjing, China) with a stimulation frequency of 2.0 Hz and a stimulation intensity of 1.0 mA; (2) EA 3 mA group (*n =* 10): the treatment was the same as for the EA 1 mA group with a stimulation intensity of 3 mA; (3) Mox 43°C group (*n =* 10): fine moxibustion made for animal experiments was ignited and placed 20 mm ± 5 mm above the acupoints. A surface thermometer (Testo 905-T2, Testo, Germany) was used to confirm the temperature (43 °C ± 1 °C); (4) Mox 46 °C group (*n =* 10): the treatment was the same as for the Mox 43 °C, with a temperature confirmed to be 46 °C± 1 °C; (5)Model Control (MC) group (*n =* 10): no treatment was applied but they were monitored similarly to the experimental treatment groups. Another ten normal rats were used for the Normal Control (NC) group.

The acupoints of bilateral ST37 were selected, and EA and Mox treatments were each applied for a total of 10 min, once daily, for seven consecutive days. The ST37 acupoints of rats are located in the hind legs, approximately 10 mm below the head of the fibula at the distolateral aspect of the knee. Positions of rat acupoints were determined according to Experimental Acupuncture (Li Z, Chief Editor, Chinese Medicine Press, 2007).

***Animal surgery***

After anesthetizing with an intraperitoneal injection of urethane (10%, 1.0-1.2 g/kg), SD rats were fixed on the operating table in a prone position. The spinal cord at L1-L3 was exposed by removing the overlying musculature along the dorsal midline, and then spinal clamps were fixed on the vertebral plate. Spinal dura mater was carefully removed under a microscope to prevent damage to the medulla. After the surgery, a groove was sutured with the flap and then covered with liquid paraffin at 38 °C in order to keep the medulla wet. Following the insertion of micropipettes, 2% agar gel was placed over the surface of the medulla, not only to prevent the medulla from drying but also to stabilize the recordings by keeping the medulla fixed during respiration.

***Extracellular recordings of WDR neuronal activity***

The positions of the recorded sites were as follows: 0.5-1.5 mm lateral to the posterior median fissure of the spinal cord and 500-1500 μm beneath the surface of the medulla. During the recording, glass micropipettes (cusp: 5 μm, impedance: 8-12 MΩ) were inserted into nuclear groups to target WDR neurons. Through the micropipettes, single-unit activities were channeled into an 8301-micropipette amplifier (MEZ8301, Nihon Kohden) and Power Labdata collection system (PL4097, AD Instruments, Australia) for the amplification of cell discharge and signal management. The standard recording procedures were as follows: after neuronal discharges were stabilized, background activity was first recorded for 10 s. After 30 s of EA and Mox stimulation, the background activity was recorded again for 20 s.

Each acupuncture needle (0.25×25 mm) was connected to a HANS-100 pain relieving apparatus with a stimulation frequency of 2.0 Hz and a stimulation intensity of 1 mA and 3 mA. Mox stimulation at the temperatures of 43 °C± 1 °C and 46 °C± 1 °C were randomly applied. Both of EA and Mox were applied to Shangjuxu (ST37, bilateral) acupoints on the same side of the receptor field.

***Abdominal withdrawal reflex scores before and after treatment***

The abdominal withdrawal reflex (AWR) scoring criteria are shown in Table 1, using a procedure described procedure[12]. Rats in the treatment group were fasted starting the afternoon of the previous day. Vaseline was smeared on the surface of the balloon which was then slowly inserted into 5 cm into the rat anus, according to the physical curve of the colorectum, and retained for 5 min. The test was initiated when the rats became adapted. After air was added into the balloon with a syringe, the rat rectum was stimulated and different degrees of contraction reactions were observed. The pressure (mmHg) during behavior responses, scored as 1, 2, 3, and 4, was recorded and expressed as the threshold of sensitivity (Table 1). Each score was tested three times, and each rat was tested by two persons who had not participated in the research design. Means were calculated (6 values in total). Three-minute intervals were given between each of the two tests to allow for full adaptation of rats.

***Toluidine blue staining assay***

Toluidine blue staining was used to stain MCs. First, de-paraffinization and hydration of 4-μm paraffin-embedded sections was achieved by soaking samples in xylenes I and II for 20 min each and then anhydrous graded ethanol was applied (90%, 80%, and 70% for 5 min each). Then, toluidine was added to the tissue sections and stained for 20 min. Samples were washed in distilled water for 10 s and 0.5% acetic acid was added to differentiate color. Slides were observed under a light microscope until the cytoplasm turned purple-red. Samples were dehydrated with 95% ethanol for 1 min and then with anhydrous ethanol for 1 min two times. Tissues were cleared twice with xylene for 20 min each. The appropriate amount of neutral resin was added and sections were sealed. The sample slides (× 400) and counted MCs were observed. Cytoplasmic granules were stained purple-red and nuclei were stained blue. Clear, smooth, and intact cytoplasmic membranes with clear indicated stable MCs. Broken cytoplasmic membranes with purple-red granules around cells indicated degranulated MCs. MCs were counted in three non-overlapping random views and means were obtained for total and degranulated MCs. Degranulation rates were calculated as follows: degranulation rate (%) = degranulated MC count/total MC count × 100%.

***Immunohistochemical assay***

The expressions of 5-HT, 5-HT3R and 5-HT4R in the colonic tissue of D-IBS rats was detected by immunohistochemical (IHC) staining. The sections were exposed to 0.01 mol/L citrate buffer (pH 6.0), microwaved at 30% power for 20 min for heat fixation, and then cooled to room temperature. The sections were washed 3 times with phosphate buffered saline (PBS) for 3 min and exposed to 0.3% H2O2 for 20 min at room temperature to inhibit endogenous peroxidases. Following a final PBS wash (3 min× 3 min), the samples were exposed to 20% normal goat serum and incubated for 30 min. Antibodies were added drop-wise (5-HT 1:100, 5-HT3R 1:50, 5-HT4R 1:80, Santa Cruz, CA, United States), and the sections were incubated at 37 °C for 2 h. The sections were washed with PBS 3 times for 3 min, incubated in horseradish peroxidase/rabbit reagent at 37 °C for 30 min, and PBS washed 3 times for 3 min. The sections were then incubated in 3,3-diaminobenzidine (DAB) chromogenic reagent for 8-12 min and dyed with hematoxylin lining and blue in the presence of hot water. After drying, the sections were sealed with neutral gum for further observation under a light microscope. A semi-quantitative analysis of the staining was performed using the medical image quantitative analysis system (MIQAS, Shanghai Qiuwei Biomedical Technology Co.). Positive results were indicated by the presence of brown or tan particles in the stained colonic tissue cells. In each slice, three positive areas were counted and assessed for optical density (OD) in a high power field to calculate an IHC positive index (positive area × OD/total area) for 5-HT, 5-HT3R and 5-HT4R.

***Statistical analysis***

All statistical analyses were performed using SPSS 19.0 (SPSS Inc. Chicago, IL). AWR scores for rats are presented as interquartile ranges. Differences in means were compared by one-way ANOVA testing. Non-normal data were compared using a non-parametric test. All two-sided *P* values < 0.05 were considered statistically significant.

**RESULTS**

***Effects of EA with different current intensities on the activation of WDR neurons in the dorsal horn of the spinal cord***

Ten WDR neurons were recorded in the dorsal horn of the spinal cord for 11 control rats and 12 model rats, and the effects of EA with different current intensities on the WDR neurons were observed. It was found that the background activities of these neurons were activated by EA. Within the range of 1 mA and 3 mA, the degree of neuronal activation was increased concurrently with the increase in current intensity. When EA with 1 mA was applied, the degree of activation in the WDR neurons of normal rats was 0.84 ± 0.74 spikes/s, while that of model rats increased to 2.73 ± 0.65 spikes/s. There were statistically significant differences between them (*P* < 0.001). When the intensity increased to 3 mA, the degree of activation in the WDR neurons of normal rats was 1.91 ± 1.48 spikes/s, while that of model rats increased to 6.44 ± 1.26 spikes/s. There were also statistically significant differences between the two groups (*P* < 0.001). It is evident that EA with different intensities on ST37 acupoints has increased activation effects on WDR neurons in the dorsal horn of the spinal cord in model rats with visceral hypersensitivity, compared to normal rats (Figure 1).

***Effects of Mox at different temperatures on the activation of WDR neurons in the dorsal horn of the spinal cord***

Ten WDR neurons in the dorsal horn of the spinal cords of 11 normal rats and 13 model rats were recorded, respectively, and the effects of Mox at different temperatures on the WDR neurons were observed. The background activities of these neurons wereactivated by Mox. Within the range of 43 °C and 46 °C, the degree of neuronal activation was increased concurrently with the increase in temperature. When Mox at 43 ℃ was applied, the degree of activation in the WDR neurons of normal rats was 1.76 ± 0.81 spikes/s, while that of model rats increased to 4.14 ± 1.83 spikes/s. There were statistically difference between them (*P* = 0.001). When the temperature increased to 46 °C, the degree of activation in the WDR neurons of normal rats was 5.19 ± 2.03 spikes/s, while that of model rats increased to 7.91 ± 2.27 spikes/s. There were also statistically differences between the two groups (*P* = 0.01). It is evident that Mox applied at different temperatures to ST37 acupoints has increased activation effects on WDR neurons in the dorsal horn of the spinal cord in model rats with visceral hypersensitivity, as compared with normal rats (Figure 2).

***AWR scores comparisons***

AWR scores of the Mox 43 °C, Mox 46 °C , EA 1 mA groups and the EA 3 mA group were all significantly different from the model group at CRD pressure of 20, 40, 60, and 80 mmHg (*P* < 0.01). AWR scores of the NC group were not significantly different from the Mox 43 °C, Mox 46 °C and EA 1mA groups at CRD pressure of 20, 40, 60, and 80 mmHg (20 mmHg: *P*Mox43°C= 0.265, *P*Mox46°C = 0.265, *P*Acu1mA= 0.097; 40 mmHg: *P*Mox43°C= 0.693, *P*Mox46°C= 0.401, *P*Acu1mA= 0.416; 60 mmHg: *P*Mox43°C= 0.119, *P*Mox46°C= 0.262, *P*Acu1mA= 0.069; 80 mmHg: *P*Mox43°C= 0.063, *P*Mox46°C= 1.000, *P*Acu1mA= 0.170）. AWR scores of the EA 3 mA group at 20mmHg, 40 mmHg and 80 mmHg were not significantly different from the NC group (20 mmHg: *P* = 0.265, 40mmHg: *P* = 0.107, 80 mmHg: *P* = 0.063), whereas AWR scores of the EA 3mA group at 60 mmHg were different from the NC group (60 mmHg: *P* = 0.016). These data indicated that Mox at 46°C and EA at 1 mA treatments were able to decrease visceral hypersensitivity or increase the pain threshold significantly, and they were superior to the EA 3 mA group. (Figure 3)

***Change of MC activity in colon***

Compared with the NC group, the MC counts in the colon were significantly increased in the Mox at 43 °C and 46 °C groups, and EA at 1 mA and 3 mA groups (*P*Mox43°C<0.001, *P*Mox46°C< 0.001, *P*Acu1mA= 0.007, *P*Acu3mA< 0.001). The MC degranulation rates in the colon were also significantly increased in the Mox at 43 °C and Mox at 46 °C and EA at 1 mA and 3 mA groups compared with the NC group (*P* < 0.001). Compared with the MC group, the MC degranulation rates in the colon were significantly decreased in the Mox 46 °C group (*P* < 0.001), and there were differences in MC degranulation rates between the Mox 46 °C group and the Mox 43 °C group (*P* = 0.005) (Figures 4, 5).

***5-HT expression in colon***

Compared with the MC group, 5-HT expression in the colon was significantly decreased in NC group, the Mox at 43 °C and 46 °C groups and the EA at 1 mA, 3 mA groups (*P* < 0.001), and the expression in the Mox at 43°C and 46°C groups was significantly lower than the EA at 1 mA, 3 mA groups (*P*Mox43°C *vs* Acu1mA= 0.007, *P*Mox43°C *vs* Acu3mA= 0.014, *P*Mox46°C *vs* Acu1mA< 0.001, *P*Mox46°C *vs* Acu3mA< 0.001). Compared with the NC group, 5-HT expression in the colon was not significantly different in the Mox46°C group (*P* = 0.332) (Figures 6, 7).

***5-HT3R expression in colon***

Compared with the MC group, 5-HT3R expressions in the colon was significantly decreasedin the NC group, the Mox at 43 °C and 46 °C groups and the EA at 3 mA group (*P*NC< 0.001, *P*Mox43°C< 0.001, *P*Mox46°C< 0.001, *P*Acu3mA= 0.008), and the expression in the Mox at 43°C and 46°C groups was significantly reduced compared to the EA at 1 mA and 3 mA groups (*P*Mox43°C *vs* Acu1mA< 0.001, *PMox43°C vs* Acu3mA= 0.002, *P*Mox46°C *vs* Acu1mA< 0.001, *P*Mox46°C *vs* Acu3mA< 0.001)(Figures 8, 9).

***5-HT4R expression in colon***

Compared with the MC group, 5-HT4R expressions in the colon was significantly decreased in the NC group, the Mox at 43 °C and 46 °C groups and the EA at 1 mA and 3 mA groups (*P*Mox43°C < 0.001, *P*Mox46°C< 0.001, *P*Acu1mA= 0.003, *P*Acu3mA< 0.001). Compared with the NC group, 5-HT4R expressions in the colon was not significantly different in the Mox at 43 °C and 46 °C groups and the EA at 3 mA group (*P*Mox43°C= 0.184, *P*Mox46°C= 0.448, *P*Acu3mA= 0.178), whereas there was a statistically significant in EA 1 mA group (*P*Acu1mA= 0.025)(Figure 10, 11).

**DISCUSSION**

Studies have proven that acupuncture and moxibustion have analgesic effects[13,14]. Receptors in skin or muscles are activated by the mechanical stimulation of acupuncture and the heat stimulation of moxibustion, which affects nervous system and the biological activity of tissues and cells to relieve pains[15,16]. With the help of the supraspinal cord, afferent impulses induced by acupuncture and moxibustion can transmit along peripheral A or C fibers to the dorsal horn of the spinal cord, where they will converge and interact with the visceral nociceptive afferent impulse. Therefore, acupuncture and moxibustion can inhibit the response of neurons in the dorsal horn of the spinal cord activated by visceral nociceptive afferent impulses[11,17]. Afferent impulses of organs or deep tissues can sensitize related segmental neurons, which makes the impulse responses from the body surface more intense. Meanwhile, the size and function of acupoints can change with the visceral functions[18], and in pathological conditions, the function of an acupoint area in reflecting and treating disease can be greatly enhanced to reach the status of acupoints area sensitization[19]. Visceralgia and sensitization interact with each other. Therefore, the dorsal horn of the spinal cord is the key for regulation of visceral hypersensitivity. The function of acupoints is not only related to the sensitization in the condition of visceral hyperalgesia, but is also associated with the convergence of body surface afferent and visceral nociceptive afferents in the spinal cord or supraspinal cord[20]. Acupuncture and moxibustion transmit signals through the activation of receptors in acupoint areas or through bioactive substances, and the stimulation of acupuncture in the body surface can converge with visceral nociceptive stimuli in the spinal cord to regulate the body functions.

Our study found that while stimulating ST37 in model rats with visceral hypersensitivity, with the increase of EA intensities from 1 mA to 3 mA and Mox temperature from 43 °C to 46 °C, the activation reaction of WDR neurons in the dorsal horn of the spinal cord was increased. Meanwhile, the intestinal pain threshold of model rats with visceral hypersensitivity was markedly inhibited. The abnormal activation of mast cells that induce gut motility and secretion in the colon and abnormal expression of 5-HT, 5-HT3R and 5-HT4R were also significantly inhibited. This indicates that the analgesic effects of EA and/or Mox on the target tissues of model rats with visceral hypersensitivity not only have a positive correlation with their intensities, but are also are associated with the fact that WDR neurons can converge and integrate the stimulation induced by EA and Mox. It has been reported that needling the acupoints near the pain spot or the adjacent vertebrae would achieve better analgesic effects[21]. Furthermore, the analgesic effects were achieved when Aδ fibers were activated, and they became become stronger when type-C fibers were activated[22]. In this study, ST37 was selected as the location for EA and Mox application, and ST37 is located in the receptive field of WDR neurons that involved convergence and integration of visceral nociceptive afferent impulses[23]. We applied EA with 1 mA and 3 mA intensities, and Mox at 43 °C and 46 °C, to ST37 of normal rats and model rats respectively. It was found that EA with 3 mA and Mox at 46 °C can achieve more significant activation of WDR neurons in the dorsal horn of the spinal cord. Stimulation with low intensities, such as 1 mA EA and 43 °C Mox, can only activate type A neural fibers, whereas stimulation with high intensities, such as 3 mA EA and 46 °C Mox, can also activate type C fibers, thereby inducing greater analgesic effects.

Abnormal activation of mass cells in the colon is one of the main pathological characteristics of visceral hypersensitivity (including gut motility, abnormal secretion and visceral pain)[24,25]. In this study, the MC counts and degranulation rates in the colons of all model rats (model group, 1 mA EA group, 3 mA EA group, 43 °C Mox group, 46 °C Mox group) were greatly increased compared with those of normal rats, which indicates that with visceral hypersensitivity, the counts of MCs in the colon are not only significantly increased, but they also presenting abnormal activation (degranulation). Furthermore, degranulated MCs may release large quantities of active substances, such as histamine and 5-HT. According to several studies, as an important agent of regulating the brain-gut axis and the intestinal neural system, 5-HT can regulate visceral perception and visceral neural reflex[26]. The activation of 5-HT3R can induce cellular the pathways for non-specific positive ion movement, allowing the entry of Na+ and Ca2+ and the exiting of K+. These pathways of positive ion movement can depolarize postsynaptic neurons, transmit signals around and induce the release of excitatory or inhibitory neurotransmitters. In this way, smooth muscles may were contracted and relaxed abnormally[27]. In addition, 5-HT can influence gastrointestinal mobility through 5-HT4R, as activated 5-HT4R can play an active role in regulating gut mobility and visceral hyperalgesia through the inductionof a release of acetylcholine or neurotransmitters for P motor nerves[28,29].

In our study, we observed that after applying EA with different intensities and Mox at varying temperatures, degranulation rate of MC in the colons of rats with visceral hypersensitivity were reduced to declined in varying degrees, which demonstrates that EA and Mox, especially the Mox at 46 °C, have an inhibitory function towards the abnormally activated MCs in areas with visceral hypersensitivity. At the same time, it was also found that the expression levels of 5-HT, 5-HT3R and 5-HT4R in model rats were much higher than those in normal group, as were the AWR scores. After stimulation of EA with 1 mA and 3 mA and Mox at 43 °C and 46 °C was performed, compared to the model group, the expression of 5-HT, 5-HT3R and 5-HT4R was decreased to varying degrees. Mox was more effective than EA in reducing the expression. Meanwhile, AWR scores for all treatment groups (especially the Mox 46 °C) were decreased markedly compared with the model group. These results indicate that in colons of rats with visceral hypersensitivity, MCs are activated abnormally and 5-HT, 5-HT3R and 5-HT4R have increased expression. Stimulating ST37 with EA with different intensities and Mox at varying temperatures can activate the WDR neurons in the dorsal horn of the spinal cord and thus relieve pains in the target organs (colons). Among four different stimulations, Mox at 46 °C has the best analgesic effects. Additionally, we also found that the analgesic effects were achieved by EA and Mox through WDR neurons of the spinal cord, which may affect the abnormal MC activation and high expression of 5-HT, 5-HT3R and 5-HT4R by converging and integrating the visceral nociceptive afferent impulse with stimulation induced by EA and Mox at the ST37 acupoints. These results may reveal one of the mechanisms of analgesic effects of acupuncture in treating IBS with visceral hypersensitivity.

There are differences between stimulation by EA with different current intensities and Mox at different temperatures in the relief of visceral hypersensitivity. The analgesic effects of Mox at 46 °C are much greater than those of Mox at 43 °C, EA at 1 mA and EA at 3 mA. A potential mechanism is that WDR neurons from the dorsal horn of the spinal cord may converge and integrate the stimulation induced by EA and Mox in the segmental receptor field with visceral nociceptive afferent impulses and then influence the MC activation reaction of the colon and the high expression of 5-HT, 5-HT3R and 5-HT4R.

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**COMMENTS**

***Background***

The pathogenesis of irritable bowel syndrome is complex and has not been fully elucidated, recently visceral hypersensitivity was considered to be one of the specific indicators for irritable bowel syndrome (IBS), and it can, to some extent, induce the symptoms, including urgent defecation, flatulence and abdominal pain. Acupuncture and Moxibustion can both regulate visceral hypersensitivity in IBS, however, the underlying mechanisms remain unclear.

***Research frontiers***

Previous studies have shown that acupuncture can greatly regulate visceral hypersensitivity in IBS, this study attempted to provide experimental evidence for electro-acupuncture (EA) and moxibustion (Mox) in treating IBS with visceral hypersensitivity.

***Innovations and breakthroughs***

This study attempted to observe the convergence of afferent impulse induced by EA with different current intensities and Mox at varying temperatures in wide dynamic range neurons in the dorsal horn of the spinal cord, and its influence on activation of mast cells and expression changes of 5-HT, 5-HT3R and 5-HT4R in colon.

***Applications***

To provide experimental evidence for EA and Mox in treating IBS with visceral hypersensitivity, and the results may reveal one of the mechanisms of analgesic effects of acupuncture in treating IBS with visceral hypersensitivity.

***Peer-review***

This manuscript is a well-conducted study with moderate relevance and novelty.

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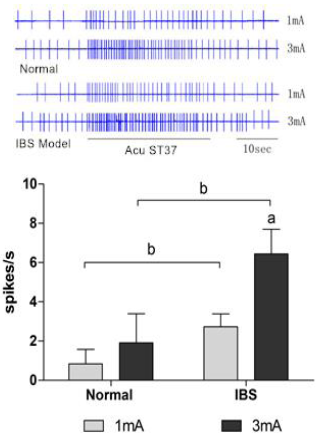
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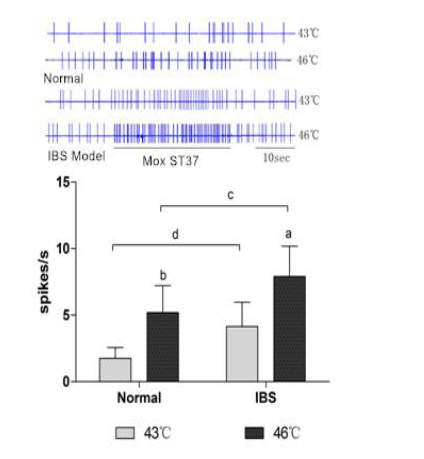
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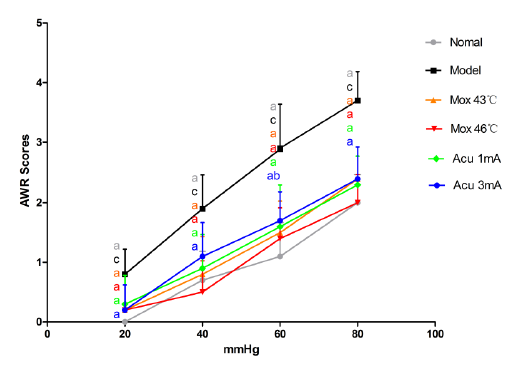


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**Figure 1 Effects of electro-acupuncture with different current intensities on the activation of wide dynamic range neurons in the dorsal horn of the spinal cord.** b*P* < 0.01, *vs* the NC group; d*P* < 0.01, *vs* EA 1 mA group. IBS: Irritable bowel syndrome.



**Figure 2 Effects of moxibustion with different temperatures on the activation of wide dynamic range neurons in the dorsal horn of the spinal cord.** a*P* < 0.05, b*P* < 0.01, *vs* Mox 43 ℃ group; c*P* < 0.05, d*P* < 0.01, *vs* the NC group. Mox: moxibustion.



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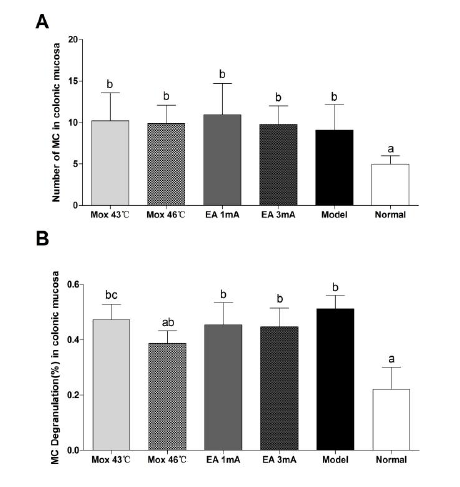
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**Figure 3 abdominal withdrawal reflex scores comparison.** b*P* < 0.01, *vs* the mast cells group; c*P* < 0.05, d*P* < 0.01, *vs* the NC group.

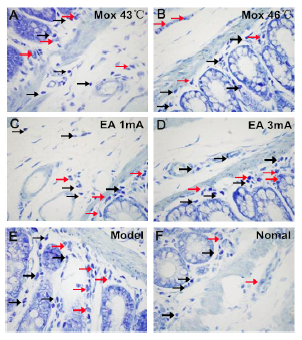


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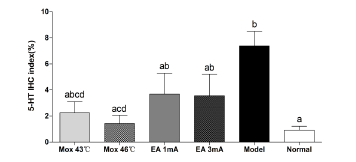
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**Figure 4 Change of mast cells activity in the colon**. A: Number of MC in colonic mucosa, a*P* < 0.05, b*P* < 0.01, *vs* the MC group; B: MC degranulation(%) in colonic mucosa. b*P* < 0.01, *vs* the NC group; d*P* < 0.01, *vs* the MC group; e*P* < 0.05, *vs* the Mox 46 ℃ group. MC: Mast cells.



**Figure 5** **Change of mast cells activity in the colon (black arrows indicate mast cells, red arrows indicate mast cell degranulation).** A: Mox 43 ℃； B: Mox 46 ℃; C: EA 1mA; D: EA 3mA; E: Model; F: Nomal.



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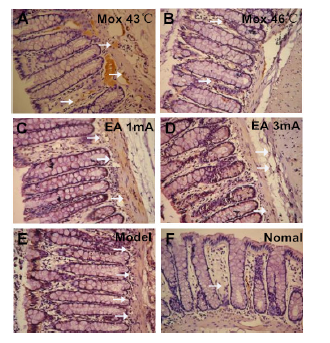
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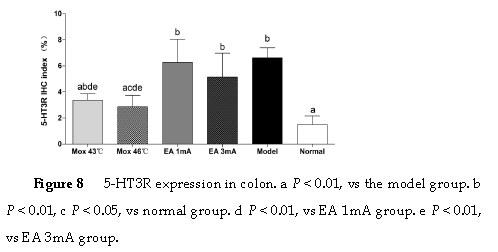
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**Figure 6 5-HT expression in the colon.** b*P* < 0.01, *vs* the MC group; c*P* < 0.05, d*P* < 0.01, *vs* the NC group; e*P* < 0.01, *vs* the Mox 46 ℃ group. MC: Mast cells.



**Figure 7 5-hydroxytryptamine expression in the colon (white arrows indicate positive expression of 5-hydroxytryptamine).** A: Mox 43 ℃； B: Mox 46 ℃; C: EA 1mA; D: EA 3mA; E: Model; F: Nomal.



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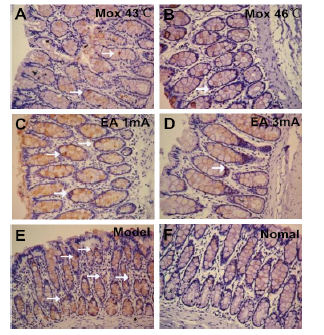
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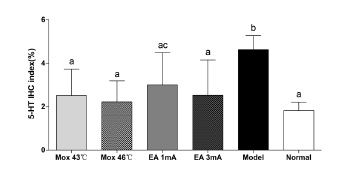
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**Figure 8 5-hydroxytryptamine 3 receptor expression in the colon.** b*P* < 0.01, *vs* the MC group; c*P* < 0.05, d*P* < 0.01, *vs* the NC group; e*P* < 0.05, f*P* < 0.01, *vs* the Mox 46 ℃ group.



**Figure 9** **5-hydroxytryptamine 3 receptor expression in the colon (white arrows indicate positive expression of 5-hydroxytryptamine 3 receptor).** A: Mox 43 ℃； B: Mox 46 ℃; C: EA 1mA; D: EA 3mA; E: Model; F: Nomal.



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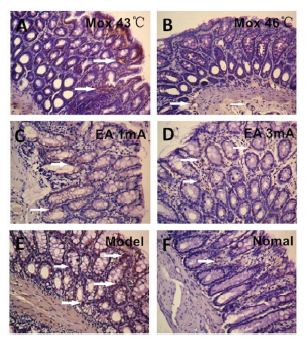
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**Figure 10 5-hydroxytryptamine 4 receptor expression in colon.** b*P* < 0.01, *vs* the MC group; c*P* < 0.05, d*P* < 0.01, *vs* NC group. MC: Mast cells.



**Figure 11 5-hydroxytryptamine 4 receptor expression in colon (white arrows indicate positive expression of 5-hydroxytryptamine 4 receptor).** A: Mox 43 ℃； B: Mox 46 ℃; C: EA 1mA; D: EA 3mA; E: Model; F: Nomal.

**Table 1 withdrawal reflex scoring criteria**

|  |
| --- |
| Score 0 No behavioral response to colorectum |
| Score 1 Immobile during distension of CR and occasional clicking the head at onset of the stimulus |
| Score 2 A mild contraction of abdominal muscles, but no lifting of abdomen off the plattorm |
| Score 3 A strong contraction of abdominal muscles and lifting of abdomen off the platform, no lifting of pelvic structure off the platform |
| Score 4 Arching body and lifting of pelvic structure and scrotum |