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# Analgesic action of suspended moxibustion in rats with chronic visceral hyperalgesia correlates with enkephalins in the spinal cord<sup>☆</sup>

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## Abstract

Rats that modeled chronic visceral hyperalgesia received suspended moxibustion at bilateral *Tianshu* (ST25) and *Shangjuxu* (ST37) once daily over a period of 7 days. Results show that suspended moxibustion significantly depressed abdominal withdrawal reflex scores and increased enkephalin concentration in the spinal cord. The experimental findings suggest that spinal enkephalins contributed to the analgesic effect of suspended moxibustion in rats with chronic visceral hyperalgesia.

**Key Words:** suspended moxibustion; chronic visceral hyperalgesia; enkephalins; irritable bowel syndrome

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## INTRODUCTION

Moxibustion is an alternative or complementary therapy, which relies on the heating power of moxa to warmly stimulate the organism. It is a therapeutic method for the prevention and treatment of diseases through the actions of meridians and acupoints<sup>[1]</sup>. A number of studies have proved its analgesic effect for the treatment of pain, such as primary dysmenorrhea<sup>[2]</sup>, knee osteoarthritis<sup>[3-4]</sup>, rheumatoid arthritis<sup>[5]</sup>, and cancer pain<sup>[6-7]</sup>. Our previous study indicated that herb-partition moxibustion was a potentially valuable therapeutic remedy for the treatment of patients with irritable bowel syndrome, especially in alleviating visceral pain<sup>[8]</sup>. However, the mechanism of moxibustion analgesia that underlies acupuncture analgesia is not well reported. Moreover, our previous experiment has shown that suspended moxibustion could increase the pain threshold by reducing 5-hydroxytryptamine concentrations in the colon of rats with chronic visceral hyperalgesia, and increase the concentration of dynorphin and endomorphin in the spinal cord<sup>[9-11]</sup>. This is evidence that the analgesic effect of moxibustion may be associated with the nervous system. Thus, we hypothesize that suspended moxibustion achieves its analgesic effect in treating chronic visceral

pain by modulating the concentrations of enkephalins in the spinal cord. In the present study, we focused on the effect of moxibustion in rats with chronic visceral hyperalgesia on enkephalins in the spinal cord.

## RESULTS

### Quantitative analysis of experimental animals

A total of 40 experimental rats were randomly divided into a normal group ( $n = 10$ ) and an experimental group ( $n = 30$ ). A rat model of chronic visceral hyperalgesia was established by mechanical colorectal distension stimulation. The 30 model rats in the experimental group were randomly assigned to three subgroups: model, suspended moxibustion and sham moxibustion. The latter two subgroups were treated with warm or non-warm suspended moxibustion at bilateral *Tianshu* (ST25) and *Shangjuxu* (ST37). All rats were included in the final analysis, with no loss.

### Analgesic effect of suspended moxibustion in rats with chronic visceral hyperalgesia

The abdominal withdrawal reflex scores were analyzed to evaluate the pain threshold for chronic visceral hyperalgesia in the irritable bowel syndrome rats within 90 minutes after treatments were implemented (the scoring criteria is shown in

supplementary Table 1 online). Results show that abdominal withdrawal reflex scores in rats with chronic visceral hyperalgesia were significantly decreased after moxibustion treatments ( $P < 0.01$ ; Table 1).

Table 1 Abdominal withdrawal reflex scores of rats in response to graded colorectal distension stimulation at 2.666, 5.332, 7.998, and 10.664 kPa

Group	Colorectal distension pressure (kPa)	
	2.666	5.332
Normal	0.15±0.08	1.12±0.25
Model	1.94±0.18 <sup>a</sup>	3.25±0.36 <sup>a</sup>
Suspended moxibustion	0.19±0.07 <sup>b</sup>	1.55±0.29 <sup>b</sup>
Sham moxibustion	1.83±1.02	3.37±0.82

Group	Colorectal distension pressure (kPa)	
	7.998	10.664
Normal	2.43±0.16	3.15±0.20
Model	3.66±0.52 <sup>a</sup>	3.87±0.10 <sup>a</sup>
Suspended moxibustion	2.38±0.21 <sup>b</sup>	3.03±0.24 <sup>b</sup>
Sham moxibustion	3.52±0.43	3.67±0.95

Data are expressed as mean ± SD of ten rats in each group. <sup>a</sup> $P < 0.01$ , vs. normal group; <sup>b</sup> $P < 0.01$ , vs. model group (least significant difference-*t* tests).

### Suspended moxibustion increased the enkephalin concentrations in the spinal cord of rats with chronic visceral hyperalgesia

After the abdominal withdrawal reflex scores were evaluated, the enkephalin concentrations in the spinal cord, as detected by enzyme linked immunosorbent assay (ELISA), were significantly increased after colorectal distension stimulation ( $P < 0.01$ ), and were further increased after suspended moxibustion treatment ( $P < 0.01$ ). No significant difference was detected in the enkephalins concentrations between the model group and the sham moxibustion group (Figure 1).

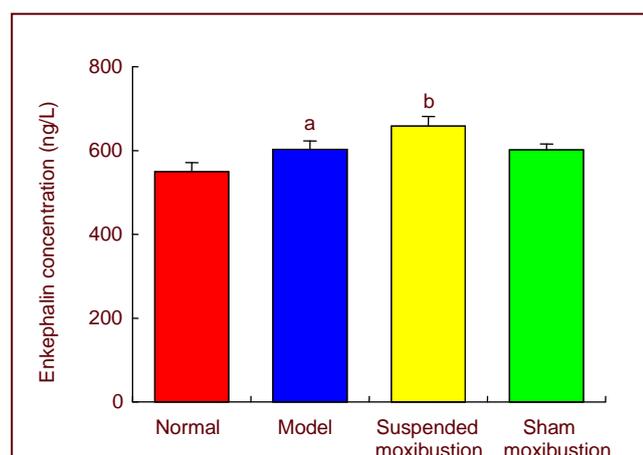


Figure 1 Effects of suspended moxibustion on enkephalin concentrations in the spinal cord of rats. Data are expressed as mean ± SD of ten rats in each group. <sup>a</sup> $P < 0.01$ , vs. normal group; <sup>b</sup> $P < 0.01$ , vs. model group (least significant difference-*t* tests).

## DISCUSSION

The irritable bowel syndrome model was established successfully by applying continuous colonic stimulation to Sprague-Dawley rats from their neonatal period to adulthood, and chronic visceral hyperalgesia was consistent with the pathological appearance of irritable bowel syndrome<sup>[12-13]</sup>. ST25 and ST37 acupoints are commonly applied to manage gastrointestinal diseases<sup>[14-15]</sup>. ST25 and ST37 are two key acupoints that were chosen in this study based on our clinical treatments of patients with irritable bowel syndrome since the 1980s<sup>[8]</sup>. Moreover, we also showed that activation of ST25 and ST37 acupoints could reduce chronic visceral hyperalgesia in irritable bowel syndrome model rats<sup>[16]</sup>. Compared with the model group, abdominal withdrawal reflex scores were significantly decreased after suspended moxibustion treatment, indicating that suspended moxibustion treatment could effectively suppress pain in irritable bowel syndrome rats, which is similar to previous studies<sup>[9, 11]</sup>.

Enkephalins are neurotransmitters which work to regulate nociception in the body<sup>[17-18]</sup>. In the present study, care was taken to investigate the concentrations of enkephalins in the spinal cord of irritable bowel syndrome rats, induced by colorectal distension stimulation, including treatment with the moxibustion. Compared with normal controls, enkephalin concentrations in the spinal cord were higher in the model group. This is evidence that pain signals led to the release of endogenous enkephalins in the spinal cord of irritable bowel syndrome rats. Further increased enkephalins in the moxibustion-treated rats are shown, and no significant difference was found between the model group and the sham moxibustion group. The present study provides data suggesting that enkephalins participate in moxibustion-treated regulation of nociception. Other studies have demonstrated the efficacy of electro-acupuncture in suppressing pain by increasing the enkephalin concentration in the spinal cord<sup>[19-21]</sup>. We speculated that, similar to acupuncture analgesia, moxibustion analgesia is a comprehensive process, and the spinal cord is a primary integration center. The moxibustion signal and pain signal converge and interact in the spinal cord, which results in the local release of enkephalins. These afferent pathways propagate to the midbrain, triggering a sequence of excitatory and inhibitory mediators in the spinal cord, leading to pre- and postsynaptic inhibition and suppression of pain transmission.

In summary, the present study provides the first demonstration that moxibustion could release enkephalins in the spinal cord in irritable bowel syndrome rats. Through the action of enkephalins in the spinal cord, moxibustion could relieve chronic visceral hyperalgesia in irritable bowel syndrome rats, which is similar to the mechanism of acupuncture analgesia.

## MATERIALS AND METHODS

### Design

A randomized, controlled, animal study.

### Time and setting

This experiment was performed at the Animal Experimental Center, Shanghai University of Traditional Chinese Medicine, China, from July to September 2009.

### Materials

A total of 40 neonatal male Sprague-Dawley rats at the age of 5 days were obtained from the Experimental Animal Center, Shanghai University of Traditional Chinese Medicine, China (certificate No. TJLA-2006-270). The animals were maintained in a plastic cage containing corn chip bedding with controlled temperature ( $22 \pm 2^\circ\text{C}$ ), in  $60 \pm 5\%$  humidity, and a 12-hour light-dark cycle, with a maximum of five rats per cage. All rats were used strictly in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Rats were given adaptive feeding for 3 days before the experiment.

### Methods

#### **Establishment of chronic visceral hyperalgesia models**

Neonatal rats in the experimental groups were subjected to daily mechanical colorectal distension from 8 days to 21 days after birth. A lactoprene balloon (length 20.0 mm, diameter 3.0 mm) with 0.5 mL of air was inserted slowly into the descending colon for 1 minute and then deflated and withdrawn, twice a day, with a 30-minute interval. Significant differences in abdominal withdrawal reflex score between the normal and the experimental groups indicated success in establishing the model<sup>[22]</sup>.

#### **Suspended moxibustion treatment**

Following successful model establishment in rats at the age of 37 days, suspended moxibustion was given to bilateral ST25 (about 10 mm lateral to the navel) and ST37 (about 10 mm below to the *Housanli* point (ST36)), igniting a refined moxa stick (0.5 cm in diameter made of refined mugwort floss; Nanyang Hanyi Co., Ltd., Nanyang, China; 2 cm high from the acupoints) for 10 minutes, once a day for 7 consecutive days. Rats were not anesthetized before treatment with suspended moxibustion, and they were held in a supine position on one gloved hand. The rats in the sham moxibustion group received treatment with a non-igniting moxa stick. The rats in the normal and model groups were the same as the treated-rats regarding the hand grip position.

#### **Abdominal withdrawal reflex scores**

The abdominal withdrawal reflex scores were evaluated according to the scale described by Al-Chaer *et al.*<sup>[22]</sup>. When the balloon was inserted into the descending colon, colorectal distension stimulation was produced by rapidly inflating the balloon at strengths of 2.666, 5.332, 7.998, and 10.664 kPa for a period of 20 seconds. Each score was tested three times, and each rat was tested by two different persons who were not

involved in this research project. There were 3-minute intervals between the two tests, to allow the rats to adapt. The mean values of all tested values were calculated and considered the final score of each rat.

#### **Concentrations of enkephalins as detected by ELISA assay**

After abdominal withdrawal reflex scores were evaluated, the rats were sacrificed by intraperitoneal anesthesia using sodium pentobarbital (80 mg/kg), and the segments of the spinal cord ( $L_4-S_1$ ) were removed from the rats and homogenized in phosphate buffered saline (10%, ratio of colon weight and phosphate buffered saline volume), and then centrifuged at 4 000 r/min at  $4^\circ\text{C}$  for 30 minutes. The supernatant was separated for assessment. The enkephalin concentrations in the supernatants were assayed by ELISA<sup>[23]</sup>. ELISA kits were used strictly according to the manufacturer's instructions (Bionewtrans Pharmaceutical Biotechnology Co., Ltd., Franklin, MA, USA), with the results expressed per wet weight of tissue samples (ng/L). The sample (50  $\mu\text{L}$ ) was incubated with 50  $\mu\text{L}$  biotin-antibody (biotinylated anti-rat enkephalin 1 mg/mL) for 60 minutes at  $37^\circ\text{C}$ , then added with 80  $\mu\text{L}$  horse radish peroxidase (1: 1 600) for another 30 minutes at  $37^\circ\text{C}$ . The incubation reactions were terminated with 50  $\mu\text{L}$  stop solution (2 M  $\text{H}_2\text{SO}_4$ ). Absorbance was read with an enzyme-labeled meter (Multiskan Ascent mark, Finland) within 30 minutes at 450 nm. Enkephalins in the spinal cord (ng/L) = concentration  $\times$  dilution times of the sample.

#### **Statistical analysis**

Data were statistically analyzed by SPSS 10.0 statistical package (SPSS, Chicago, IL, USA), measurement data was expressed as mean  $\pm$  SD. One-way analysis of variance was used if the data were in accordance with normal distribution, least significant difference-*t* test was used together to compare inter-class variation. A  $P < 0.01$  level was considered a statistically significant difference.

**Author contributions:** Huang Wu, Tao Yi, and Li Qi designed the study. Li Qi and Tao Yi implemented the experiments. Xiaopeng Ma, Huirong Liu, and Xiaomei Wang analyzed the data. Tao Yi, Li Qi, and Xiaopeng Ma wrote and revised the manuscript. Huang Wu supervised the whole research and edited the manuscript.

**Conflicts of interest:** None declared.

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**Ethical approval:** This study was approved by the Animals Ethics Committee of Yueyang Hospital of Chinese Integrative Medicine affiliated to Shanghai University of Traditional Chinese Medicine in China.

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**Supplementary information:** Supplementary data associated with this article can be found, in the online version, by visiting [www.nrronline.org](http://www.nrronline.org), and entering Vol. 7, No. 3, 2012 after selecting the "NRR Current Issue" button on the page.

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