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Electroacupuncture at LI11 promotes jejunal motility via the parasympathetic pathway

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Abstract

Background: Gastrointestinal motility disorder has been demonstrated to be regulated by acupuncture treatment. The mechanisms underlying the effects of acupuncture stimulation of abdominal and lower limb acupoints on gastrointestinal motility have been thoroughly studied; however, the physiology underlying the effects of acupuncture on the forelimbs to mediate gastrointestinal motility requires further exploration. The aim of this study was to determine whether electroacupuncture (EA) at LI11 promotes jejunal motility, whether the parasympathetic pathway participates in this effect, and if so, which somatic afferent nerve fibres are involved.

Methods: A manometric balloon was used to observe jejunal motility. The effects and mechanisms of EA at LI11 were explored in male Sprague-Dawley rats with or without drug administration (propranolol, clenbuterol, acetylcholine, and atropine) and with or without vagotomy. Three types of male mice ($\beta_1\beta_2$ receptor-knockout [$\beta_1\beta_2^{-/-}$] mice, M_2M_3 receptor-knockout [$M_2M_3^{-/-}$] mice and wild-type [WT] mice) were also studied by using different EA intensities (1, 2, 4, 6, and 8 mA). A total of 72 rats and 56 mice were included in the study.

Results: EA at LI11 increased the contractile amplitude of jejunal motility in the majority of both rats and mice. However, EA at LI11 did not enhance jejunal motility in rats administered atropine, rats that underwent vagotomy, and $M_2M_3^{-/-}$ mice (at all intensities). In WT mice, EA at LI11 significantly increased jejunal motility at all intensities except 1 mA, and a plateau was reached at intensities greater than 4 mA.

Conclusion: Our results suggest that EA at LI11 promotes jejunal motility primarily by exciting the parasympathetic pathway, and that A δ -fibres and C-fibres may play important roles in the process.

Keywords: Electroacupuncture (EA), LI11, Jejunal motility, Parasympathetic pathway, Sympathetic pathway

Background

Gastrointestinal motility disorder consists of multiple clinical symptoms and occurs in numerous diseases, including diarrhoea, constipation and irritable bowel syndrome (IBS) [1, 2]. Functional bowel disorders have become a common research focus in recent decades, owing to the rapid advances in neurogastroenterology and the development of many new methods to study gastrointestinal motility [3].

An important role of the somatic-autonomic reflex in gastrointestinal motility has been demonstrated, whereas the autonomic nervous system has been reported to be

highly involved in modulating visceral organ function [4–8]. Further studies have shown that small bowel contractile activity is under nervous system control via parasympathetic excitatory and sympathetic inhibitory nerve fibres directly act on smooth muscle cells [9, 10].

Many studies have provided reliable evidence of the effectiveness of acupuncture therapy for treating gastrointestinal motility disorder, primarily through autonomic nerve reflexes [4, 11, 12]. In animal models, acupuncture stimulation of abdominal skin inhibits gastrointestinal motility primarily by exciting sympathetic pathways [13], whereas acupuncture at hind paws enhances gastric motility mainly via parasympathetic pathways [14, 15]. Electroacupuncture (EA) is a variant of manual acupuncture (MA) in which electrical stimulation is applied through

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needles. Owing to the ease of modulating its stimulation frequency and intensity, EA is recognized as a quantifiable treatment and is widely used in clinical and experimental research [16]. For this reason, we chose EA as the stimulation method in this study.

Previous research has focused on stimulation of the abdomen or the hind paws, whereas few studies have used the forepaws. LI11 (Quchi), located at the midpoint between the lateral end of the transverse cubital crease and the lateral humeral epicondyle [14], is traditionally used to regulate gastrointestinal motility, because there sufficient supporting clinical and experimental evidence of its efficacy [17–26]. However, in contrast to other acupoints for regulating gastrointestinal motility, such as ST36 (Zusanli) and ST25 (Tianshu), the more evidence from basic research regarding the use of LI11 in acupuncture is needed.

We hypothesized that the immediate effect of EA stimulation at LI11 would be promoting jejunal motility, primarily by exciting parasympathetic pathways and inhibiting sympathetic pathways.

Methods

Animals

Male Sprague-Dawley rats (180–230 g; Model Animal Research Center of Nanjing Medical University, China) and male mice (22–28 g), including $\beta_1\beta_2^{-/-}$ mice (B6.129X1- $\beta_1\beta_2^{\text{tm1}ul/NJU}$, J003810; donated by the Jackson Laboratory, USA), $M_2M_3^{-/-}$ mice (B6.129X1- $M_2M_3^{\text{tm1}ul/NJU}$, D0407; from Kumamoto University, Japan), and wild-type counterparts (WT mice; purchased from the Model Animal Research Center of Nanjing University, China) were used in this study. Gene knockout in mice ($\beta_1\beta_2^{-/-}$ mice and $M_2M_3^{-/-}$ mice) was verified with PCR, and their genetic background is shown in the Additional file 1.

The animals were housed under a temperature of 22 °C and a relative humidity of 40%–60% at the SPF Experimental Animal Center, Nanjing University of TCM. They were housed under a 12 h/12 h light/dark cycle and were given free access to food and water. Animals underwent an adaptation period for seven days before inclusion in experiments. All experimental manipulations were undertaken in accordance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, China.

Drugs

All animals in the study were anaesthetized with urethane (U2500; Sigma, St. Louis, MO, USA). The beta adrenoceptor agonist clenbuterol hydrochloride (clen; C5423; Sigma, St. Louis, MO, USA) and the beta adrenoceptor antagonist propranolol hydrochloride (prop; P0084; Sigma) were administered to the rats. Next, the muscarinic receptor

antagonist atropine hydrochloride (atropine; A6625; Sigma) and the agonist acetylcholine hydrochloride (ACh; A0132; Sigma) were administered. Penicillin antibiotic (B151226; Shandong Lukang Pharmaceutical Co., Ltd., Jinjing, China) was administered after surgery. The concentration, doses, and administration methods were 1) urethane: 20%, 8 mL/kg for rats and 5 mL/kg for mice, I.P.; 2) clenbuterol: 0.2%, maintenance dose of 80 $\mu\text{L}/\text{kg}/\text{min}$, intravenous (I.V.); 3) propranolol: 0.4%, initial dose of 1.0 mL/kg and maintenance dose of 40 $\mu\text{L}/\text{kg}/\text{min}$, I.V.; 4) acetylcholine: 0.1%, maintenance dose of 20 $\mu\text{L}/\text{kg}/\text{min}$, I.V.; 5) atropine: 0.2%, initial dose of 0.8 mL/kg and maintenance dose of 40 $\mu\text{L}/\text{kg}/\text{min}$, I.V.; and 6) penicillin: 8×10^5 IU dissolved in 2 mL saline, 0.4 mL/d per rat, intramuscular (I.M.).

Recording of the jejunal motility

All animals in the study were fasted for 12 h and given free access to water prior to anaesthetization with urethane. After supplementary anaesthesia, the rats in the drug-administered groups underwent endotracheal intubation to maintain the respiratory tract unobstructed and the left internal jugular venous catheter accessible for drug administration. A small incision (length: 5–8 mm in rats, 2–3 mm in mice) was made below the xiphoid, and a small balloon (diameter: approximately 2 mm) made of flexible rubber was inserted into the jejunum to approximately 3–5 cm (rats) or 0.8–1.2 cm (mice) distal to the duodenal suspensory ligament. The balloon, which was connected to a polyethylene tube (length: 10 cm), was then filled with 0.05–0.1 mL warm water to maintain the jejunal baseline pressure at approximately 0.3 kPa. The jejunal pressure was recorded with a transducer (YPJ01; Chengdu Instrument Factory, China) through the balloon and tube. The signal was collected with a biological signal-sampling system (RM6240; Chengdu Instrument Factory) for further analysis. The baseline pressure was maintained at 0.28–0.32 kPa. During the experiment, an electric heating board was used to maintain the temperature of the animals at 37 ± 0.5 °C.

EA stimulation

LI11 (Quchi) is located midway between the lateral end of the transverse cubital crease and the lateral humeral epicondyle. A pair of stainless steel acupuncture needles (diameter: 0.3 mm) were inserted to a depth of 3 mm into the muscle layer at the right LI11. The needles were connected to a Han EA therapeutic stimulator (LH₄02A; Beijing Huawei Industrial Development Corporation, China). The frequency of EA was set at 2/15 Hz for each stimulation.

Vagotomy

The animals were anaesthetized with urethane, and a small incision was made in the midline of the abdomen.

Animals then underwent bilateral vagotomy by dissection of the ventral and dorsal branches of the vagus nerve. The animals in the sham control group underwent the same procedure but without vagus nerve dissection. After suturing, the animals received penicillin antibiotic shots (0.2 mL each rat per day, I.M.) and were allowed three days to recover before the experimental session. Control group animals did not undergo any treatment before experiments. Twenty-four rats were used in this experiment.

Study design

To verify our hypothesis, we sought to 1) demonstrate whether EA at LI11 could promote jejunal motility; 2) explore the effects of EA at LI11 on the sympathetic pathway and the parasympathetic pathway, and 3) explore which somatic afferent nerve fibres participate in the stimulation process associated with EA at LI11.

In the first experiment, we divided animals into four groups (rats with EA, rats without EA, mice with EA and mice without EA) and then recorded the jejunal pressure to describe the characteristics of jejunal motility.

In the second experiment, we first used drugs to excite or inhibit a specific pathway before EA stimulation. Then, we cut the gastro-vagal nerves of the rats to evaluate the role of the parasympathetic pathway. Lastly, $\beta_1\beta_2$ adrenoceptor double-knockout ($\beta_1\beta_2^{-/-}$) mice, M_2M_3 muscarinic double-knockout ($M_2M_3^{-/-}$) mice, and wild-type mice were used to further verify the results.

In the last experiment, we divided animals into three groups (WT group, $\beta_1\beta_2^{-/-}$ group and $M_2M_3^{-/-}$ group) and used different intensities of EA to explore the mechanism by which somatic afferent nerve fibres participate in the stimulation process associated with EA at LI11. The procedure and details of the experiments are shown in Fig. 1. There were 8 animals per group in all experiments.

Assessment

Jejunal pressure during EA (dur-EA) was compared with the jejunal pressure before EA was performed (pre-EA)

[27]. The response was identified as being enhanced if the dur-EA pressure was >105% of the pre-EA pressure. The formula used to calculate the percentage change is given below (1).

$$\text{Percentage change} = \frac{\text{dur-EA}}{\text{pre-EA}} \times 100\% \quad (1)$$

Statistical analysis

Results are expressed as the mean \pm SEM (standard error of the mean). Data were analysed using SPSS 19.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) software programs. Paired-sample *t* tests (within the group) and one-way ANOVA (between groups) were used for statistical analyses. $P < 0.05$ was considered statistically significant. The data curves for different intensities were fitted with eq. (2) (X: log of intensity; Y: response, increasing as X increases. Top and bottom: plateaus in the same units as Y. LogEC50: same log units as X).

$$Y = \text{Bottom} + \frac{(\text{Top}-\text{Bottom})}{1 + 10^{X-\text{LogEC50}}} \quad (2)$$

Results

Characteristics of jejunal motility of normal animals with or without EA at LI11

After the injection of warm water into the balloon and stabilization of jejunal motility, we observed that the jejunal pressure was maintained in rats at approximately 0.8–1.0 kPa (Fig. 2a) and in mice at approximately 0.4–0.5 kPa, after injecting 0.05 mL warm water into the balloon (Fig. 2c). EA at LI11 induced increases in the contractile amplitude of jejunal motility in both mice and rats. In mice, the amplitude increased by approximately 0.05–0.3 kPa, and for rats, it increased by approximately 0.01–0.05 kPa (see Fig. 2b and d). The results suggested that EA at LI11 promotes jejunal motility.

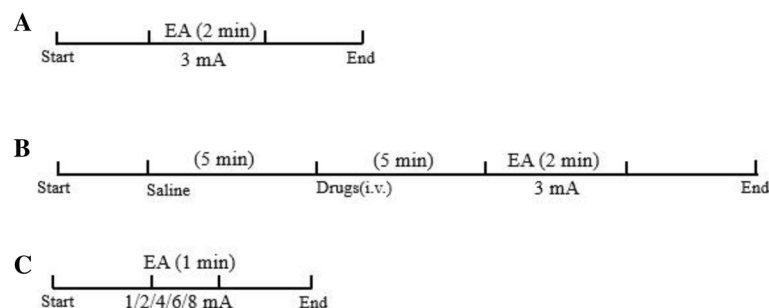
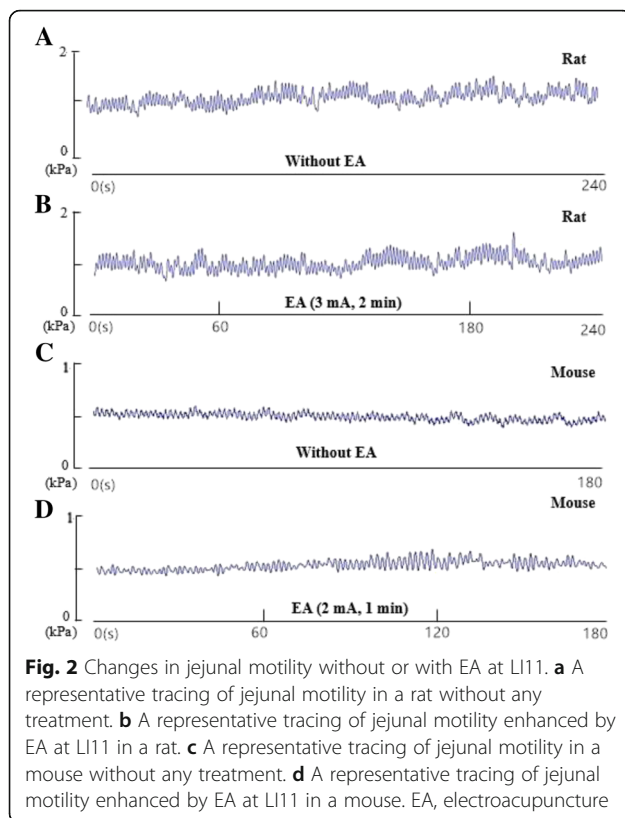


Fig. 1 The experimental procedure. **a** Intervention timeline in rats stimulated by ordinary EA. **b** Intervention timeline in rats with drug (clenbuterol, propranolol, acetylcholine or atropine) administration. **c** Intervention timeline in three kinds of mice (wild-type, $\beta_1\beta_2^{-/-}$, $M_2M_3^{-/-}$) with different intensities of electroacupuncture (1, 2, 4, 6, and 8 mA)



The role of the sympathetic pathway in the effect of EA at LI11

We used the beta adrenoceptor agonist clenbuterol and antagonist propranolol in rats and then performed EA at LI11 at an intensity of 3 mA. We divided rats into three groups: a control group (no administration), a clenbuterol group and a propranolol group (8 rats per group). Jejunum movement was markedly inhibited by the beta receptor agonist but was excited by propranolol.

As shown in Fig. 3Ab and Ac, we found that EA induced excitement of jejunum movement in animals in all groups, corresponding to changes greater than 105%, whereas no significant differences were observed among groups. To further substantiate whether the sympathetic pathway was involved in the effect of EA at LI11, we applied EA stimulation of 2 mA to the LI11 of $\beta_1\beta_2^{-/-}$ mice and found no significant differences between WT and $\beta_1\beta_2^{-/-}$ mice (8 mice per group, Fig. 3Bb and Bc). These data suggested that the sympathetic pathway may not play a key role in the effect of EA at LI11.

The role of the parasympathetic pathway in the effect of EA at LI11

Next, to determine whether the parasympathetic pathway was involved in the effect of EA at LI11 in regulating jejunal motility, we used the muscarinic receptor agonist ACh and antagonist atropine. We divided rats

into three groups (control group, ACh group and atropine group) with 8 rats per group.

The jejunal motility of the animals increased after ACh administration but decreased with atropine administration. We found that the internal pressure of the jejunum of rats in the ACh and control groups increased after EA stimulation. However, there were no obvious differences in jejunal motility between pre-EA and dur-EA after administration of atropine (from 0.24 ± 0.04 to 0.25 ± 0.04 in 8 rats, $P > 0.05$); hence, EA at LI11 did not enhance jejunal motility in the presence of atropine (Fig. 4Ab and Ac).

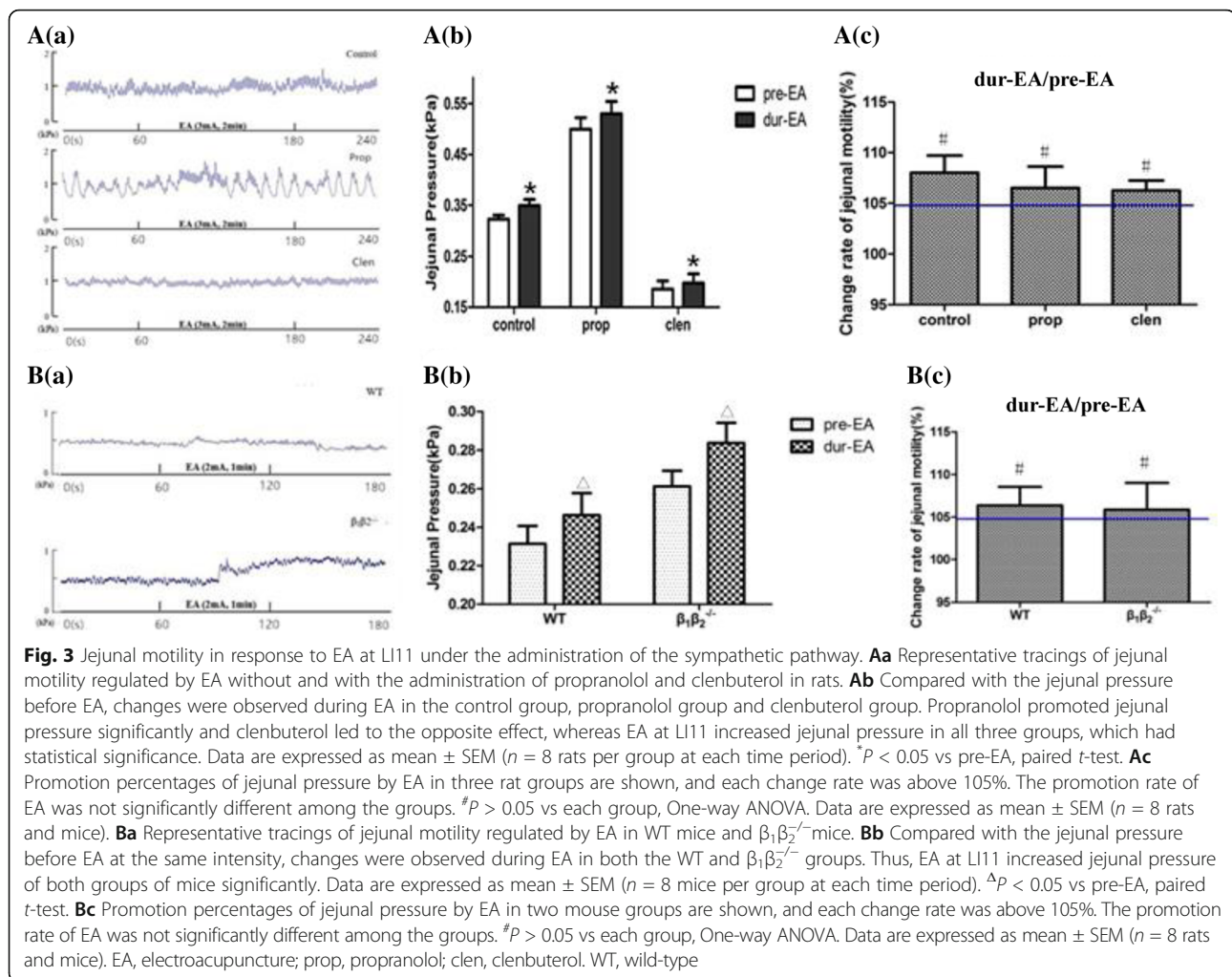
We then used rats with gastric vagotomy. We divided rats into three groups including a control group (no administration), a sham control group (subjected to the same procedure as the vagotomy group except for vagus nerve dissection) and a vagotomy group (8 rats per group). The experiment was performed three days after surgery. The results (see Fig. 4Ba, Bb and Bc) showed that movement of the jejunum was markedly inhibited and was not excited by EA stimulation when the bilateral gastro-vagal nerves were cut. The rats in the sham control group still exhibited enhanced jejunal motility after EA, as did the control group.

Furthermore, we used $M_2M_3^{-/-}$ mice to determine the role of the parasympathetic pathway in the effect of EA at LI11. There were 8 mice in the WT group and in the $M_2M_3^{-/-}$ group. The $M_2M_3^{-/-}$ group also did not exhibit enhanced jejunal motility, thus further suggesting that the parasympathetic pathway may mediate the effect of EA at LI11 (see Fig. 4Cb and Cc). Together, these results demonstrated that the parasympathetic pathway plays an important role in the promotion effect of EA at LI11.

Effects of EA at LI11 of different intensities on jejunal motility in mice

To gain further insight into the mechanism through which somatic afferent nerve fibres participate in the stimulation process associated with EA at LI11, different intensities of EA (1, 2, 4, 6, and 8 mA) were applied to three groups of mice ($\beta_1\beta_2^{-/-}$, $M_2M_3^{-/-}$ and WT mice). In the WT group, we observed that the promotion of jejunal motility by EA at LI11 with different intensities exceeded 105%, except for 1 mA stimulation (Fig. 5a and d). The $\log EC_{50}$ value of EA stimulation in WT mice was 1.71 ± 0.59 mA, and the promotion of jejunal motility increased in response to increasing EA intensity up to 4 mA. When the intensity exceeded 4 mA, a plateau in jejunal motility was reached (Fig. 5d).

The $\log EC_{50}$ value of EA stimulation in $\beta_1\beta_2^{-/-}$ mice was 4.60 ± 0.41 mA, and the value in $M_2M_3^{-/-}$ mice was 1.24 ± 1.77 mA. Compared with WT mice, the rate of change of jejunal pressure caused by EA at LI11 was significantly lower with intensities of 4 mA to 8 mA in $M_2M_3^{-/-}$ mice, and a similar trend was observed for



2 mA. However, the $M_2M_3^{-/-}$ mice showed markedly different results as compared with those for other groups of mice subjected to EA of the same intensity, because EA at any intensity did not alter jejunal motility of these mice (Fig. 5c and f). No differences in the effects of EA were found between WT and $\beta_1\beta_2^{-/-}$ mice stimulated at intensities of 1, 2, or 4 mA.

There was a sudden increase in the rate of change of jejunal motility when the EA intensity was increased to 4 mA. However, the motility subsequently reached a plateau and did not further change when the EA intensity was increased to 6 mA.

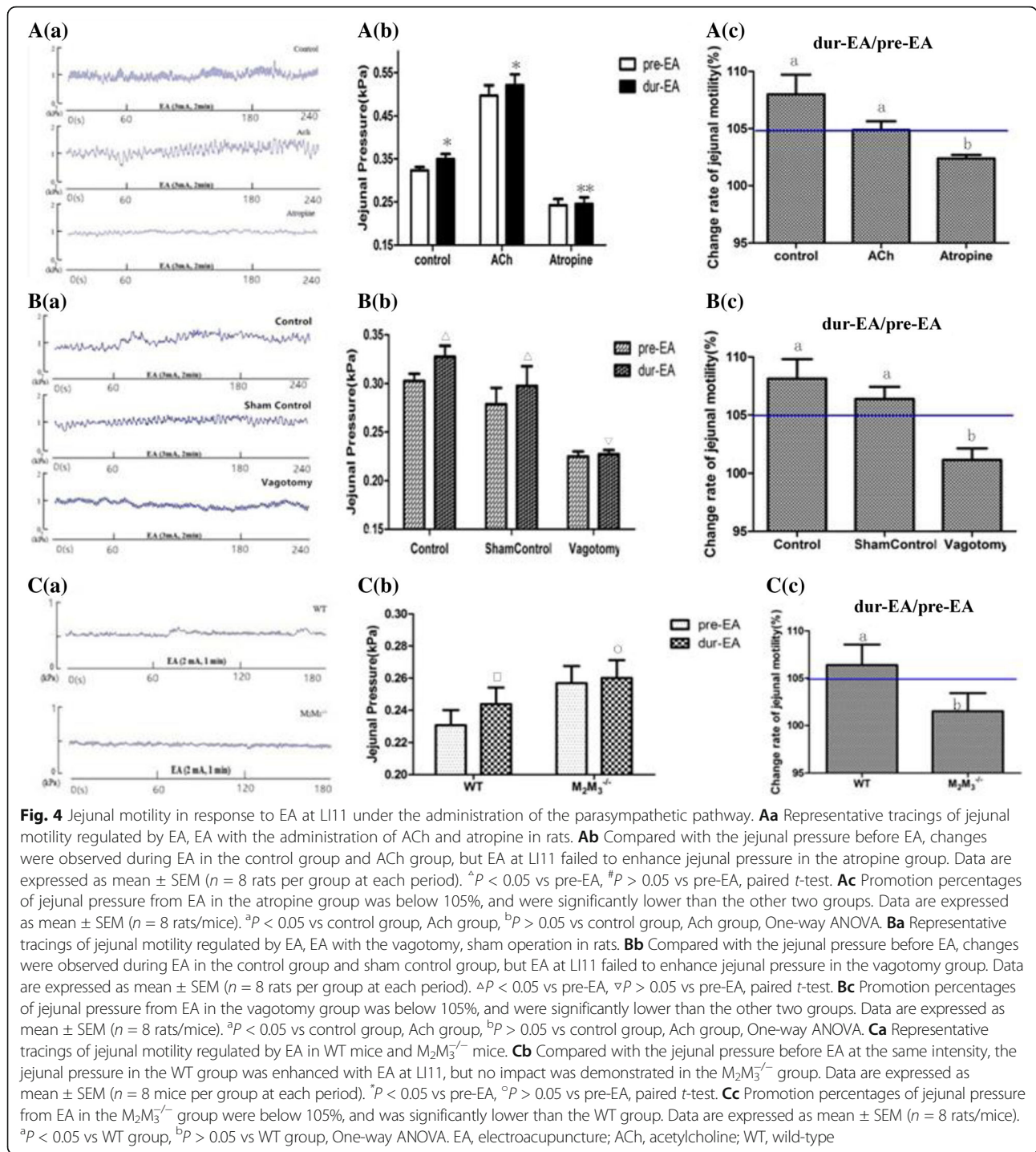
In general, these data further demonstrated that the parasympathetic pathway plays an important role in the jejunal motility promotion effect of EA at LI11.

Discussion

Gastrointestinal motility disorders occur in many gastrointestinal and other systemic diseases. Current drug therapy is not particularly effective at controlling the symptoms, and people with the disorder require long-

term drug therapy. This therapy increases patients' economic burden and is associated with increased risks of possible drug side effects [28–31]. Acupuncture has been reported to be an effective method of complementary and alternative medicine for the treatment of gastrointestinal motility disorders in numerous clinical studies [32–35]. LI11 (Quchi) is a useful acupoint on the arm that is often used to treat gastrointestinal diseases. It has been studied primarily for mediating gastrointestinal motility [17–26].

Along the gastrointestinal tract, normal peristalsis and homeostatic sensory and motor mechanisms are under the control of extrinsic parasympathetic and sympathetic pathways. By exploring the response to vagal stimulation of the jejunum and ileum in cats, Kewenter has found clear evidence that splanchnic sympathetic fibres exert an inhibitory effect on the ileum at the intramural ganglionic, but not directly on smooth muscle cells [36]. Although Sato et al. have found that abdominal stimulation inhibits gastrointestinal motility, stimulation of the hind paw enhances gastrointestinal motility in rats [4–6]. On the basis



of these discoveries, Zhu Bing et al. [14, 15, 37–39] have studied the effects of acupuncture stimulation and its physiological mechanisms and have demonstrated that acupuncture stimulation at abdominal points inhibits gastrointestinal motility but that acupuncture at hind paw points enhances gastrointestinal motility. These authors have also defined “homotopic” and “heterotopic”

acupoints as part of a new theoretical approach to interpret acupuncture effects and mechanisms. According to this approach, afferent innervation of a homotopic acupoint is in the same spinal cord segment from which the efferent branch innervates visceral organs, whereas afferent innervation is in a different segment for heterotopic acupoints. Acupuncture stimulation at homotopic points

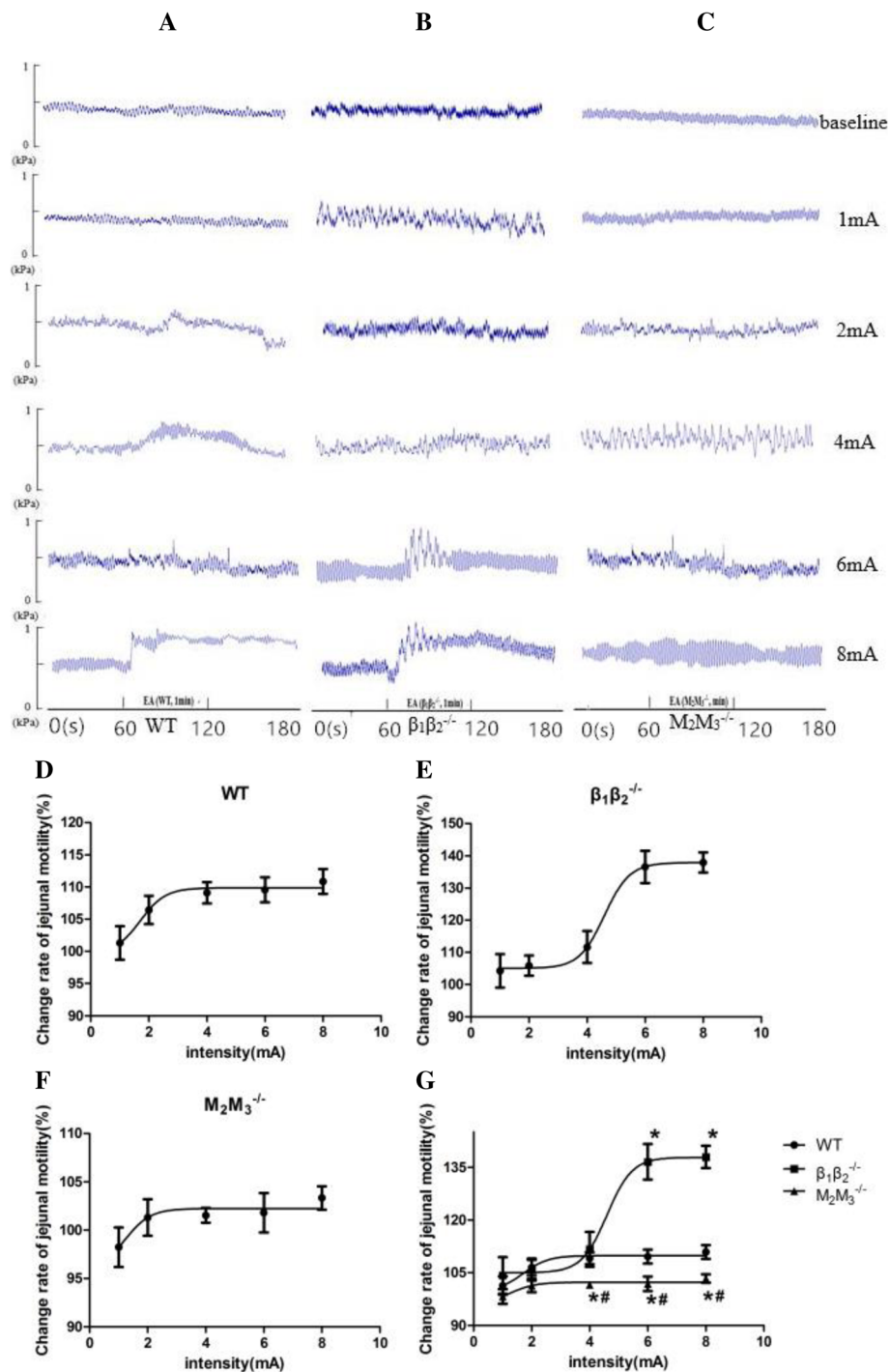


Fig. 5 The effect of different intensities of EA at LI11 on jejunal motility in WT, $\beta_1\beta_2^{-/-}$ and $M_2M_3^{-/-}$ mice. **a-c** Representative tracings of jejunal motility with different intensities of EA in WT, $\beta_1\beta_2^{-/-}$ and $M_2M_3^{-/-}$ mice, respectively. **d-f** The fitting curves of the EA effect with different intensities on jejunal motility in WT, $\beta_1\beta_2^{-/-}$ and $M_2M_3^{-/-}$ mice, respectively, showing the relationships between different intensities of EA (1, 2, 4, 6, and 8 mA) and the rate of change rate in jejunal pressure. **g** The integrate fitting curve showed the enhanced effect of EA with different intensities on jejunal motility in three groups. Data are expressed as mean \pm SEM ($n = 8$ mice). * $P < 0.05$ vs WT mice, # $P < 0.05$ vs $\beta_1\beta_2^{-/-}$ mice, independent t -test. WT, wild-type

inhibits gastrointestinal motility via the sympathetic pathway; in contrast, acupuncture at heterotopic points enhances gastrointestinal motility via the parasympathetic

pathway. Our previous study has shown that EA at ST25 inhibits gastric and jejunal motility via the sympathetic pathway; however, EA at ST37 excites gastric and jejunal

motility via the parasympathetic pathway [13, 40]. These results support the “homotopic and heterotopic acupoints” theory. We then turned our focus to the gastrointestinal effect of acupuncture at forelimb points such as LI11 (Quchi) and its underlying mechanism, because LI11 is an effective acupoint in regulating gastrointestinal motility. In fact, many acupuncturists select this point for treating gastrointestinal motility disorders in clinical practice [17–22]. The experimental exploration of the effect of acupuncture at LI11 in regulating gastrointestinal motility has not received much attention in the past. Qin [14], however, has reported that acupuncture at LI11 (containing afferents from the C5 spinal dorsal horn) is a heterotopic acupoint to the jejunum (T9–12); this treatment increases the amplitude of peristalsis waves and enhances jejunal motility in normal rats as well as constipated and diarrheic rats. In our study, we used an antagonist and agonist for both adrenoceptors and muscarinic receptors to identify the roles of the sympathetic and parasympathetic pathways in normal rats. In addition, we used gene knock-out mice ($\beta_1\beta_2^{-/-}$ mice and $M_2M_3^{-/-}$ mice) for further verification. As shown in Fig. 2, we found a clear promotion effect of EA at LI11 on jejunal motility, in experiments using both rats and mice. Further explorations of the underlying neural mechanisms are shown in Figs. 3 and 4. There were three notable results of this study: 1) EA stimulation did not enhance jejunal motility in the presence of atropine in rats (Fig. 4a); 2) after vagotomy, the effect of EA on jejunal motility disappeared in rats (Fig. 4b); and 3) EA stimulation at LI11 was less effective in $M_2M_3^{-/-}$ mice than in WT and $\beta_1\beta_2^{-/-}$ mice (Fig. 4c).

To clarify the effect and mechanism of EA at LI11 on jejunal motility, we used both an antagonist and agonist of adrenoceptors and muscarinic receptors to determine the roles of the sympathetic and parasympathetic pathways in normal rats. We used intravenous administration, and thus, the drugs acted systemically on the entire body rather than on only the target nerves. Therefore, the roles of the target nerves could not be specifically verified [41–46]. Because of this limitation, gene knock-out animals were used in our study. These mice have frequently been used in studies of neurobiological mechanisms of acupuncture, and their reliability has been demonstrated in previous related studies [47]. The results of the gene knockout study also fully supported our scientific hypothesis. Notably, in the experiments with different EA intensities (Fig. 5), the rate of change of jejunal motility in the $\beta_1\beta_2^{-/-}$ mice greatly exceeded that of the WT mice. The former group of mice showed a large amplitude change, from 4 mA to 6 mA stimulation intensity, and the maximum stimulation after entering the plateau region was 2 mA higher than that of the WT animals. We propose that the effect of

stimulation at LI11 is primarily due to parasympathetic-M receptors. The mutual antagonistic effects between sympathetic and parasympathetic nerves may explain why the rate of change in beta receptor knockout animals subjected to acupuncture is stronger than that of WT mice.

In this study, we observed that the parasympathetic pathway is involved in EA at LI11, but we did not perform further studies of the sympathetic pathway. No sufficient evidence was found to demonstrate that LI11 stimulation inhibits sympathetic nerve activity, thus representing a limitation of our study that should be addressed in further research. However, our previous work has shown that stimulating ST37 (Shangjuxu) excites parasympathetic nerves, and stimulating ST25 (Tianshu) excites sympathetic nerves [40, 48]. All these results together support the “homotopic and heterotopic acupoints” theory proposed by Zhu Bing et al.

To address the mechanism of the effects of EA stimulation at LI11, we sought to characterize the somatic afferents of EA stimulation by using different stimulation intensities. Somatic afferent nerve fibres are composed of A α - (group I), A β - (group II), A δ - (group III) and C-fibres (group IV) [37, 49]. The mean stimulation thresholds for inducing firing in mouse A δ - and C-fibres have been reported to be approximately 2 mA and 3 mA, respectively [37]. Thus, we chose different EA stimulation intensities (1, 2, 4, 6, and 8 mA) to test the responses in three types of mice ($\beta_1\beta_2^{-/-}$, $M_2M_3^{-/-}$ and WT mice). In normal mice (the WT mice group), EA at LI11 significantly increased jejunal motility at all intensities except 1 mA, and when the intensity exceeded 4 mA, the effect plateaued. These results verify that A δ -fibres are activated by the stimulation when the intensity is maintained at 2 mA and that 4 mA can active C-fibres. The $M_2M_3^{-/-}$ group differed from the WT and $\beta_1\beta_2^{-/-}$ groups in that the rate of change remained under 105% for all stimulation intensities. This result suggests that activation of muscarinic receptors plays a key role in the effect of EA at LI11, thus further supporting further our hypothesis.

Conclusion

In summary, we clearly demonstrated the important role of the parasympathetic pathway in the promotion effect of EA stimulation at LI11 on jejunal motility. The intensity-dependent manner of EA stimuli indicates that A δ -fibres and C-fibres may participate in the response to EA stimulation from 2 mA to 4 mA. LI11, a heterotopic point, also promotes jejunal motility under EA stimulation, similarly to the points on the hind paw. This study provides further evidence that acupuncture regulates gastrointestinal function via the somatic-autonomic reflex.

Additional file

Additional file 1: Genetic background of gene knockout mice.
(DOCX 195 kb)

Abbreviations

EA: Electroacupuncture; IBS: Irritable bowel syndrome; MA: Manual acupuncture

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Availability of data and materials

Data are all contained within the paper.

Authors' contributions

BX conceived and designed the experiments; YW, YL, NZ performed the experiments; XH and XS wrote the paper; ZY supervised the study. All authors have read and approved the final version of manuscript.

Authors' information

Xuanming Hu, Ph.D candidate, Major Research Direction: basic research of acupuncture treatment.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

The authors declare that they consent for publication.

Ethics approval and consent to participate

All procedures had the approval of the Animal Ethics Committee of the Nanjing University of Chinese Medicine.

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References

- Lauro A, Zanfi C, Dazzi A, di Gioia P, Stanghellini V, Pironi L, et al. Disease-related intestinal transplant in adults: results from a single center. *Transplant Proc.* 2014;46:245–8.
- Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *JAMA.* 2015;313:949–58.
- Törnblom H, Simrén M, Abrahamsson H. Gastrointestinal motility and neurogastroenterology. *Scandinavian J Gastroenterology.* 2015;50:685–97.
- Kametani H, Sato A, Sato Y, Simpson A. Neural mechanisms of reflex facilitation and inhibition of gastric motility to stimulation of various skin areas in rats. *J Physiol.* 1979;294:407–18.
- Sato A, Schmidt RF. Muscle and cutaneous afferents evoking sympathetic reflexes. *Brain Res.* 1966;2:399–401.
- Sato A, Kaufman A, Koizumi K, Brooks CM. Afferent nerve groups and sympathetic reflex pathways. *Brain Res.* 1969;14:575–87.
- Caulfield MP. Muscarinic receptors—characterization, coupling and function. *Pharmacol Ther.* 1993;58:319–79.
- Noguchi E. Acupuncture regulates gut motility and secretion via nerve reflexes. *Auton Neurosci.* 2010;156:15–8.
- Bayliss WM, Starling EH. The movements and innervation of the small intestine. *J Physiol.* 1899;24:99–143.
- Kock NG. An experimental analysis of mechanisms engaged in reflex inhibition of intestinal motility. *Acta Physiol Scand Suppl.* 1959;47:1–54.
- Jiang J, Yi Y, Zhan L. Acupuncture: a good choice to patients with intractable slow-transit constipation. *Int J Color Dis.* 2015;30:721–2.
- Han G, Leem J, Lee H, Lee J. Electroacupuncture to treat gastroesophageal reflux disease: study protocol for a randomized controlled trial. *Trials.* 2016;17:246.
- Yu Z, Zhang N, Lu CX, Pang TT, Wang KY, Jiang JF, et al. Electroacupuncture at ST25 inhibits jejunal motility: role of sympathetic pathways and TRPV1. *World J Gastroenterol.* 2016;22:1834–43.
- Qin QG, Gao XY, Liu K, Yu XC, Li L, Wang HP, et al. Acupuncture at heterotopic acupoints enhances jejunal motility in constipated and diarrheic rats. *World J Gastroenterol.* 2014;20:18271–83.
- Gao X, Qin Q, Yu X, Liu K, Li L, Qiao H, et al. Acupuncture at heterotopic acupoints facilitates distal colonic motility via activating M3 receptors and somatic afferent C-fibers in normal, constipated, or diarrhoeic rats. *Neurogastroenterol Motil.* 2015;27:1817–30.
- Shen EY, Lai YJ. The efficacy of frequency-specific acupuncture stimulation on extracellular dopamine concentration in striatum—a rat model study. *Neurosci Lett.* 2007;415:179–84.
- Xiong F, Wang Y, Li SQ, Tian M, Zheng CH, Huang GY. Electroacupuncture decreases the urinary bladder pressure in patients with acute gastrointestinal injury. *J Huazhong Univ Sci Technol[Med Sci].* 2015;34(5):775–81.
- Wu JN, Zhang BY, Zhu WZ, Du RS, Liu ZS. Ministered acupressure for treating adult psychiatric patients with constipation: a randomized controlled trial. *Zhongguo Zhen Jiu.* 2014;34(6):521–8.
- Xu X, Zheng C1, Zhang M, Wang W, Huang GA. randomized controlled trial of acupuncture to treat functional constipation: design and protocol. *BMC Complement Altern Med.* 2014;29:14:423.
- Chang XR, Yan J, Shen J, Liu M, Wang XJ. Observation on the therapeutic effect of needling method for harmonizing spleen-stomach on diabetic gastroparesis. *Zhongguo Zhen Jiu.* 2007;27(4):258–60.
- Wang YL, Cao X, Liu ZC, Xu B. Observation on the therapeutic effect of electroacupuncture on simple obesity of gastrointestinal heat pattern/syndrome. *World J Acupuncture-Moxibustion (WJAM).* 2013;23:1–5.
- Qin QG, Wang HP, Liu K, Zhao YX, Ben H, Gao XY, et al. Effects of acupuncture on intestinal motility: Agonism and antagonism. *World Chin Med.* 2013;8(3):262–6.
- Jang JH, Lee DJ, Bae CH, Ha KT, Kwon S, Park HJ, et al. Changes in small intestinal motility and related hormones by acupuncture stimulation at Zusanli (ST 36) in mice. *Chin J Integr Med.* 2017;23(2):215–20.
- Feng H, Yu Z, Xu B. Roles of TRPV1 receptor in electroacupuncture regulating the jejunal motility of mice: an experimental study. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* 2014;34(7):859–63.
- Yu Z, Xia YB, Lu MX, Lin J, Yu WJ, Xu B. Influence of electroacupuncture stimulation of "tianshu" (ST 25), "quchi" (LI 11) and "shangjuxu" (ST 37) and their pairs on gastric motility in the rat. *Zhen Ci Yan Jiu.* 2013;38(1):40–7.
- Fang JQ, Zhang LL, Shao XM, Lian LL, Yu XJ, Dong ZH, et al. Effects of transcutaneous electrical acupoint stimulation combined with general anesthesia on changes of gastric dynamics in controlled hypotension dogs. *Zhen Ci Yan Jiu.* 2011;36(6):397–402.
- Pang T, Lu C, Wang K, Liang C, Yu Z, Zhu B, et al. Electroacupuncture at ST25 inhibits Cisapride-induced gastric motility in an intensity-dependent manner. *Evid Based Complement Alternat Med.* 2016;2016:3457025.
- Koloski NA, Talley NJ, Boyce PM. The impact of functional gastrointestinal disorders on quality of life. *Am J Gastroenterol.* 2000;95:67–71.
- Quigley, Eamonn MM. Probiotics in functional gastrointestinal disorders: what are the facts? *Curr Opin Pharmacol.* 2008;8:704–8.
- Schmulsom M, Corazziari E, Ghoshal UC, Myung SJ, Gerson CD, Quigley EM, et al. A four-country comparison of healthcare systems, implementation of diagnostic criteria, and treatment availability for functional gastrointestinal disorders a report of the Rome foundation working team on cross-cultural, multinational research. *Neurogastroenterol Motil.* 2014;26:1368–85.
- Kaminski A, Kamper A, Thaler K. Antidepressants for the treatment of abdominal pain-related functional gastrointestinal disorders in children and adolescents. *Cochrane Database Syst Rev.* 2011;(7).
- Lima FA, Ferreira LE, Pace FH. Acupuncture effectiveness as a complementary therapy in functional dyspepsia patients. *Arq Gastroenterol.* 2013;50:202–7.

33. Zhang X, Jin HF, Fan YH, Lu B, Meng LN, Chen JD. Effects and mechanisms of transcutaneous electroacupuncture on chemotherapy-induced nausea and vomiting. *Evid Based Complement Alternat Med.* 2014;2014:860631.
34. Ouyang H, Xing J, Chen J. Electroacupuncture restores impaired gastric accommodation in vagotomized dogs. *Dig Dis Sci.* 2004;49:1418–24.
35. Glickman-Simon R, Tessier J. Guided imagery for postoperative pain, energy healing for quality of life, probiotics for acute diarrhea in children, acupuncture for postoperative nausea and vomiting, and animal-assisted therapy for mental disorders. *Explore (NY).* 2014;10:326–9.
36. Pahlin PE, Kewenter J. Sympathetic nervous control of cat ileocecal sphincter. *Am J Phys.* 1976;231:296–305.
37. Li YQ, Zhu B, Rong PJ, Ben H, Li YH. Neural mechanism of acupuncture-modulated gastric motility. *World J Gastroenterol.* 2007;13:709–16.
38. Zhao YX, Cui CX, Qin QG, Gao JH, Yu XC, Zhu B. Effect of manual acupuncture on bowel motility in normal kunming mouse. *J Tradit Chin Med.* 2015;35:227–33.
39. Zhu B, Xu WD, Rong PJ, Ben H, Gao XY. By C-fiber reflex inhibition by electroacupuncture with different intensities applied at homotopic and heterotopic acupoints in rats selectively destructive effects on myelinated and unmyelinated afferent fibers. *Brain Res.* 2004;1011:228–37.
40. Yu Z, Cao X, Xia Y, Ren B, Feng H, Wang Y, et al. Electroacupuncture stimulation at CV12 inhibits gastric motility via TRPV1 receptor. *Evid Based Complement Alternat Med.* 2013;2013:294789.
41. Mason RP, Giles TD, Sowers JR. Evolving mechanisms of action of beta blockers: focus on nebivolol. *J Cardiovasc Pharmacol.* 2009;54(2):123–8.
42. Pertwee RG. Cannabinoids and the gastrointestinal tract. *Gut.* 2001;48:859–67.
43. Bertaccini G, Coruzzi G. Receptors in the gastrointestinal tract. *Pharmacol Res Commun.* 1987;19:87–118.
44. Allescher HD. Medicamentous modification of gastrointestinal motility and secretion. *Z Gastroenterol.* 1991;29:27–30.
45. Michel MC. Therapeutic modulation of urinary bladder function: multiple targets at multiple levels. *Annu Rev Pharmacol Toxicol.* 2015;55:269–87.
46. Nunes EJ1, Randall PA, Podurgiel S, Correa M, Salamone JD. Nucleus accumbens neurotransmission and effort-related choice behavior in food motivation: effects of drugs acting on dopamine, adenosine, and muscarinic acetylcholine receptors. *Neurosci Biobehav Rev.* 2013;37:2015–25.
47. Gao X, Zhao Y, Su Y, Liu K, Yu X, Cui C, et al. β 1/2or M2/3Receptors Are Required for Different Gastrointestinal Motility Responses Induced by Acupuncture at Heterotopic or Homotopic Acupoints. *PLoS One.* 2016; 11(12):e0168200c.
48. Yuan M, Li Y, Wang Y, Zhang N, Hu X, Yin Y, et al. Electroacupuncture at ST37 enhances Jejunal motility via excitation of the parasympathetic system in rats and mice. *Evid Based Complement Alternat Med.* 2016;2016:3840230.
49. Ohsawa H, Yamaguchi S, Ishimaru H, Shimura M, Sato Y. Neural mechanism of pupillary dilation elicited by electro-acupuncture stimulation in anesthetized rats. *J Auton Nerv Syst.* 1997;64:101–6.

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