OPEN ACCESS

Electroacupuncture ameliorates abnormal defaecation and regulates corticotrophin-releasing factor in a rat model of stress

Yuxue Zhao, Changxiang Cui, Xiaochun Yu, Juanjuan Xin, Fengyan Lu, Junhong Gao, Bing Zhu

Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing, China

Correspondence to
Professor Bing Zhu, and Professor Junhong GaoInstitute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, 16 Nanxiaojiestreet, Dongzhimennei, Beijing 100700, China; zhubing@mail.cintcm.ac.cn; drjunhonggao@aliyun.com

ABSTRACT

Objective To examine the effect of electroacupuncture (EA) treatment on abnormal defaecation in a rat model of chronic heterotypic stress (CHS) and investigate the underlying mechanisms.

Methods 20 male Sprague-Dawley rats were randomly divided into three groups: normal (n=6), CHS (n=7), and CHS+EA (n=7). Rats in the CHS group and CHS+EA groups received four different types of stressors for 7 days. For rats in the CHS+EA group, EA was applied at ST36 in the bilateral hind legs for 30 min before each stress-loading session. Rats in the normal group did not receive stressors or EA treatment. The faecal pellets of each rat were collected and weighed at a fixed time every day. Protein expression of corticotrophin-releasing factor (CRF) in the hypothalamus and colorectal tissues was measured by Western blotting at the end of the experiment on the 7th day.

Results After 7 consecutive days of CHS, the number of faecal pellets, faecal wet weight, and faecal water content were significantly increased in the CHS group compared with the normal group (p=0.035, p=0.008 and p=0.008, respectively). All three parameters were significantly decreased in CHS+EA versus CHS groups (p=0.030, p=0.011 and p=0.006, respectively). Stress significantly increased CRF expression in both the hypothalamus and colorectal tissues. The excessive CRF responses seen following CHS were significantly suppressed by EA treatment.

Conclusions EA treatment can ameliorate stress loading induced abnormal defaecation in rats and decrease protein expression of CRF centrally (hypothalamus) and peripherally (colorectal tissues), suggesting a potentially therapeutic role for EA in stress-related responses.

INTRODUCTION

Abnormal defaecation is one of the most common gastrointestinal (GI) conditions in the general population and can severely affect quality of life.[1] Disorders of colonic transit may contribute to the symptoms of defecatory conditions closely related to functional bowel disorders (FBD). Accumulating evidence suggests a major role of stress in the development of FBD, especially for constipation- or diarrhoea-predominant irritable bowel syndrome (IBS).[2 3] Stress has an extensive effect on colonic function, with altered motor function and stimulation of defaecation.[3] In daily life, people encounter various types of psychological and physical stress, which may contribute to accelerated colonic transit and lead to abnormal defaecation symptoms.[4]

Corticotropin-releasing factor (CRF) is a principal hypothalamic mediator of the autonomic, neuroendocrine, and behavioural responses to stress. Evidence suggests that stress activates the production and release of CRF from the hypothalamus, and accelerates colonic motility at the same time.[5 6] CRF is also known to play a major role in the regulation of GI functions. Central or peripheral injection of CRF delays gastric emptying and stimulates colonic motility.[3] Administration of a CRF antagonist can abolish stress-induced increases in colonic motility,[6 7] which suggests that stress influences GI motility via CRF signalling.

Acupuncture has been widely practised clinically for thousands of years.[8] It has been used to treat various diseases,

including GI disorders and psychological conditions. Electroacupuncture (EA) is a modification of traditional acupuncture, in which electrical current is applied to the metal needles instead of simple manual stimulation. A series of studies has revealed that acupuncture can antagonise stress-induced GI disorders. EA has been shown to improve stress-induced impairment of gastric motility.[9 10] In particular, EA at ST36 ameliorates delayed gastric emptying and accelerated colonic transit induced by restraint stress (RS) in rodents, and also accelerates the recovery of GI function in humans after colorectal surgery.[11 12]

The aim of the present study was to examine the effect of EA treatment on abnormal defaecation in a rat model of stress and investigate the underlying mechanisms, which may relate to endogenous CRF expression. We choose chronic heterotypic stress (CHS) as our stress model to avoid the habituation and restoration that may be produced in conventional stress models.[3] We hypothesised that EA at ST36 would down-regulate hypothalamic and colorectal expression of CRF, which may act as a principal regulator of stress mediating the excessive colorectal motility that is known to arise from CHS. Our objective, therefore, was to determine whether EA can ameliorate abnormal defaecation via inhibition of central and peripheral CRF signals.

METHODS
Animals
Twenty male specific pathogen-free (SPF) grade Sprague-Dawley rats (120–150 g) were obtained from the China Academy of Military Science (license no.

SCKX (JUN) 2007-004). All animals were kept individually in polypropylene cages under controlled conditions of temperature (22–25°C) and light (6:00 on, 18:00 off) and fed with standard rodent chow and tap water ad libitum. The animal experiments were performed in accordance with the National Institutes of Health's 'Guide for the Care and Use of Laboratory Animals' and were approved by the Institutional Animal Care and Use Committee of China Academy of Chinese Medical Sciences (reference no. 2015010801). All efforts were made to minimise suffering.

Study design
The 20 rats were randomly divided into three groups: a normal group (n=6), a CHS group (n=7) and a CHS+EA group (n=7). Rats in the normal group did not receive any intervention. Rats in the CHS and CHS+EA groups each received heterotypic stressors for a week (figure 1A), as previously reported.[4] Rats in the CHS+EA group additionally received EA treatment before each stress loading (figure 1A). The faecal pellets of each rat were collected and weighed every day. The body weight of each rat was also recorded at the same time. On the 7th day, at the end of the experiment, all animals were euthanased with a lethal dose of 30% intraperitoneal urethane (3 mL/rat). After euthanasia, samples were rapidly collected from the hypothalamus and distal colon/rectum (3 cm proximal to the anus). All of the samples were placed into liquid nitrogen immediately and then frozen and stored at −80°C pending detection of CRF expression. All morning experiments were started at 08:30, and all afternoon experiments started at 13:30.
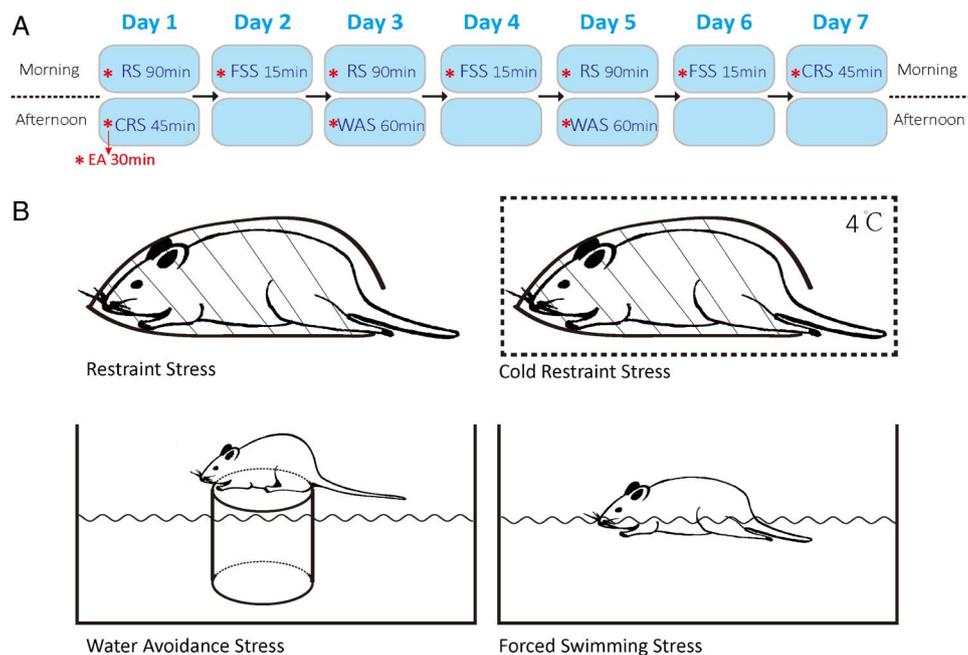
Figure 1 Flow chart illustrating the experimental design (A) and types of stressor applied to establish the chronic heterotypic stress (CHS) rat model, namely restraint stress (RS), cold restraint stress (CRS), water avoidance stress (WAS), and forced swimming stress (FSS). EA, electroacupuncture.

Chronic heterotypic stress model

Rats in the CHS and CHS+EA groups were exposed to different sorts of stressors each day (morning or/and afternoon) for 1 week (figure 1A). Based on a previous study,[4] four kinds of stressors were applied, as illustrated in figure 1B: RS, cold restraint stress (CRS), water avoidance stress (WAS), and forced swimming stress (FSS), respectively. For RS, rats were placed in a custom-made canvas bag (12 cm in length and 6 cm in width) in the prone position for 90 min. Animals were allowed to move their hind limbs but not their heads or trunks. For CRS, rats were placed in the same position in the same bag, but maintained at a temperature of 4°C for 45 min. For WAS, rats were placed on a circular platform (5.5 cm in diameter) in the middle of a plastic tank (51 cm in length, 39 cm in width, and 39 cm in height) for 60 min. The plastic tank was filled with tap water to a depth of 25 cm and the circular platform was positioned 2 cm above the water level. For FSS, rats were placed into the same plastic tank filled to the same depth (25 cm) with water, but without any platform, for 15 min, such that they had to keep swimming to avoid sinking. Thereafter, the rats were dried and warmed. During all four stress-loading sessions, rats in the normal group were kept individually in polypropylene cages.

EA treatment

Stainless steel needles (30 gauge in diameter, 40 mm long; Huatuo, Suzhou, China) were inserted to a depth of 5 mm through the skin and into the underlying muscles at ST36 bilaterally. ST36 is located 5 mm lateral to and 10 mm below the anterior tubercle of the tibia in rats. The needle handles were connected to an EA apparatus (HANS-200A, Nanjing, China) and stimulation was applied (10 Hz frequency, 1 mA intensity, 0.5 ms pulse width) before each stress-loading for 30 min (figure 1A).

Body weight measurement

The body weight of each animal was measured every day for 7 consecutive days to monitor general health, and overall body weight growth rate and the average daily weight gain during the whole experimental period were calculated. Baseline body weight was measured on day 1 in the morning before stress loading, and all repeat measurements were made in the afternoon after stress loading. The body weight growth rate and average daily weight gain were calculated using the following formulae:[13]

Body weight growth rate = (body weight on day 7 − body weight on day 1)/body weight on day 1 × 100%; and Average daily weight gain = (body weight on day 7 − body weight on day 1)/7 days, respectively

Measurement of defecatory function

The daily stools of each rat expelled over a 24 hour period were studied for 7 successive days. Defecatory function was evaluated through three major indicators, namely faecal pellet number, faecal wet weight, and faecal water content. After they were counted and their total wet weight was determined, the faecal pellets of each rat were well dried and weighed in order to calculate faecal water content, according to the following formula:

$$\text{Faecal water content}(\%) = \text{faecal wet weight} - \text{faecal dry weight})/\text{faecal wet weight} \times 100\%.$$

Western blotting

Western blotting was performed to measure CRF expression in the hypothalamus and colorectal tissues, taking glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal control. Protein samples were denatured in sodium dodecyl sulfate (SDS) sample buffer (125 mmol/L Tris-HCl, pH 6.8, 50% glycerol, 2% SDS, 5% mercaptoethanol, and 0.01% bromophenol blue), subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto Immobilon-FL transfer membranes (Millipore). Blotted membranes were blocked with 5% skimmed milk in Tris-buffered saline containing 0.05% Tween-20 for 2 hours and subsequently incubated overnight at 4°C with primary rabbit anti-goat-CRF antibodies diluted 1:200 (catalogue no. sc-10718, Santa Cruz Biotechnology Inc, California, USA). After three washes in Tris-buffered saline containing 0.05% Tween 20, the membranes were incubated with anti-rabbit IgG antibody-horseradish peroxidase (HRP) diluted 1:4000 (catalogue no. sc-2030, Santa Cruz Biotechnology Inc) for 1 hour. Protein expression of CRF was quantified using the Odyssey infrared imaging system (Li-Cor Biosciences) and was reported relative to each sample's GAPDH content.

Statistical analysis

All data were expressed as mean±SD, and analysed by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) V.13.0 (SPSS Inc, Chicago, Illinois, USA). The post-hoc test of least significant difference was used to compare mean values between groups. $p < 0.05$ was considered statistically significant.

RESULTS

Body weight

As shown in figure 2A, CHS significantly suppressed body weight growth rate compared with the normal group on day 3 (6.3±2.0% vs 13.1±0.7%, p=0.002), day 5 (20.5±0.6% vs 26.8±1.7%, p=0.033), and day 7 (30.8±0.5% vs 44.3±3.6%, p=0.047), respectively. EA treatment significantly improved body weight growth rate relative to the untreated CHS group on day 3 (11.7±0.8% vs 6.3±2.0%, p=0.014), day 5

(24.0±0.5% vs 20.5±0.6%, p=0.004), and day 7 (34.5±0.8% vs 30.8±0.5%, p=0.031), respectively.

As shown in figure 2B, CHS significantly inhibited average daily weight gain compared with the normal group (5.63±0.10 g vs 7.70±0.52 g, p=0.039). EA treatment significantly increased average daily weight gain compared with the CHS group (6.51±0.14 g vs 5.63±0.10 g, p=0.003).

Defecatory function

As shown in figure 3A, the number of faecal pellets was markedly increased after CHS compared with the normal group on day 7 (54.6±2.66 vs 43.3±2.69, p=0.035). EA significantly reduced the number of faecal pellets compared with the CHS group after 7 days of intervention (44.0±3.56 vs 54.6±2.66, p=0.030).

As shown in figure 3B, faecal wet weight was significantly increased after CHS compared with the normal group on day 7 (8.21±0.42 g vs 5.69±0.50 g, p=0.008). EA significantly reduced the faecal wet weight compared with the CHS group after 7 days of intervention (5.96±0.56 g vs 8.21±0.42 g, p=0.011).

As shown in figure 3C, the faecal water content was significantly elevated after CHS compared with the normal group on day 7 (46.6±0.90% vs 36.3±3.37%, p=0.008). EA significantly reduced the faecal water content compared with the CHS group after 7 days of intervention (35.95±2.91% vs 46.6±0.90%, p=0.006).

Expression of CRF in hypothalamus and colorectal tissues

As shown in figure 4A, the relative expression of CRF in the hypothalamus was significantly increased after CHS compared with the normal group (0.32±0.03 vs 0.19±0.01, p=0.018). EA significantly inhibited hypothalamic CRF expression compared with the untreated CHS group (0.21±0.04 vs 0.32±0.03, p=0.041).

As shown in figure 4B, expression of CRF in the distal colon and rectum was significantly increased after CHS compared with the normal group (0.52±0.10 vs 0.27±0.02, p=0.019). EA significantly suppressed colorectal CRF expression compared with the CHS group (0.31±0.07 vs 0.52±0.10, p=0.048).

DISCUSSION

Excessive and uncontrollable stress is implicated in the pathogenesis of various diseases.[14] In particular, accumulation of stress in daily life is strongly associated with GI disorders.[4] [15] Exposure to acute or chronic stress has been shown to delay gastric emptying, alter intestinal transit, accelerate colonic motility, and stimulate defaecation in rodents. A growing body of evidence demonstrates that increased faecal weight and excessive defecatory activity can be induced by various stressors.[16] [17] In the present study, male Sprague-Dawley rats received CHS for 7 days. As a result, defaecation was significantly activated, reflected by an increased number of faecal pellets and higher faecal wet weight. The faecal water content was also increased, but diarrhoea was not observed, indicating that CHS alters intestinal transit and faecal characteristics but is not severe enough to cause diarrhoea.

Stressors also have an effect on general conditions, especially body weight. Chronic mild stress is known to cause significant weight loss in mice, compared to controls,[18] and stress exposure generally leads to a gradual reduction in body weight gain.[19] In the present study, the body weights of each animal were monitored for 7 consecutive days. Our data demonstrate that CHS inhibits both body weight growth rate and average daily weight gain during stress exposure, when compared with healthy control rats.

CRF, as a principal mediator of the hypothalamic-pituitary-adrenal (HPA) axis, plays a vital role in the GI response to stress.[5] [14] Central and peripheral injection of CRF mimics GI responses to stress, such as delayed gastric emptying and
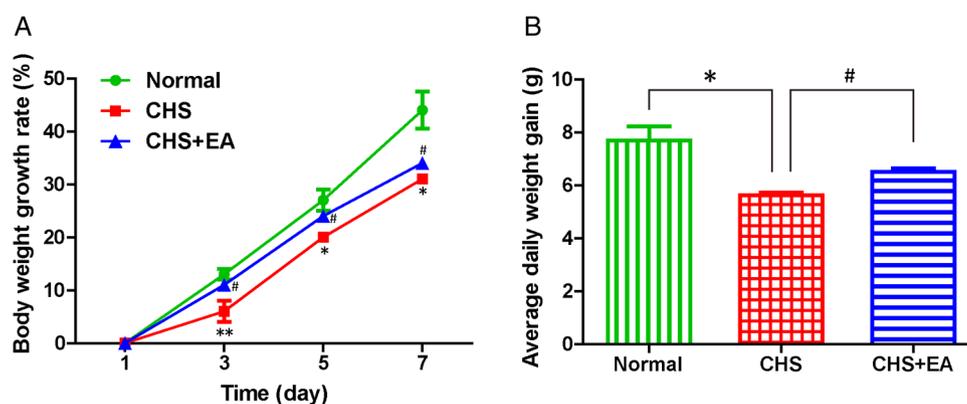
Figure 2 Body weight growth rate (A) and average daily weight gain (B) in six healthy rats (Normal group) and 14 rats exposed to chronic heterotypic stress and left untreated (CHS group, n=7) or treated with electroacupuncture before each stress loading (CHS +EA, n=7). Data are presented as mean±SD. *p<0.05, **p<0.01 vs Normal group; #p<0.05 vs CHS group.
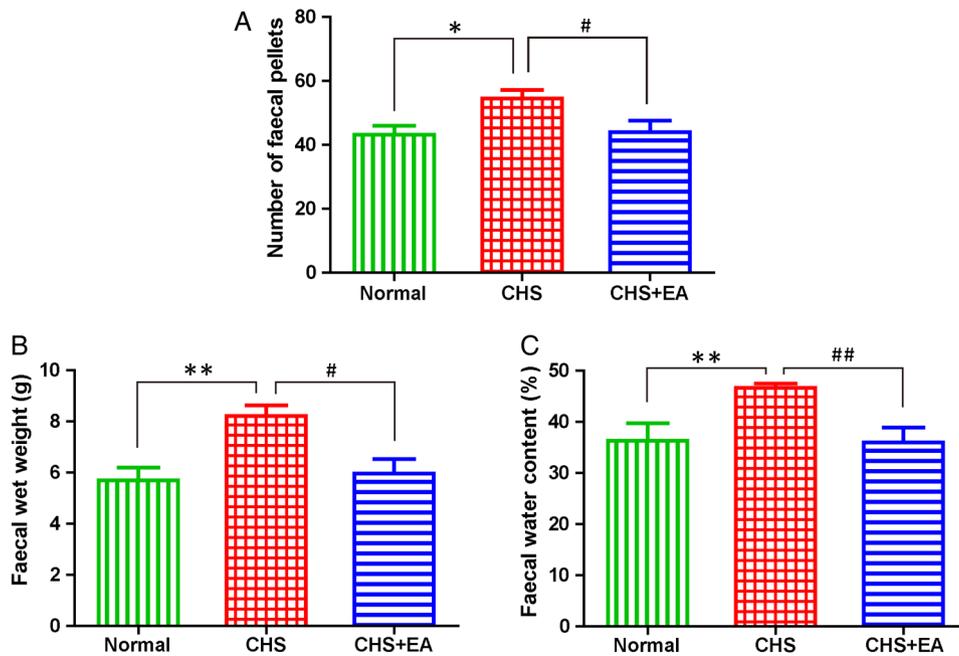
Figure 3 Number of faecal pellets (A), faecal wet weight (B), and faecal water content (C) in six healthy rats (Normal group) and 14 rats exposed to chronic heterotypic stress and left untreated (CHS group, n=7) or treated with electroacupuncture before each stress loading (CHS+EA, n=7). Data are presented as mean±SD. *p<0.05, **p<0.01 vs Normal group; #p<0.05, ##p<0.01 vs CHS group.
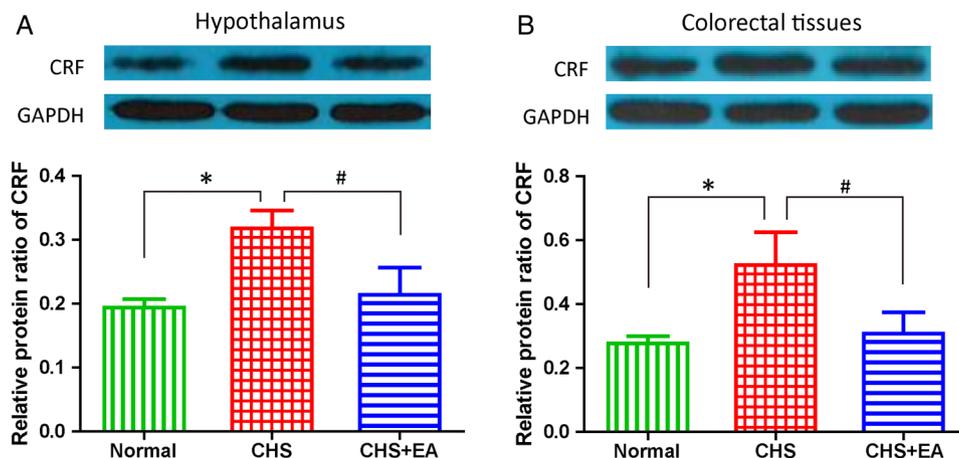
Figure 4 Protein expression of corticotrophin-releasing factor (CRF) in the hypothalamus (A) and colon (B) measured by Western blotting, including representative gels, in six healthy rats (Normal group) and 14 rats exposed to chronic heterotypic stress and left untreated (CHS group, n=7) or treated with electroacupuncture before each stress loading (CHS+EA, n=7). Data are presented as mean±SD. *p<0.05 vs Normal group; #p<0.05 vs CHS group. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

stimulation of colonic motility, and administration of a CRF receptor antagonist has been shown to abolish the effects of exogenous stressors on GI motility.[6] Chronic and acute stress significantly activate CRF-immunoreactive neurons, altere CRF peptide level, and induce CRF mRNA expression in the para-ventricular nucleus (PVN) of the hypothalamus.[3 5] In the present study, CHS significantly activated hypothalamic and colonic CRF expression, suggesting a central and peripheral role of CRF in mediating the stress response.

Acupuncture has historically been used to treat various GI disorders.[20] EA, a particular form of acupuncture where an electrical current passes between the acupuncture needles, was used in the present study because of its standardised stimulation parameters, which means it is more reproducible than manual acupuncture.[21] Previous experimental research has shown the regulatory effect of acupuncture on GI motility. Our previous studies in normal rodents demonstrate that acupuncture at ST36 augments gastric transit,[22] jejunal motility, and colonic movement via a somato-parasympathetic pathway.[23] In contrast, the effect of acupuncture on colonic motility appears to be quite different under stress conditions. For example, electrical stimulation at ST36 attenuates accelerated

colonic transit induced by CHS.[3] EA at ST36 or auricular acupuncture improves GI motility in models of GI disease by elevating 5-hydroxytryptamine (5-HT, serotonin) concentrations in colonic tissues and in the brain.[24–26] In the present study, particular attention was paid to the effect of EA on abnormal defaecation in CHS rats, which has rarely been reported before. The acupuncture point we chose to stimulate was ST36, which is commonly used for the treatment of GI

Figure 5 Hypothetical paradigm illustrating putative mechanisms of action underlying the effect of electroacupuncture (EA) at ST36 on stress-induced abnormal defaecation. Chronic heterotypic stress activates corticotrophin-releasing factor (CRF)-synthesising neurons in the paraventricular nucleus of the hypothalamus, which project to the sacral parasympathetic nucleus of the spinal cord. The parasympathetic efferent fibres innervate the colon and thereby impact colonic motor function, leading to stimulation of colonic transit and defaecation. Somatosensory information from EA stimulation at ST36 is sent to the nucleus tractus solitarius (NTS) via somatic afferent fibres. Meanwhile, visceral signals from the affected colon are conveyed to the NTS through visceral afferent fibres. Inputs from the colon and ST36 are integrated in the NTS, which in turn trigger anti-stress outputs via activation of γ-aminobutyric acid (GABA)-ergic and glutaminergic neurons. Consequently, accelerated colonic transit and excessive defaecation are attenuated due to suppressed CRF signalling.

symptoms in clinical acupuncture practice. After 7 days of continuous EA treatment, the excessive defaecation observed following CHS was significantly attenuated, with a decreased number of faecal pellets and reduced faecal weight.

The colon is innervated by the pelvic nerves, the parasympathetic vagus nerve, and the adrenergic sympathetic nerves from the thoracic and lumbar segments of the spinal cord.[27] The PVN of the hypothalamus, the locus coeruleus, and Barrington's nucleus are believe to be the brain regions where CRF and stress activate colonic motility and transit.[28] CRF-synthesising neurons therein project to the sacral parasympathetic nucleus (SPN) of the spinal cord, which gives rise to efferent nerves that synapse with neurons in the intramural plexi, the smooth muscle of the colon, and the internal anal sphincter.[29] The activation of CRF neurons in the PVN facilitates the autonomic signals and stress responses that influence colonic motility. In normal rodents, EA at ST36 promotes colonic motility via the sacral parasympathetic efferent pathway. In contrast, EA at ST36 inhibits excessive colonic motility in CHS rats via an independent sympathetic mechanism.[11] This may be attributable to the activation of γ-aminobutyric acid (GABA) and glutamate in the dorsal motor nucleus of the vagus, which is adjacent to the nucleus tractus solitarius (NTS).[11] Although not directly investigated in the present experiment, these mechanisms are likely to, at least partially, underlie the effect of EA on disordered defaecation related to abnormal colonic motility.

Meanwhile, the present study indicates that EA contributes to the maintenance of general health, with restored body weight gain and increased weight growth rate during the experiment, such that animals remained in a relatively good condition. Previous studies have shown that oxytocin (OXT) attenuates the activation of HPA activity and CRF expression in the PVN following acute stress and CHS.[1–3] Furthermore, central administration of OXT inhibits the accelerated colonic motility induced by stress exposure. Therefore, OXT is likely to play an important role in the inhibition of stress-induced colonic motility, by suppressing CRF expression and HPA activity.[3] Electrical stimulation at ST36 activates OXT-immunopositive cells and increases OXT expression in the hypothalamus, the central nervous system and the periphery following CHS. Furthermore, several studies reveal that EA can modulate the HPA axis by inhibiting hypothalamic corticotrophin-releasing hormone (CRH) expression, and decreasing serum or plasma concentrations of adrenocorticotrophic hormone (ACTH) and cortisol.[4–6] In the present study, EA at ST36 suppressed over-expression of CRF in the hypothalamus, distal colon, and rectum. Although not measured herein, this may be attributable to the activation of OXT expression in the hypothalamus, as well as the inhibition of HPA activity following EA treatment.

Moreover, the OXT system interacts with GABA/glutamate. OXT in the PVN has been shown to inhibit HPA activity through the recruitment of GABAergic neurons.[7] Central OXT inhibits stimulated CRF induced by stress via GABA receptors in the PVN. The GABAergic system is also involved in the anti-stress response mediated by OXT.[8] Based on previous and present studies, we hypothesise a neural mechanism (illustrated in figure 5) in which CHS activates CRF-synthesising neurons in the PVN, which project to the SPN. Thus, stimulation of the parasympathetic nerves innervating the colon could impact colonic motor function, leading to accelerated colonic transit and stimulation of defaecation. On the other hand, sensory information from EA at ST36 could be transmitted to the NTS via somatic afferents, while visceral signals from the affected colon could be conveyed to the NTS through visceral afferents. Inputs from the colon and muscle tissue at ST36, respectively, might be integrated in the NTS, which in turn may send anti-stress outputs via the activation of GABA neurons. At the same time, the putatively increased OXT level in the PVN induced by EA could attenuate elevated CRF expression, inhibit activity of the HPA, and upregulate GABAergic neurons,[7 8] thereby forming a positive feedback loop augmenting the putative anti-stress effect. Consequently, excessive defaecation could be attenuated by EA via interaction between CRF, OXT, and the HPA and GABAergic systems.

CONCLUSION

In the present study, we investigated the effects of EA on CHS-induced abnormal defaecation and altered general condition, as well as the influence of EA on the expression of CRF hypothalamus and colorectal tissues, in the rat. We found that EA at ST36 significantly increased the body weight growth rate and average daily weight gain otherwise suppressed by CHS, indicating that EA can impact the animals' general condition under stress. Furthermore, EA at ST36 significantly decreased the excessive defaecation induced by CHS, demonstrating that EA can modulate the accelerated colorectal motility that is observed in response to stress. In addition, EA at ST36 significantly inhibited over-expression of CRF in the hypothalamus and colorectal tissues, suggesting that EA can modulate CRF both centrally and peripherally.

REFERENCES

1 Bravo JA, Dinan TG, Cryan JF. Alterations in the central CRF system of two different rat models of comorbid depression and functional gastrointestinal disorders. *Int J Neuropsychopharmacol* 2011;14:666–83.

2 Zheng J, Dobner A, Babygirija R, *et al*. Effects of repeated restraint stress on gastric motility in rats. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R1358–65.

3 Yoshimoto S, Babygirija R, Dobner A, *et al*. Anti-stress effects of transcutaneous electrical nerve stimulation (TENS) on colonic motility in rats. *Dig Dis Sci* 2012;57:1213–21.

4 Suda K, Setoyama H, Nanno M, *et al*. Involvement of parasympathetic pelvic efferent pathway in psychological stress-induced defecation. *World J Gastroenterol* 2013;19:1200–9.

5 Meng QY, Chen XN, Tong DL, *et al*. Stress and glucocorticoids regulated corticotropin releasing factor in rat prefrontal cortex. *Mol Cell Endocrinol* 2011;342:54–63.

6 Tsukamoto K, Nakade Y, Mantyh C, *et al*. Peripherally administered CRF stimulates colonic motility via central CRF receptors and vagal pathways in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R1537–41.

7 Nakade Y, Fukuda H, Iwa M, *et al*. Restraint stress stimulates colonic motility via central corticotropin-releasing factor and peripheral 5-HT3 receptors in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2007;292:G1037–44.

8 Yang ES, Li PW, Nilius B, *et al*. Ancient Chinese medicine and mechanistic evidence of acupuncture physiology. *Pflugers Arch* 2011;462:645–53.

9 Song J, Yin J, Sallam HS, *et al*. Electroacupuncture improves burn-induced impairment in gastric motility mediated via the vagal mechanism in rats. *Neurogastroenterol Motil* 2013;25:807–e635.

10 Zhang N, Song G, Chen J, *et al*. Ameliorating effects and autonomic mechanisms of needle-less transcutaneous electrical stimulation at ST36 on stress-induced impairment in gastric slow waves. *J Gastroenterol Hepatol* 2015;30:1574–81.

11 Iwa M, Nakade Y, Pappas TN, *et al*. Electroacupuncture elicits dual effects: stimulation of delayed gastric emptying and inhibition of accelerated colonic transit induced by restraint stress in rats. *Dig Dis Sci* 2006;51:1493–500.

12 Zhang Z, Wang C, Li Q, *et al*. Electroacupuncture at ST36 accelerates the recovery of gastrointestinal motility after colorectal surgery: a randomised controlled trial. *Acupunct Med* 2014;32:223–6.

13 Abubakar MB, Abdullah WZ, Sulaiman SA, *et al*. The effects of exposure to petrol vapours on growth, haematological parameters and oxidative markers in Sprague-Dawley male rats. *Malays J Med Sci* 2015;22:23–31.

14 Taché Y, Bonaz B. Corticotropin-releasing factor receptors and stress-related alterations of gut motor function. *J Clin Invest* 2007;117:33–40.

15 Keightley PC, Koloski NA, Talley NJ. Pathways in gut-brain communication: evidence for distinct gut-to-brain and brain-to-gut syndromes. *Aust N Z J Psychiatry* 2015;49:207–14.

16 Tan W, Zhou W, Luo HS, *et al*. The inhibitory effect of melatonin on colonic motility disorders induced by water avoidance stress in rats. *Eur Rev Med Pharmacol Sci* 2013;17:3060–7.

17 Taché Y, Million M. Role of corticotropin-releasing factor signaling in stress-related alterations of colonic motility and hyperalgesia. *J Neurogastroenterol Motil* 2015;21:8–24.

18 Zhao J, Jung YH, Jang CG, *et al*. Metabolomic identification of biochemical changes induced by fluoxetine and imipramine in a chronic mild stress mouse model of depression. *Sci Rep* 2015;5:8890.

19 Lee B, Shim I, Lee H, *et al*. Fucoidan prevents depression-like behavior in rats exposed to repeated restraint stress. *J Nat Med* 2013;67:534–44.

20 Takahashi T. Acupuncture for functional gastrointestinal disorders. *J Gastroenterol* 2006;41:408–17.

21 Gao LL, Guo Y, Sha T, *et al*. Differential effects of variable frequencies of manual acupuncture at ST36 in rats with atropine-induced inhibition of gastric motility. *Acupunct Med* 2016;34:33–9.

22 Li YQ, Zhu B, Rong PJ, *et al*. Effective regularity in modulation on gastric motility induced by different acupoint stimulation. *World J Gastroenterol* 2006;12:7642–8.

23 Yuxue Z, Changxiang C, Qingguang Q, *et al*. Effect of manual acupuncture on bowel motility in normal Kunming mouse. *J Tradit Chin Med* 2015;35:227–33.

24 Li H, Wang YP. Effect of auricular acupuncture on gastrointestinal motility and its relationship with vagal activity. *Acupunct Med* 2013;31:57–64.

25 Zhu X, Liu Z, Qu H, *et al*. The effect and mechanism of electroacupuncture at LI11 and ST37 on constipation in a rat model. *Acupunct Med* 2016;34:194–200.

26 Li H, Hu S, Zhang J, *et al*. Effects and mechanisms of auricular electroacupuncture on visceral pain induced by colorectal distension in conscious rats. *Acupunct Med* 2014;32:472–7.

27 Smith ME, Morton DG. *The digestive system: systems of the body*. 2nd edn. Edinburgh: Churchill Livingstone Publishers, 2010:172–3.

28 Kresse AE, Million M, Saperas E, *et al*. Colitis induces CRF expression in hypothalamic magnocellular neurons and blunts CRF gene response to stress in rats. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G1203–13.

29 Reyes BA, Valentino RJ, Xu G, *et al*. Hypothalamic projections to locus coeruleus neurons in rat brain. *Eur J Neurosci* 2005;22:93–106.