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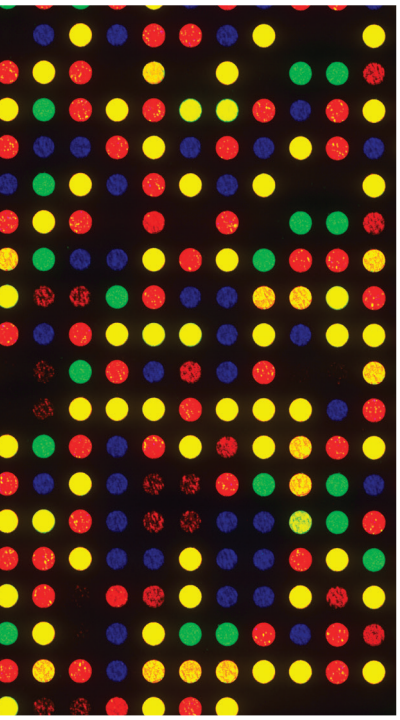
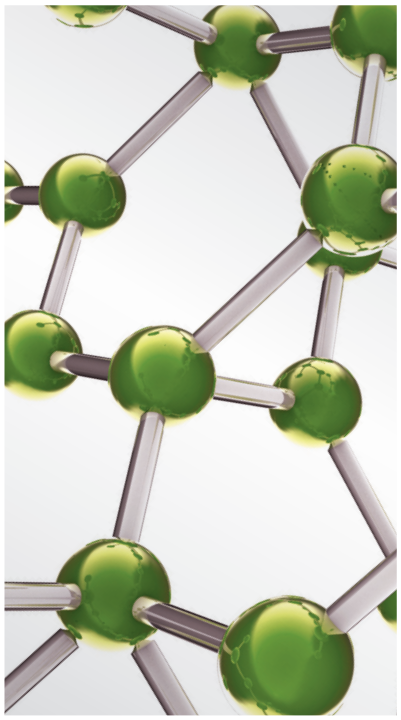
Acupoint Sensitization, Acupuncture Analgesia, Acupuncture on Visceral Functional Disorders, and Its Mechanism

Guest Editors: Xiaochun Yu, Bing Zhu, Zhixiu Lin, Haifa Qiao, Jian Kong, and Xinyan Gao



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Editorial Board

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Editorial

Acupoint Sensitization, Acupuncture Analgesia, Acupuncture on Visceral Functional Disorders, and Its Mechanism

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Our special issue, which had opened for 6 months in the second half of 2014, focused on acupoint sensitization, acupuncture analgesia, acupuncture for visceral modulation in gastrointestinal systems, acupuncture for modulation of brain function, acupoint combination treatment of insomnia and gastrointestinal disorders, and nonspecific and specific effects of acupuncture based on stimulation intensity.

Of these papers in press, S. Chen et al. reported that the location and tenderness of Diji (SP8) were not the same in healthy subjects as in dysmenorrheal patients, suggesting dynamic and sensitivity of acupoints under different pathological status. S. Feng et al. did data mining analysis on acupoints or combinations for treatment of vascular dementia and gave suggestions for acupoint selection based on the most commonly used formulas. L. Dai et al. performed basic research in a sciatic nerve injury rat model and found that deep EA stimulation is better in improving neuromuscular function and benign regulation of apoptosis-related factors than shallow EA. J. Wang et al., based on their previous study that hippocampal mAChR-1 participating in MARK signaling was involved in EA induced cumulative analgesia in neuropathic pain rats, observed in their present study that EA2W was closely related to the cumulative analgesia via intracellular ERK and p38 MARK signaling. P. Rong et al. observed that, in anesthetized rats, EA on ST36-ST37 could enhance nucleus ventralis posterior lateralis thalami neuronal discharges which were fired by CRD-induced visceral pain. Their study indicates that acupoints may be sensitized under visceral disorders. Y. Jin et al. conducted a single blinded, randomized, controlled trial on acupuncture treatment of functional dyspepsia and found that acupuncture manipulation exhibited better effects on improving dyspeptic symptoms, mental status, and quality of life in patients with FD than nonacupoint without manipulation.

In general, we have papers involving clinical trials, data mining analysis or study protocol, and basic research in press, which thoroughly meet the expectation of our initial call for papers of this issue.

Acknowledgments

We thank all authors for their excellent contributions and reviewers for their valuable help. The Lead Guest Editor would like to thank the five Guest Editors for their dedicated cooperation. We hope the special issue will bring readers useful academic reference in their research.

Xiaochun Yu
Bing Zhu
Zhixiu Lin
Haifa Qiao
Jian Kong
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Research Article

Influences of Deqi on Immediate Analgesia Effect of Needling SP6 (Sanyinjiao) in Patients with Primary Dysmenorrhea in Cold and Dampness Stagnation Pattern: Study Protocol for a Randomized Controlled Trial

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Deqi, according to traditional Chinese medicine, is a specific needle sensation during the retention of needles at certain acupoints and is considered to be necessary to produce therapeutic effects from acupuncture. Although some modern researches have showed that Deqi is essential for producing acupuncture analgesia and anesthesia, the data are not enough. It is a paper of a multicenter, randomized controlled study protocol, to evaluate the influences of Deqi on acupuncture SP6 in Cold and Dampness Stagnation pattern primary dysmenorrhea patients, in terms of reducing pain and anxiety, and to find out the relationship between Deqi and the temperature changes at SP6 (Sanyinjiao) and CV4 (Guanyuan). The results of this trial will be helpful to explain the role of Deqi in acupuncture analgesia and may provide a new objective index for measuring Deqi in the future study. This trial is registered with ChiCTR-TRC-13003086.

1. Introduction

Deqi, according to records in both ancient and modern books of traditional Chinese medicine (TCM), is a specific needle sensation which usually occurs during retention of acupuncture needles at certain acupoints, and both patients and acupuncturists can feel the Deqi sensation. It is regarded as a necessary factor for producing effects from acupuncture following traditional Chinese medicine theory. Patients can feel Deqi as multiple sensations at the needling acupoints and along the meridians sometime, such as soreness, numbness, distension, or a minimal muscular contraction around the needle [1–3] and also objective physiological changes, such as the skin temperature changes at the acupoints [4] or the response of brain [5, 6]. For acupuncturists, they can feel a change of the mechanical behavior of the tissues surrounding the needle, such as an increase of the force necessary to pull the needle out of the tissue (pullout force) [7]. Although some modern research showed that Deqi is essential for producing acupuncture analgesia and anesthesia [8], new evidence to confirm the conclusion is still urgently needed [9, 10].

Primary dysmenorrhea (PD) refers to painful menstrual cramps without any evident pathology. It is characterized by crampy suprapubic pain with radiation into the lower quadrants, the lumbar area, and the thighs [11]. PD is a common gynecological complaint and significantly affects study, work, sports, and social activities [12–18]. So far nonsteroidal anti-inflammatory drugs (NSAIDs) or oral contraceptive

pills (OCPs) are widely advocated as standard treatments for women with PD [19, 20]. However, acupuncture as nonpharmacological approaches has great potential value.

Acupuncture is one of the main treatment modalities of TCM. Several trials [21, 22] have already demonstrated the encouraging results of acupuncture as a nonpharmacological option for the treatment of PD. Two systematic reviews also demonstrated the effect [23] and cost-effectiveness [24] of this therapy. The acupoint SP6 (Sanyinjiao) is found to be one of the most commonly used points encountered when searching ancient Chinese medical classics, Chinese acupuncture textbooks, and clinical trials using acupuncture-related therapies for PD [25, 26]. Previous randomized controlled trials have showed that acupuncture at SP6 (Sanyinjiao) can relieve the pain of PD immediately [27–29] especially for PD patients with Cold and Dampness Stagnation pattern [30], which is the most common pattern in PD patients [31, 32]. Langevin's study [7] also showed that, compared with the nonacupoint control group, the average pullout force in SP6 (Sanyinjiao) was greater, which suggested that SP6 (Sanyinjiao) might be especially sensitive in Deqi.

In this study, we will perform a randomized controlled trial using SP6 to investigate the influences of Deqi on immediate analgesia effects in PD patients with Cold and Dampness Stagnation pattern. The primary objective of this trial is to evaluate the influences of Deqi on acupuncture therapy, in terms of pain reduction (measured on a 0–100 mm visual analogue scale for pain (VAS-P)) achieved before and after intervention (i.e., before and after 30 minutes of treatment). The secondary objectives are as follows: (1) to evaluate the influences of anxiety reduction on a 0–100 mm visual analogue scale for anxiety (VAS-A) and (2) to evaluate the influences of temperature changes at SP6 (Sanyinjiao) and CV4 (Guanyuan) acupoints monitor by a digital infrared thermographic imaging device (only in the Dongzhimen Hospital).

2. Methods

2.1. Ethics. The trial protocol is in accordance with the principle of the Declaration of Helsinki [33] and has been approved by the Ethic Committee of Beijing University of Chinese Medicine (Beijing, China, Approval number 2012-040). Each participant will be notified regarding the study protocol. Written informed consent will be obtained from each participant.

2.2. Settings and Participants. A target sample of 96 participants will be recruited in acupuncture clinic from the following four hospitals: Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University, Huguosi Hospital of Traditional Chinese Medicine affiliated to Beijing University of Chinese Medicine, and Hebei Medical University. The trial will be conducted from March 2013 to March 2015.

2.2.1. Sample Size. This is a study without similar references as we know and 40 patients per group is acceptable for calculating sample size for further studies. Assuming a dropout rate of 20%, we will plan to enroll a total of 96 participants with 48 in each group.

2.2.2. Recruitment of Participants. Participants will be recruited from the four hospitals mentioned above in outpatient clinics. Posters will be used outside the acupuncture clinics. The posters will contain brief introductions about the inclusion/exclusion criteria, the free acupuncture treatments offered to eligible participants, and the contact information of the researchers.

2.2.3. Inclusion Criteria. Participants who meet all the following requirements will be allowed for enrollment:

(1) nulliparous women, aged between 18 and 30 years old, diagnosed with PD (according to the criteria of the Primary Dysmenorrhea Consensus Guideline [34]), and in Cold and Dampness Stagnation pattern (based on a revised Chinese national guideline [30]),

(2) moderate to severe primary dysmenorrhea (PD) (≥40 mm on VAS-P) for three consecutive menstrual cycles,

(3) duration of PD (self-reported pain) varying from 6 months to 15 years,

(4) written informed consent.

2.2.4. Exclusion Criteria. Exclusion criteria are as follows:

(1) secondary dysmenorrhea (e.g., endometriosis and fibroids),

(2) irregular/infrequent menstrual cycles (i.e., beyond the typical range of 21- to 35-day cycle),

(3) complication with severe diseases (e.g., cerebral, liver, kidney, or hematopoietic system diseases), mental defects, or asthma,

(4) pregnancy,

(5) use of analgesic medication in 24 hours before treatment,

(6) having a professional acupuncture background,

(7) having potentially poor treatment compliance (e.g., unstable working and living situation or difficulty in following up).

2.3. Randomization and Blinding. The central randomization will be performed by the Center for Evidence-Based Chinese Medicine affiliated to Beijing University of Chinese Medicine in China, using complete randomization to generate the random allocation sequence. Once a participant is included, the researcher will contact the randomization center for the group allocation of the participant, and the acupuncturist will be informed by telephone. Participants will be allocated at a 1 : 1 ratio (Figure 1).

The participants will be informed that they will have a 50% chance of being allocated in either of the two acupuncture groups, and both groups will be potentially effective;

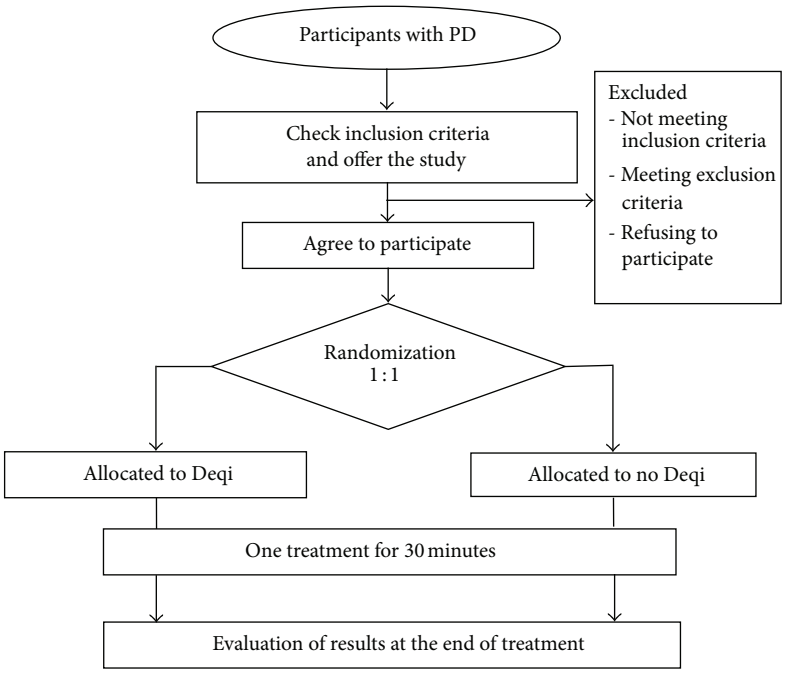

Figure 1: Flow diagram for the study. Work scheme with description of assessment visits and times.

hence, participants will be blinded to the group allocation. The patients will also be informed of the possible risks associated with acupuncture (hematoma or fainting). The acupuncturist cannot be blinded due to the specific nature of intervention. Outcome assessors and personnel who will deal with data collection and data analysis will be blinded throughout the entire trial.

2.4. Interventions. All participants, when their VAS-P score of menstrual pain is equal to or more than 40 mm on the first day of menstruation, will each receive an acupuncture treatment for 30 minutes at bilateral SP6 acupoints. The SP6 lies on the tibial aspect of the leg, is posterior to the medial border of the tibia, and is 3 B-cun (proportional bone cun) superior to the prominence of the medial malleolus [35].

(a) Deqi Group. The treatment will be performed after sterilizing the skin on the areas where the needles will be inserted, with participants lying face up. Using single-use sterile needles (Zhongyan Taihe, Wuxi Jiajian Medical Instrument Co. Ltd., Jiangsu, China) of 0.30 mm calibre and 40 mm length, a needle will be vertically inserted 1 cun in depth and will be manipulated by lifting-thrusting and twirling methods for 30 seconds to achieve Deqi.

(b) No Deqi Group. Single-use sterile needles (Zhongyan Taihe, Wuxi Jiajian Medical Instrument Co. Ltd., Jiangsu, China) of 0.18 mm calibre and 13 mm length will be used. The treatment will be performed after sterilizing the skin on the areas where the needles will be inserted, with participants lying face up. A needle will be vertically inserted 0.1 cun in depth. No manipulation will be performed after insertion of needles to avoid Deqi.

2.5. Outcome Measures

2.5.1. Primary Outcome Measures. The changes of pain will be measured by visual analogue scale for pain (VAS-P), before and after the treatment. The validity and reliability of the VAS-P scale have been proven [36–38] and it was employed in our previous similar studies [29, 30]. The scale measures a continuous quantitative variable varying from 0 mm (no pain) to 100 mm (worst pain ever).

2.5.2. Secondary Outcome Measures

(1) The changes of the anxiety are measured by visual analogue scale for anxiety (VAS-A), before and after the treatment. This scale has been used in a PD study [39]. The scale measures a continuous quantitative variable varying from 0 mm (no anxiety) to 100 mm (almost death).

(2) The changes of temperature at SP6 (Sanyinjiao) and CV4 (Guanyuan) acupoints (located at the lower abdomen, 3 B-cun inferior to the centre of the umbilicus, on the anterior median line [35], e.g., in the skin area corresponding to the uterus) are measured by a digital infrared thermographic imaging device (USA, FLIR Systems Inc., SC640) before and after treatment. It is demonstrated that acupuncture can affect skin temperature at the needling acupoint, and it is feasible to detect the changes of the skin temperature by using a thermographic camera at a certain acupoint during acupuncture treatment [40].

(3) Adverse events: the possible side effects and adverse reactions during the treatment will be recorded in the case report form (CRF).

Table 1: Data collection at different assessment points. Example of outcome measurements.

Variable	T0	T1
VAS-P	X	X
VAS-A	X	X
ADCAS		X
Temperature at SP6, CV4	X	X
Side effects and adverse reactions		X
Sociodemographic data	X	

Note: VAS-P: visual analogue scale for pain (0–100); VAS-A: visual analogue scale for anxiety (0–100); ADCAS: Acupuncture Deqi Clinical Assessment Scale; SP6: Sanyinjiao; CV4: Guanyuan; T0: before acupuncture treatment; T1: after acupuncture treatment.

2.6. Data Collection. The data required for evaluating the influences of Deqi on acupuncture therapy will be collected at baseline and the completion of intervention. Data will be obtained via physical measurements. Data collection instruments and the study timeline are summarized in Table 1.

A case report form (CRF) has been designed, to include the variables of interest, which will be completed by the corresponding researcher at each research center. After the trial finished, the information obtained will be input to an electronic database, for subsequent statistical analysis.

2.7. Data Storage and Confidentiality. All CRFs will be stored in a locked cabinet at a study office in School of Acupuncture, Moxibustion and Tuina, Beijing University of Chinese Medicine, and will have a unique identification number. Data will be input to an electronic database, and the access to the database is restricted to the study team.

2.8. Statistical Analysis. All analyses will be performed using SPSS 17.0 (SPSS Inc., Chicago, IL). Potential differences across study groups on demographic and clinical history variables will be compared by means of analysis of covariance (ANCOVA). Repeated-measures analysis of variance (ANOVA) will be utilized for the analysis of VAS-P and VAS-A scores and temperatures at SP6 (Sanyinjiao) as well as CV4 (Guanyuan) acupoint. $P < 0.05$ will be denoted as significant.

Although most participants of the Deqi group will experience Deqi, there will probably be some exceptions that participants in the group will not experience Deqi. Therefore, we will redivide all participants into the real Deqi group and unreal Deqi group according to a self-designed Acupuncture Deqi Clinical Assessment Scale (ADCAS) grade after treatment. We will perform a secondary analysis with similar statistical method to explore whether the real Deqi has influences on the effect of acupuncture. We will compare the first and second analysis results.

3. Discussion

The trial is designed to illustrate Deqi effect on acupuncture treatment. It will estimate Deqi state of solo acupoint and compare analgesic right after needling. All participants will be randomly divided into Deqi or no Deqi group. Unlike other impact factors of acupuncture treatment, Deqi is a group of specific subjective sensations which can only be felt during acupuncture by both patients and acupuncturists. It is impossible to randomly allocate participants into a Deqi or no Deqi group, so there have been no Deqi randomized controlled trials (RCTs). However, whether Deqi can be achieved during acupuncture can be predicted to a certain extent; therefore, we will randomly divide all participants into Deqi or no Deqi group. We also employ manipulation technique to promote Deqi in the Deqi group, while in the no Deqi group we avoid Deqi according to TCM theory and experts' experience.

In this study, we will use an important self-designed instrument—Acupuncture Deqi Clinical Assessment Scale (ADCAS). The scale shows the intensity of participants' sensation, ranging from 0 to 4: 0 (no), 1 (slight), 2 (mild), 3 (obvious), and 4 (strong). It will be firstly used as an instrument for redividing all the included participants in reanalysis. Actually, some researchers and their colleagues have attempted to qualify and quantify sensations of Deqi [41–47] and designed several Deqi scales or questionnaires like the Vincent scale, the Park questionnaire, the MGH Acupuncture Sensation Scale (MASS), and Southampton Needling Sensation Questionnaire (SNSQ). Some of the scales or questionnaires have been used in Deqi trials [48–50] and MASS even has a Chinese edition [51]. However, though Deqi sensation can be felt by patients and acupuncturists, all the current scales take no consideration of the Deqi sensation felt by acupuncturists' hands, which decreases the reliability of those scales.

Additionally, all the previous scales or questionnaires derived from a pain questionnaire—McGill Pain Questionnaire (MPQ) [52], which are not based on TCM theory. Thus, we designed this new Deqi questionnaire based on the previous instruments and especially followed TCM theory and rigorous methodology, such as selecting the items included in the Deqi questionnaire and testing reliability and validity. In fact, the reliability and validity of ADCAS were evaluated by a study with 73 PD patients needled on SP6, in which the disease and selected acupoint were the same as in this study, and it had a good result (the paper will be published later).

As a subjective sensation, Deqi has no objective measure instrument by far. This trial will explore the possibility of acupoint temperature changing as a sign of Deqi and will be devoted to the provision of a tool to determine Deqi.

This trial, in accordance with the STRICTA [53] and good clinical practice guidelines (GCP), will be helpful in explaining the role of Deqi in acupuncture analgesia, provide new objective evidence for Deqi, and improve the understanding of complicated mechanisms of acupuncture in practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yu-qi Liu and Peng Zhang as well as their affiliations contributed equally to this work.

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Research Article

Acupuncture for Functional Dyspepsia: A Single Blinded, Randomized, Controlled Trial

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In order to investigate the therapeutic potential of acupuncture on patients with functional dyspepsia (FD), patients were randomized to receive acupuncture at classic acupoints with manipulations (treatment group) versus acupuncture at nonacupoints without manipulation (control group) once every other day, three times a week, for one month and were followed up for three months. The primary outcomes included dyspeptic symptoms, quality of life, and mental status. The secondary outcomes included the fasting serum gastrin concentration, and frequency and propagation velocity of gastric slow waves. Sixty patients with FD were included, among whom, four dropped out. After one month's treatment, patients with FD showed significant improvements in primary (in both groups) and secondary (in the eight patients of the treatment group) outcomes as compared with baseline ($P = 0.0078$ to <0.0001); treatment group has better outcomes in all primary outcome measures ($P < 0.0001$ except for SDS ($P = 0.0005$)). Improvements on dyspeptic symptoms persist during follow-up (better in the treatment group). Acupuncture with manual manipulation had better effects on improving dyspeptic symptoms, mental status, and quality of life in patients with FD. These effects may be related to the increased frequency and propagation speed of gastric slow waves and serum gastrin secretion.

1. Introduction

Functional dyspepsia (FD) is dyspepsia without evidence of an organic disease that is likely to explain the cause [1]. Based on the Rome III criteria, symptoms of FD may include bloating, belching, early satiety, abdominal distension, nausea, or indigestion during the last three months with symptom onset at least six month ago. These symptoms are categorized into epigastric pain syndrome and postprandial distress syndrome [2]. Pathophysiological factors which may cause FD include genetic predispositions, early family environment, psychosocial factors, abnormal gastric motility, visceral hypersensitivity, inflammation, and bacterial flora [3]. Particularly, gastrointestinal motor abnormalities, altered visceral sensation, and psychosocial factors have all been identified as major pathophysiological changes in FD [3, 4]. The prevalence of FD varies between 11% and 29.2% [5]. In the United States, FD was found in 29.2% of the population, and in the United Kingdom the prevalence was 23.8% [6, 7].

FD greatly decreases patients' quality of life as the symptoms, particularly abdominal pain and indigestion, cause emotional distress, problems with food and drink, and impaired vitality [8]. Patients with FD usually require extensive diagnostic procedures and long-term medical care which in turn place heavy economic burden on patients and the society. Management of FD mainly includes lifestyle modification, *H. Pylori* treatment, acid suppression therapy, prokinetics, antidepressants, and antiflatulents. Despite these

treatment options, treatment for FD often remains unsatisfactory [9]. The management of FD is challenging especially when initial drug therapy fails, which is not uncommon [10]. Furthermore, besides side effects, traditional drug therapy has been strikingly shown to have little to no efficacy [11]. For example, benefits from *H. Pylori* treatment were found to be minimal [12]; acid suppression therapy was found to be suboptimal with no apparent effects on dysmotility-like dyspepsia [13].

From a traditional Chinese medicine (TCM) perspective, FD is characterized by disrupted qi flow inside the middle energizer due to external pathogenic factors [14]. The middle energizer refers to spleen and stomach which are responsible for food transformation and transportation. Kidney is responsible for bone health and the generation of bone marrow; excessive physical work consumes kidney energy. Meanwhile, excessive mental work consumes blood and causes imbalance of emotion. Blood is controlled by heart and emotion is regulated by liver. Thus, treatment in TCM including acupuncture should aim to facilitate qi and blood circulation in meridians related to these organs and thus normalizes patient's status of health. With the guidance of these diagnostic and therapeutic principles, therapeutic effectiveness of acupuncture for abdominal pain, abdominal distension, bloating, nausea, and others was well documented in various TCM classics and has been reported in research studies [15–20].

Besides normalization of qi and blood in the affected meridians, modern understandings of these results also lie in pathophysiological research studies, in which researchers found that acupuncture in patients with FD could accelerate solid gastric emptying [17], increase plasma level of neuropeptide Y but not motilin [18], and induce deactivation of the brainstem, anterior cingulate cortex (ACC), insula, thalamus, and hypothalamus in the human body [21]. In addition, acupuncture was also found to enhance normal gastric myoelectrical regularity in both healthy people and patients with diabetic gastric dysrhythmia [22, 23], alters the frequency of gastric slow waves in healthy volunteers [24], and accelerates solid gastric emptying in diabetic gastroparesis [25].

Acupuncture seems to be a promising treatment for FD; however, the aforementioned clinical trials did not investigate the effects of acupuncture on emotional symptoms [15–25], the prevalence of which has been found to be high in patients with FD [26]; placebo effect which is common in both patients with FD and acupuncture procedures will likely add more uncertainties in the therapeutic effectiveness of acupuncture [27, 28], and, finally, not all of the studies performed acupuncture procedures based on TCM principles including the meridian theories, such as an emphasis on Deqi sensations.

In the present study, we aimed to determine (i) the effect of acupuncture on dyspeptic symptoms, quality of life, and mental status in patients with FD; (ii) the effect difference between classic acupuncture based on TCM principles and acupuncture on nonacupoints; and (iii) effects of classic acupuncture on serum gastrin concentration and frequency and propagation velocity of gastric slow waves.

2. Material and Methods

2.1. Study Design and Setting. This was a single blinded, randomized, controlled trial of manual acupuncture on classic acupoints versus nonclassic acupoints performed at the Department of Acupuncture at Guang An Men Hospital, one of the top teaching hospitals for TCM education in China. Hospital ethics committee approved the study protocol. Participants were recruited through advertisements on local newspapers, posters, and hospital website and signed informed consent before study participation.

An investigator who was not involved in acupuncture procedures and data analyses was responsible for the generation of a random number table, based on which, participants were allocated to receive either classic acupoint (treatment group) or nonclassic acupoint (control group) acupuncture treatments. Participants were blinded to acupuncture procedures.

2.2. Participants. For inclusion, patients have to fulfill the Rome III diagnostic criteria for FD. For the last three months with symptom onset at least six months ago, patient has one or more of the following: (1) bothersome postprandial fullness; (2) early satiation; (3) epigastric pain; (4) epigastric burning; and (5) no evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms. In addition, the following criteria were also met: no mental disorders; would otherwise be healthy; age 18 to 70; nonpregnant; and one week prior to and during participation, cessation of all medication related to the gastrointestinal system, which may include but not be limited to gastric suppression drugs, prokinetics, *H. Pylori* eradication agents, and antidepressants.

Eight patients in the treatment group also signed in for assessment of gastrin concentration and frequency and propagation velocity of gastric slow waves. To measure the differences between patients with FD and healthy adults in these assessments, eight healthy volunteers were also included to match with these eight patients with FD in the present study.

2.3. Treatment Protocol. All patients included were randomized into two groups: classic acupoint (treatment) or nonacupoint (control) groups. For the treatment group, acupoints ST36 and KI3 were used in every group members; additional acupoints of GB4, PC6, and HT7 may also be used based on pattern recognition of symptoms. Based on TCM theories, ST36 was used to invigorate functions of the stomach and spleen; KI3, the Yuan-source acupoint, was used to invigorate functions of the kidney. ST36 and KI3 function together to restore the normal qi flow inside the stomach and spleen meridian. In addition, for patients with obvious depression, anxiety, or insomnia symptoms, GB41 was used to restore liver function, and PC6 and HT7 were used to nourish the heart to resume balance of the mind. Classic acupoints were localized according to the 2008 World Health Organization standards [29]. For the treatment group, needle insertion was perpendicular with a depth of about 25 mm. In order to

reach an optimal response which is defined as Deqi sensations including soreness, heaviness, fullness, propagation of needling sensation, and/or adjacent muscle twitching [30], moderate combined acupuncture manipulation of lifting, thrusting, and twirling with a frequency of 60–120 times/min was performed. These acupuncture manipulation techniques were performed continually to reach one to three times of Deqi sensation (with a short interval between Deqi sensations if more than once during the first two minutes); then, the needle was removed. If no Deqi sensation was obtained during the first two minutes, acupuncture needle was then left in place for 20 to 60 minutes, and one acupuncture manipulation was applied right before needle removal regardless of Deqi sensation.

For the control group, nonclassic acupoints in different dermatomes but close proximity of the aforementioned acupoints were used in the distal portion of extremities correspondingly. KI3, ST36, and GB41 are located in the L4, 5, and S1 dermatome; thus nonclassic acupoints located inside anterior thigh (L2 and L3 dermatome) were used. PC6 and HT7 are located inside the C7, 8, and T1 dermatome; thus nonclassic acupoint in the anterior antebrachium (C5 dermatome) was used. In the control group, needle insertion was perpendicular with a depth of two to three millimeters with needle retention of 20 minutes but no acupuncture manipulations.

Treatments in both groups were implemented once every other day, three to four times a week for one month. All patients were then followed up for three months. All acupuncture procedures were performed by the same acupuncturist who had more than six years' clinical experiences. Huatuo brand needles (Φ 0.35 mm × 25 mm, manufactured by Suzhou Medical Appliance manufactory, Jiangsu, China) were used for all acupuncture procedures.

2.4. Outcome Assessment. The primary outcomes of the study included dyspeptic symptoms, quality of life, and mental status. For dyspeptic symptoms, we used the four cardinal dyspeptic symptoms and their corresponding assessments as reported in the Chinese version Nepean Dyspepsia Index (NDI) [31, 32]. The intensity, frequency, and level of interference of postprandial fullness, early satiety, epigastric pain, and epigastric burning sensation were rated. Intensity of each symptom was graded and scored as the following: 0, absent; 1, mild; 2, moderate; 3, severe; 4, critical. Frequency of each symptom was also graded as follows: 0, absent; 1, occasionally (1-2 days/week); 2, sometimes (3–5 days/week); 3, frequently (every day, but intermittent symptoms), 4, continuous symptoms. Level of interference of each symptom was scored and graded as the following: 0, none; 1, mild interference; 2, moderate interference; 3, severe interference; 4, critic interference. The number in front of each grading indicates the score of the corresponding symptom; the score for each symptom in the checklist of cardinal dyspeptic symptoms was calculated by adding its scores in the corresponding frequency, severity, and level of discomfort; dyspeptic symptom sum score (DSSS) is the sum score of the four symptoms in the checklist.

Quality of life was measured by the short-form 36 (SF-36) questionnaires [33]. Mental statuses of patients were evaluated via Zung Self-Rating Depression Scale (SDS) [34] and Self-Rating Anxiety Scale (SAS) [35]. Scoring of these standardized assessments followed guidelines published in the Manual of Standardized Assessment Tools in Behavioral Medicine [36]. SF-36 measures Quality of Life (QoL) across eight domains; score of each domain = [(actual raw score − lowest possible raw score)/raw score range] × 100. For the SDS score, the following equation was used: SDS Index = Raw Score × 1.25. Grading of SDS is as the following: SDS Index less than 53 points is considered normal, 53 to 62 as mild depression, 63 to 72 as moderate depression, and 73 and higher as severe depression [36]. For the grading of SAS, the following categories were used: normal range (less than 50), mild anxiety (50 to 59), moderate anxiety (60 to 69), and severe anxiety (70 and higher) [36].

The secondary outcomes include fasting serum gastrin concentration and frequency and propagation velocity of gastric slow waves. These measurements were performed in the eight patients with FD in the treatment group before and after treatment, but only once in healthy volunteers. A fasting venous blood sample was drawn from the basilic vein prior to breakfast early in the morning. About three milliliters of the blood sample was sent to Peking Union Medical College Hospital for measurement of serum gastrin levels. Meanwhile, the participant was given 120 mL 80% (w/v) barium sulfate suspension (Qingdao Dongfeng Chemical Co. Ltd., Shandong, China). Participants were then placed in a supine position. Using Prestige digital X ray (GE, USA), gastric mucosa was observed; then, a Chinese coin of fifty cents was placed on top of the skin over the stomach of the participant, and gastric motions around the gastric antrum were video recorded for one minute while the participant was in a standing position. Frequency of gastric slow waves was directly counted as the number of waves that passed through the gastric antrum in one minute. Propagation velocity of gastric slow waves was assessed by the time interval between two consecutive waves that passed through the gastric antrum.

Safety evaluation includes possible hematoma, local infection, fainting, and severe pain during and after acupuncture. In addition, other conditions which warrant cessation of acupuncture treatment or withdrawal from the study if any were also documented and analyzed.

2.5. Statistical Analysis. The statistical analysis was performed by two independent statisticians. Results were compared between the two statisticians. Differences, if any, were discussed and the statistic test was reperformed until a consensus was reached between the two statisticians. The statisticians were blinded to treatments and study protocol. All results including baseline characteristics were based on per-protocol (PP) analyses. Statistical Analysis System (SAS), version 6.12, was used and a significance level was set at $P < 0.05$.

For comparisons of baseline values, chi square test was used to explore gender differences; t-test was used to explore

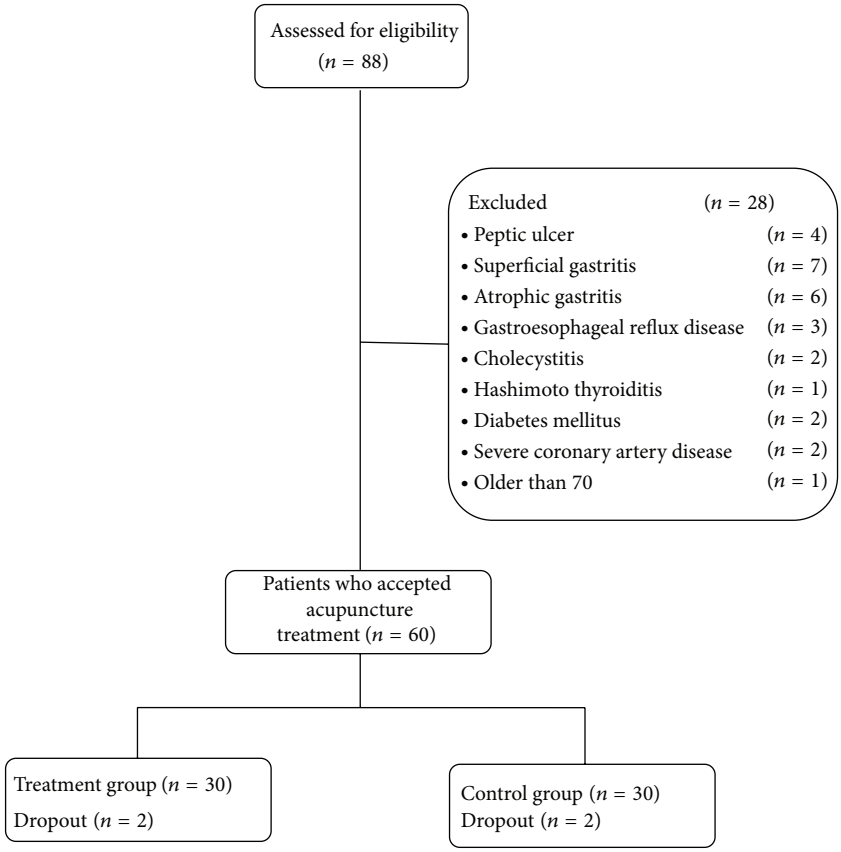

Figure 1: Flow chart of study participation.

differences in the duration of the disease; Wilcoxon rank sum test was used in all comparisons of primary and secondary outcome measures. All quantitative data including subjective scores were expressed with mean ± SD.

3. Results

From July, 2010, to January, 2011, a total of 88 patients with dyspeptic symptoms visited the Department of Acupuncture at Guang An Men Hospital in Beijing. Twenty-eight patients were excluded from the present study due to the following reasons: peptic ulcer (four patients), superficial gastritis (seven patients), atrophic gastritis (six patients), gastroesophageal reflux disease (three patients), cholecystitis (two patients), Hashimoto thyroiditis (one patient), diabetes mellitus (two patients), severe coronary artery disease (two patients), and older than 70 (one patient). Sixty patients were included and randomly assigned to either the treatment group or the control group. Of these 60 patients, 56 patients completed the study and four patients (two from each group) dropped out from the study (dropout rate: 6.67%) after the second visit. In the treatment group, one patient could not tolerate the acupuncture Deqi sensations upon needle manipulation, and the other patient in the treatment group had transportation difficulties. In the control group, the two patients directly stated to the therapist saying that the treatment was noneffective and withdrew from participation (Figure 1).

The treatment group consists of 11 males and 17 females with an age range between 23 and 65 years old and disease history of one to 40 years. The control group consists of 10 males and 18 females with an age range between 24 and 66 years old and disease history of one to 40 years. Prior to participation, no significant differences were found between these two groups in terms of gender, age, length of disease history, dyspeptic symptom sum scores, and SF-36 score (Table 1).

3.1. Primary Outcomes. At baseline, the prevalence of the four symptoms of postprandial fullness, early satiety, epigastric pain, and epigastric burning sensation in these 56 patients were 98.2%, 71.4%, 76.8%, and 58.9%, respectively; the scores for each symptom were six to nine points with a severity of disease rated moderate to severe.

After one month's treatment, as compared with baseline values, significant differences were found in both treatment and control groups in the dyspeptic symptom sum score, the scores of postprandial fullness, early satiety and epigastric pain, SDS score, and SF-36 score. Additionally, as compared with baseline, significant differences were also found in the score of epigastric burning sensation and SAS score of the treatment group but not the control group. P values were

Table 1: General characteristics of patients with FD prior to participation.

Groups	Cases (n)	Gender		Age (year)	Disease duration (years)	Dyspeptic symptom sum score	SF-36 score
		Male (n)	Female (n)				
Treatment	28	11	17	49.29 ± 10.32	12.20 ± 12.20	24.32 ± 8.28	52.51 ± 13.94
Control	28	10	18	48.25 ± 11.40	12.11 ± 10.20	24.79 ± 7.48	54.06 ± 16.41
P value		0.7825		0.7229	0.6145	0.8265	0.7043

<0.0001 for all the significant intragroup comparisons except epigastric pain, SF-36, and SDS in the control group, for which P values were 0.0078, 0.0099, and 0.0002, respectively. As compared with the control group, treatment group has better outcomes in all primary outcome measures. P values for these intergroup comparisons were all <0.0001 except for SDS ($P = 0.0005$) (Table 2).

At three months' follow-up, DSSS was recalculated for all participants. As compared with baseline values, significant differences were found in both groups in terms of DSSS ($P < 0.0001$). Meanwhile, the treatment group, as compared with the control group, had better long-term outcomes in terms of DSSS ($P < 0.0001$) (Table 3).

3.2. Secondary Outcomes. Values of preprandial serum gastrin concentration and frequency and propagation velocity of gastric slow waves in healthy volunteers and patients with FD were provided in Table 4. As compared with healthy volunteers, patients with FD had lower serum gastrin concentration and less frequent and slower propagation velocity of gastric slow waves ($P = 0.0081$, 0.0008, 0.0279, resp.) at baseline. After one month's treatment, patients with FD showed significant improvement in serum gastrin concentration and frequency and propagation velocity of gastric slow waves ($P = 0.0002$, 0.0078, and 0.0180, resp.), and no significant difference was found in these secondary outcome measures between healthy volunteers and patients with FD (Table 5).

3.3. Side Effects. No serious side effects occurred. One patient in the treatment group withdrew from the study secondarily to intolerance to the needling sensations upon acupuncture manipulation.

4. Discussion

4.1. Selection of Acupoints. The use of classic acupoint of ST36 in the present study is well-supported by former research studies [15–25], so was the use of PC6 [15–19, 24]. In previous research studies, researchers mainly considered the pathophysiological relationship between the meridians or organs of liver and spleen, heart and spleen, or spleen and kidney; acupoint of the kidney meridian is barely used for FD in these research studies [15–25]. In the present study, we used KI3 based on the analysis of all the pathophysiological relationships between and among organs and meridians related to FD symptoms. These diagnostic and therapeutic principles would be a more realistic reflection of individualized acupuncture treatment in clinical practice. The results of the present study add further credence to the use of these acupoints.

4.2. Acupuncture Manipulations. Acupuncture Deqi serves as the foundation or premise for the therapeutic effects of acupuncture treatment [30]. Although theoretical research articles highlight the importance and the components of Deqi, not many researchers emphasized Deqi in their reports of acupuncture clinical trials. The reason for lacking of information regarding Deqi may be due to the following reasons: the authors of the reports did not document it and the clinicians did not pay extra attention to the importance of Deqi during the studies. In addition, as electroacupuncture becomes more and more popular, the evaluation of Deqi is more difficult due to the mixture of electric therapy sensation with sensations from acupuncture itself. Nonetheless, report of Deqi in clinical trials reflects more the standard acupuncture treatment in clinical practice. In the present study, manual acupuncture manipulation was stopped and the needle was removed upon Deqi arrivals in the treatment group. This acupuncture treatment protocol guarantees not only the Deqi sensations thus the clinical efficacy but also safety of acupuncture treatments. The results of the present study indicate that traditional acupuncture with the emphasis of Deqi manipulations has better therapeutic results than acupuncture on nonacupoints without Deqi manipulations.

4.3. Outcome Measurement. As psychosocial factor is a common cause of FD and many patients of FD have anxiety or depression issues, measurements of these psychological symptoms are of great importance in the evaluation of clinical management of FD [1, 26]. Zung Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS) have a high reliability and validity in assessing psychological symptoms in patients [34, 35]. The improvement of SDS score and SAS score in the treatment group of the present study indicates that acupuncture has positive impacts on the psychological aspects of patients with FD. Psychological effects of acupuncture may be caused by placebo effects [28, 37]; however, as acupuncture also demonstrated therapeutic effects on psychological diseases [38, 39], we should increase our trust on the positive benefits of acupuncture on psychological symptoms of patients. To our best knowledge, no studies have explored the effects of acupuncture on psychological symptoms of FD. Thus, the present study will facilitate our understanding of the therapeutic effectiveness of acupuncture in FD.

Table 2: Scores of dyspeptic symptoms, quality of life, and mental status before and after the treatment.

Items	Groups	*N*	Baseline	After treatment	Difference	Improvement rate	*P* value
PF	Treatment	28	9.00 ± 2.09	1.57 ± 2.28	7.43 ± 2.47	82.56%	<0.0001
	Control	27	8.89 ± 2.39	6.22 ± 2.59	2.67 ± 1.88	30.03%	<0.0001
	IGC						<0.0001
ES	Treatment	19	9.74 ± 1.91	0.42 ± 1.43	9.32 ± 1.97	95.69%	<0.0001
	Control	21	8.43 ± 2.87	6.05 ± 2.52	2.38 ± 1.80	28.23%	<0.0001
	IGC						<0.0001
EP	Treatment	21	6.81 ± 2.23	0.48 ± 1.03	6.33 ± 2.31	92.95%	<0.0001
	Control	22	7.41 ± 3.02	6.32 ± 3.41	1.09 ± 1.82	14.71%	0.0078
	IGC						<0.0001
EBS	Treatment	16	6.31 ± 2.39	0.50 ± 1.55	5.81 ± 2.17	92.08%	<0.0001
	Control	17	6.71 ± 2.78	6.47 ± 3.00	0.24 ± 0.56	3.58%	0.25
	IGC						<0.0001
DSSS	Treatment	28	24.32 ± 8.28	2.50 ± 3.28	21.80 ± 8.24	89.72%	<0.0001
	Control	28	24.79 ± 7.48	19.40 ± 8.23	5.36 ± 3.29	21.62%	<0.0001
	IGC						<0.0001
SF-36	Treatment	28	52.50 ± 13.94	70.00 ± 12.54	17.00 ± 14.04	33.52%	<0.0001
	Control	28	54.00 ± 16.41	56.00 ± 13.42	2.88 ± 8.74	5.33%	0.0099
	IGC						<0.0001
SDS	Treatment	28	57.96 ± 9.55	45.60 ± 8.75	12.30 ± 9.89	21.33%	<0.0001
	Control	28	57.60 ± 11.84	54.00 ± 10.80	3.50 ± 5.92	6.07%	0.0002
	IGC						0.0005
SAS	Treatment	28	52.30 ± 10.48	42.30 ± 6.22	10.00 ± 10.22	19.11%	<0.0001
	Control	28	52.36 ± 9.67	52.20 ± 7.98	0.11 ± 4.89	0.21%	0.8533
	IGC						<0.0001

PF: postprandial discomfort; ES: early satiety; EP: epigastric pain; EBS: epigastric burning sensation; DSSS: dyspeptic symptom sum score; SF-36: short-form 36 questionnaire; SDS: Self-Rating Depression Scale; SAS: Self-Rating Anxiety Scale; IGC: intergroup comparison.

Table 3: Dyspeptic symptom sum score at baseline and during follow-up.

IND	Groups	*N*	Baseline	Follow-up	Difference	Improvement	*P* value
DSSS	Treatment	28	24.32 ± 8.28	1.68 ± 2.36	22.60 ± 8.68	93.09%	<0.0001
	Control	28	24.79 ± 7.48	16.43 ± 7.41	8.36 ± 6.58	33.92%	<0.0001
	IGC						<0.0001

DSSS: dyspeptic symptom sum score; IGC: intergroup comparison.

Quality of life is a heavy emphasis of the clinical management of all kinds of disorders. In the present study, the use of modified NDI is well-supported by its high reliability and validity in patients with dyspeptic symptoms [31, 32]. NDI measures dyspepsia symptoms and dyspepsia-specific health-related QOL (H-QOL). Outcome measurements utilizing NDI, SAS, and SDS will likely better capture the characteristics of acupuncture effects on FD. The improvement of NDI in the present study concurs with results from other acupuncture researchers regarding acupuncture treatment for FD [15–21]. Interestingly, the control group in which acupuncture was used in nonclassic acupoints also induced significant changes in dyspeptic symptoms except for epigastric burning sensations and SAS score. These results partially concur with the results reported by Ma et al. [20] and Zeng et al. [21]; however, the results differ from the results reported by Park et al. [19]. Significant superiority of classic acupoint acupuncture to nonclassic acupoint acupuncture was found in both studies by Ma et al. [20] and Zeng et al. [21]; however, they did not report changes of subcategories of NDI in both groups which makes the analysis difficult. Park et al. [19] did not find difference between classic acupoint acupuncture and nonclassic acupoint acupuncture except for pressure and cramps in upper abdomen (better results in the classic acupoint acupuncture group). The differences may be due to control group treatment. In the study by Park et al. [19], dermatome information between classic acupoint and nonclassic acupoint was not included in consideration upon the design of control group.

FD, like other diseases, is characterized by its objective physiological changes and subjective symptoms; thus, a thorough evaluation of FD should simultaneously include

Table 4: Serum gastrin concentration and frequency and propagation velocity of gastric slow waves in patients with functional dyspepsia and healthy adults.

Items	Baseline (n = 8)	After treatment (n = 8)	Healthy adults (n = 10)
Gastrin (pg/mL)	25.93 ± 5.90	44.40 ± 6.26	47.65 ± 20.21
FGSW (n/min)	2.49 ± 0.64	3.11 ± 0.14	3.11 ± 0.13
PVGSW (s)	24.25 ± 4.95	19.75 ± 2.05	19.41 ± 0.93

FGSW: frequency of gastric slow waves; PVGSW: propagation velocity of gastric slow waves.
Note: propagation velocity of gastric slow waves was assessed by the time interval between two consecutive waves that passed through the gastric antrum.

Table 5: Comparisons of serum gastrin concentration and frequency and propagation velocity of gastric slow waves before and after treatment as well as between patients with functional dyspepsia and healthy adults.

Items	Baseline versus healthy adults	Baseline versus after treatment	After treatment versus healthy adults
Gastrin	0.0081	0.0002	0.6401
FGSW	0.0008	0.0078	1.0000
PVGSW	0.0279	0.0180	0.6713

FGSW: frequency of gastric slow waves; PVGSW: propagation velocity of gastric slow waves.
Note: propagation velocity of gastric slow waves was assessed by the time interval between two consecutive waves that passed through the gastric antrum. "Baseline versus after treatment" refers to patients with functional dyspepsia only.

these two aspects. Changes in gastric motility, mainly gastric hypomotility or dysrhythmias, play an important role in the pathophysiology of FD [1–4, 13]; thus, an objective measurement of gastric motilities is of great importance in the evaluation of FD. Barium sulfate radiography of the GI system provides a direct visual observation of the frequency and propagation velocity of gastric slow waves and thus objective measurements of acupuncture effects on FD. Gastrin is a peptide hormone that stimulates the secretion of gastric acid by the parietal cells of the stomach and aids in gastric motility. Results from recent research studies indicate that abnormal gastrin level is a possible contribution factor of FD with gastric dysmotility [4, 40, 41]. The results of gastrin level in the present study are consistent with the hypothesis as patients with FD show decreased preprandial gastrin level [4, 41]. However, He et al. [40] did not find any difference in preprandial but postprandial gastrin levels (higher in patients with FD) between patients with FD and healthy volunteers. In the present study, observation of less frequent and slower propagation velocity of gastric slow waves in patients with FD via barium sulfate radiography also indicates a decreased level of gastrin. Nonetheless, changes of gastrin and gastric motility in patients with FD deserve further research. In the present study, classic acupuncture was found to increase preprandial gastrin level and enhance gastric motility of patients with FD to reach similar levels as healthy volunteers. These results are consistent with our findings in improvement of dyspeptic symptoms.

4.4. Therapeutic Mechanism of Acupuncture. Former research studies in human beings indicate that acupuncture could accelerate solid gastric emptying [18, 25] and enhance percentage of normal gastric slow waves [22, 23]. The present study showed similar results in increasing gastric motility as demonstrated by increased frequency and propagation velocity of gastric slow waves in patients with FD.

An accepted mechanism of acupuncture on the functions of the gastric system is related to its effects on the autonomic nervous systems, which Takahashi [15] summarized as follows: acupuncture at the lower limbs (ST36) causes gastric muscle contraction via stimulating the somatoparasympathetic pathway whereas acupuncture at the upper abdomen causes gastric muscle relaxation via stimulating the somatosympathetic pathway. As both main acupoints KI3 and ST36 used in the present study are located in the lower extremities, the result of enhanced gastric motility is likely to be caused by activation of the somatoparasympathetic pathway increasing the secretion of gastrin and other hormones.

Furthermore, acupuncture has also been found to induce changes in cerebral cortex activities of patients with FD [21]. Consequently, we hypothesize that effect of acupuncture on the gastrointestinal system is related to its effects on the peripheral nervous system, central nervous system, and the endocrine systems related to the GI tract. However, to prove the specific causal relationship among these systems, further research studies are needed.

5. Limitations

As blinding is difficult in acupuncture studies, the establishment of a blank control group seems impossible. Although nonclassic acupoint acupuncture procedures were used as control in the present study, they are still acupuncture procedures; thus we could not rule out the cofounding factor of needling and placebo effects in the present study. This study is performed at one clinical center with one acupuncturist on a relatively small sample; the results of the present study may not well characterize the response of patients with FD to acupuncture treatments. In addition, the analysis of the results did not include patients who dropped out; data processing based on per protocol population may

decrease the credence of the results. To better capture the response of patients with FD to acupuncture, further large scale, multicenter, randomized placebo controlled trials are warranted.

6. Conclusion

Classic acupuncture with manual manipulation could improve dyspeptic symptoms, mental status, and quality of life in patients with FD and is superior to nonclassic acupoint acupuncture without manipulations. These effects may be related to the increased frequency and propagation speed of gastric slow waves as well as increased serum gastrin secretion.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yulian Jin and Qing Zhao contributed equally to this work.

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Research Article

Discovery of Acupoints and Combinations with Potential to Treat Vascular Dementia: A Data Mining Analysis

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The prevalence of vascular dementia (VaD) is high among the elderly. Acupuncture, a popular therapeutic method in China, can improve memory, orientation, calculation, and self-managing ability in VaD patients. However, in clinical acupuncture and acupuncture research, the selection of acupoints to treat VaD remains challenging. This study aimed to discover acupoints and acupoint combinations with potential for VaD based on data mining. After database searching and screening for articles on clinical trials evaluating the effects of acupuncture on VaD, 238 acupuncture prescriptions were included for further analysis. Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shuigou (GV 26), and Shenting (GV 24) appeared most frequently in the modern literature and are potential acupoints for VaD. Combinations between Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), Shuigou (GV 26), and Zusanli (ST 36) were most frequent and represent potential combinations for VaD treatment. These results provide a reference for the selection and combination of acupoints to treat VaD in clinical acupuncture and acupuncture research.

1. Introduction

The selection of acupoints plays a critical role in the therapeutic effects of acupuncture. However, the selection of proper acupoints remains challenging, contributing to the limited therapeutic effects and application of acupuncture. Data mining has been used to discover potential acupoints from the expansive relevant literature. This method has been used to suggest acupoints on the Shaoyang Meridian for migraine treatment based on their high frequency in the literature [1]. Based on the results of data mining, a subsequent clinical trial confirmed that acupuncture was effective for the treatment of migraine and that acupoints on the Shaoyang Meridian were more effective than acupoints on other meridians [2]. Data mining has also been used to discover potential Chinese herbs for the effective treatment of specific diseases [3, 4]. These results support data mining as a promising method to discover acupoints with potential for treating diseases.

Vascular dementia (VaD) refers to cognitive impairment caused by changes in the blood circulation of the brain [5]. Its clinical manifestations include confusion or short-term memory problems, wandering, getting lost in familiar places, walking with rapid and shuffling steps, losing bladder or bowel control, laughing or crying inappropriately, difficulty in following instructions, and problem with counting money and conducting monetary transactions. At the late stage, VaD patients may have severe impairment of basic activities of daily living and lack the capacity to make appropriate decisions regarding their choices and preferences [6]. A recent population-based survey reported that the prevalence of VaD among individuals aged 65 years and older was 1.5% [7]. It has been predicted that dementia will affect 80 million people worldwide by 2040 [8]. The annual cost of care per patient is estimated to be US$17,000–55,200 for severe dementia, placing a heavy economic burden on families and society [9].

Acupuncture, a primary therapeutic method in traditional Chinese medicine (TCM), can improve memory, orientation, calculation, and self-managing ability in VaD patients [10–12]. The therapeutic effects of acupuncture are achieved via multiple pathways, including antioxidative effects, antiapoptotic effects, and neurotrophic effects [11, 13–15]. However, acupoint selection remains a challenge in the use of acupuncture to treat VaD. According to our preliminary statistics, more than 100 acupoints distributed in 13 meridians have been recorded in the modern literature for the treatment of VaD. The most effective acupoints for the treatment of VaD and the selection of acupoints for combination remain to be elucidated, representing a major limitation for clinical therapeutic effects and the application of acupuncture for VaD.

To shed some light on the selection of acupoints and acupoint combinations to treat VaD in clinical acupuncture and acupuncture research, this study aimed to discover acupoints and acupoint combinations that have potential to treat VaD via data mining.

2. Materials and Methods

The flow of information through the various phases of data mining is illustrated in Figure 1.

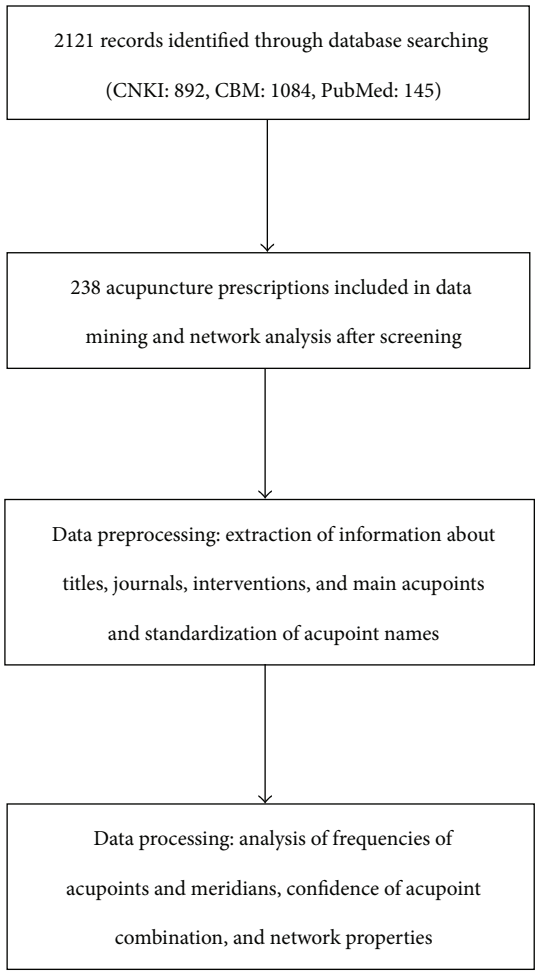


Figure 1: Flow of information through the different phases of data mining.

2.1. Inclusion Criteria for Considering Acupoint Prescriptions for Data Mining

2.1.1. Types of Studies. Clinical trials evaluating the effect of TCM acupuncture with or without randomization methods were included. Trials with or without controls were also included. The control interventions included no treatment, sham acupuncture, Western medicine, TCM herbs, nontraditional acupuncture, and TCM acupuncture containing another acupoint prescription which is different from the one in observation group. Language was restricted to Chinese and English.

2.1.2. Types of Participants. Clinical trials involving adult participants diagnosed with VaD were included.

2.1.3. Types of Interventions. Clinical trials evaluating TCM acupuncture were included. Acupuncture can be used alone or in combination with other types of interventions. TCM acupuncture involves inserting needles into traditional meridian acupoints and extraordinary acupoints. Electrical stimulation of the needles may be used. Trials using moxibustion alone or as a cointervention with acupuncture were also included.

2.1.4. Effectiveness of Acupoint Prescriptions. Acupoint prescriptions for the disease and not particular syndromes of VaD were included. There should be statistical differences in symptoms between before and after acupuncture. In a controlled trial, patients treated with acupuncture alone or in combination should receive greater benefit than patients who do not receive acupuncture therapy. The control interventions included no treatment, sham acupuncture, Western medicine, TCM herbs, nontraditional acupuncture, and TCM acupuncture containing another acupoint prescription which is different from the one in observation group. If the studies compared the therapeutic effects of different acupoint prescriptions, the most effective acupoint prescription was included.

2.2. Exclusion Criteria

2.2.1. Types of Studies. Case reports, reviews, systematic reviews, and meta-analyses were excluded.

2.2.2. Types of Participants. Trials evaluating the therapeutic effect of acupuncture for Alzheimer's disease, traumatic dementia, and other subtypes of dementia were excluded. Studies on animals were also excluded.

2.2.3. Types of Interventions. Trials stimulating Ashi points alone were excluded. Trials of dry needling or trigger point therapy, therapies that are based on principles of western anatomy and physiology, were excluded. Trials of laser acupuncture and noninvasive electrostimulation (e.g., using

electrodes on the skin rather than needles or moxibustion to stimulate the acupoints) were excluded to limit the focus to TCM acupuncture. Trials evaluating acupressure, a form of massage, were excluded as well. Finally, trials of micropuncture were excluded because micropuncture is a nontraditional acupuncture practice that is based on the principle that the head (or ear, nose, eye, abdomen, ankle, etc.) is a microsystem of the entire body.

2.2.4. Effectiveness of Acupoint Prescriptions. Acupoint prescriptions for a particular syndrome of VaD were excluded. Acupoint prescriptions with no statistical improvement of symptoms were also excluded. When the therapeutic effects of different acupoint prescriptions were compared in a study, all acupoint prescriptions except the most effective one were excluded.

2.3. Searching Methods for Identification of Studies

2.3.1. Data Sources. PubMed (http://www.pubmed.com (1966 to 2012)), the Chinese BioMedicine Database (CBM) (http://www.sinomed.ac.cn (1978 to 2012)), and China National Knowledge Infrastructure (CNKI) (http://www.cnki.net (1912 to 2012)) were searched for modern literature on acupuncture treatment for VaD.

2.3.2. Searching Strategy. The searching strategy used the following key words: (I) "acupuncture" OR "electroacupuncture" OR "moxibustion" OR "meridian" OR "acupoint"; (II) "dementia" OR "vascular dementia" OR "Alzheimer's disease." The searching strategy included literature on acupuncture treatment for Alzheimer's disease (AD) because the modern literature on acupuncture for VaD overlaps with that on AD.

2.4. Data Collection. Two reviewers independently screened the title and abstract of every record retrieved from the literature searches. All potentially relevant articles were investigated as full text in English or Chinese. In cases of disagreement, a trial was included or excluded based on discussion between the two reviewers or after a third reviewer reviewed the information. For duplicate publications, the final publication was used.

2.5. Data Preprocessing. Information about titles, journals, interventions, and main acupoints was extracted using the self-established Data Excavation Platform of Acupoint Specificity for data mining. Because acupoints have aliases, the names of acupoints were standardized according to *Fundamentals of Acupuncture* [16].

2.6. Data Processing

2.6.1. Frequencies of Acupoints. The frequencies of acupoints, meridians, and acupoints on different body parts were analyzed using the Data Excavation Platform of Acupoint Specificity.

2.6.2. Association Rules Mining. Apriori Algorithm for association rules mining [17] was adopted to analyze the frequencies and support of acupoint combinations. According to the definition of association rules mining [18], the following can be a statement of association rules mining for acupoint combination. Let $I = \{i_1, i_2, \ldots, i_m\}$ be a set of acupoints. Let D be a set of acupoint prescriptions, where each acupoint prescription T is a set of acupoints such that $T \subseteq I$. Associated with each acupoint prescription is a unique identifier, called TID. An acupoint prescription T contains X, a set of some acupoints in I, if $X \subseteq T$. The rule X-Y has *support* s in the acupoint prescription set D if s% of acupoint prescriptions in D contain $X \cup Y$.

2.6.3. Measurement of Network Properties. Community structure is a common characteristic of complex networks and is characterized by more dense internal connections within groups of nodes than with the rest of the network. In this study, a hierarchical agglomeration was adopted to detect community structure according to Clauset et al. [19]. We also focused on investigating the set of the most influential nodes in acupoint networks of VaD, defined as the nodes with the highest k-core value [20]. The k-core method is predominantly used in analyzing social networks. We employed the k-core method to obtain the cores of different acupoints. The k-core method was implemented as follows. First, all 1-degree nodes were removed, and the nodes were further pruned until no 1-degree nodes remained. The remaining nodes formed the 2-core node set. The pruning process was repeated in a similar manner for other nodes in the network assigned to the corresponding cores (denoted by ks). The nodes with the largest k-core value were defined as the network core. The degree of each acupoint was also analyzed to measure the involvement of the node in the network. The degree refers to the number of nodes to which a focal node is connected [21]. Betweenness centrality was also used to analyze an acupoint's centrality in the network. Centrality is an important concept for the analysis of networks, and betweenness centrality is one of the most prominent measures of centrality. It is used to measure the degree to which a node is in a position of brokerage by summing up the fractions of shortest paths between other pairs of vertices that pass through it [22].

3. Results

3.1. Overall Profile of Acupuncture Prescriptions. Database searching identified 892 records in CNKI, 1084 records in CBM, and 145 records in PubMed. After screening, 238 acupuncture prescriptions in 238 articles were included. Among the 238 trials, 185 are controlled clinical trials (CCTs), while the other 53 trials have no controls. The whole view on the study quality of the 185 CCTs were shown in Figure 2.

3.2. Frequencies of Acupoints and Meridians. Approximately 109 meridian-acupoints distributed over 13 meridians and 7 extraordinary acupoints have been recorded for 1400 and 133 times, respectively, in modern literature on acupuncture treatment for VaD. The most frequently used meridian was the Governor Meridian (477 times). Other frequently used meridians included the Gallbladder Meridian of Foot

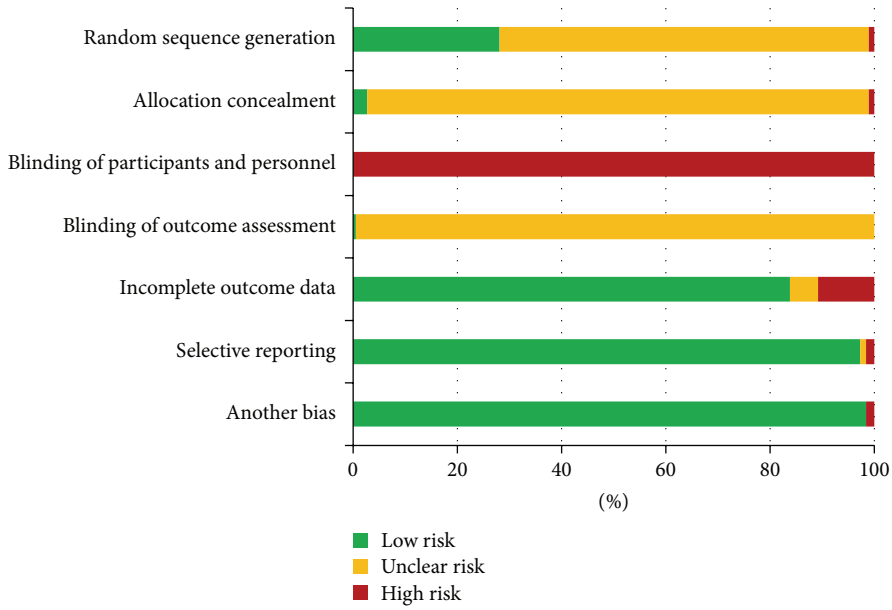


Figure 2: Whole view on the study quality of the 185 CCTs.

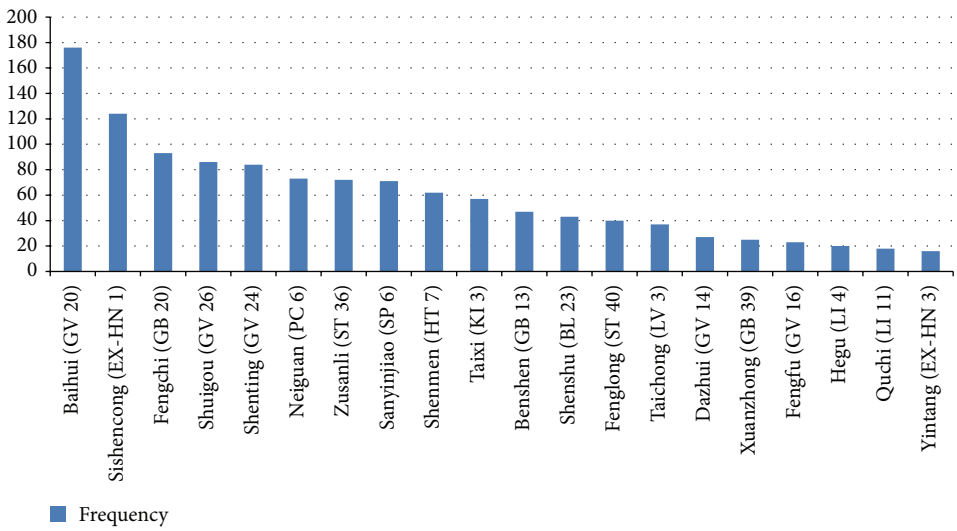


Figure 3: The 20 most frequent acupoints and their frequencies.

Shaoyang and the Stomach Meridian of Foot Yangming, which were reported for 218 and 124 times, respectively. Extraordinary acupoints were also frequently used. Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shuigou (GV 26), and Shenting (GV 24), which were among the top five acupoints in frequency, were recorded for 176, 124, 93, 86, and 84 times, respectively. The frequencies of each meridian and acupoint are shown in Table 1. The twenty most frequently used acupoints are shown in Figure 3.

3.3. Frequencies of Acupoints on Different Body Parts. Acupoints on the head, face, and neck were used most frequently, with a total number of 42 acupoints and a total frequency of 766 times, followed by acupoints on the lower limbs (383 times), upper limbs (214 times), back and lumbar (127 times), and chest and abdomen (43 times) (Figures 4(a) and 4(b)).

3.4. Frequencies of Specific Acupoints. Specific acupoints represented 78 of the 116 acupoints (67.24%). Specific acupoints have been used 1292 times, representing 84.28% of the total frequency of all acupoints (Figures 4(c) and 4(d)).

3.5. Frequencies and Support of Acupoint Combinations. Acupoint combinations between Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), Shuigou (GV 26), and Zusanli (ST 36) were used most frequently. The 15 most frequently used acupoint combinations and their support and confidence are shown in Table 2.

Table 1: Statistics of meridians and acupoints in the modern literature on acupuncture treatment for VaD.

Number	Meridian	Frequency	Number of acupoints	Acupoints and their frequencies
1	GV	477	26	Baihui (GV 20) 176, Shuigou (GV 26) 86, Shenting (GV 24) 84, Dazhui (GV 14) 27, Fengfu (GV 16) 23, Yintang (EX-HN 3) 16, Naohu (GV 17) 13, Shangxing (GV 23) 11, Mingmen (GV 4) 8, Yamen (GV 15) 5, Qiangjian (GV 18) 5, Qianding (GV 21) 4, Yaoyangguan (GV 3) 3, Zhiyang (GV 9) 3, Jinsuo (GV 8) 2, Shendao (GV 11) 1, Zhongshu (GV 7) 1, Taodao (GV 13) 1, Lingtai (GV 10) 1, Changqiang (GV 1) 1, Xuanshu (GV 5) 1, Yaoshu (GV 2) 1, Shenzhu (GV 12) 1, Jizhong (GV 6) 1, Houding (GV 19) 1, Xinhui (GV 22) 1
2	GB	218	20	Fengchi (GB 20) 93, Benshen (GB 13) 47, Xuanzhong (GB 39) 24, Shuaigu (GB 8) 7, Wangu (GB 12) 6, Toulinqi (GB 15) 6, Qubin (GB 7) 5, Xuanli (GB 6) 4, Naokong (GB 19) 4, Zuqiaoyin (GB 44) 3, Yanglingquan (GB 34) 3, Yangbai (GB 14) 3, Hanyan (GB 4) 2, Qiuxu (GB 40) 2, Fengshi (GB 31) 2, Touqiaoyin (GB 11) 2, Xuanlu (GB 5) 2, Muchuang (GB 16) 1, Zhengying (GB 17) 1, Zhongdu (GB 32) 1
3	EX-HN	133	7	Sishencong (EX-HN 1) 123, Taiyang (EX-HN 5) 4, Wailaogong (EX-UE 8) 2, Shiqizhui (EX-B 8) 1, Baxie (EX-UE 9) 1, Anmian (EX-HN 22) 1, Yiming (EX-HN 14) 1
4	ST	124	7	Zusanli (ST 36) 72, Fenglong (ST 40) 40, Touwei (ST 8) 6, Lidui (ST 45) 2, Sibai (ST 2) 2, Futu (ST 32) 1, Renying (ST 9) 1
5	BL	103	15	Shenshu (BL 23) 43, Ganshu (BL 18) 12, Tianzhu (BL 10) 10, Pishu (BL 20) 8, Feiyang (BL 58) 8, Geshu (BL 17) 7, Xinshu (BL 15) 4, Zhiyin (BL 67) 2, Kunlun (BL 60) 2, Yuzhen (BL 9) 2, Tongtian (BL 7) 1, Chengjin (BL 56) 1, Dazhu (BL 11) 1, Weizhong (BL 40) 1, Qucha (BL 4) 1
6	SP	96	5	Sanyinjiao (SP 6) 71, Xuehai (SP 10) 14, Taibai (SP 3) 8, Yinbai (SP 1) 2, Yinlingquan (SP 9) 1
7	PC	86	5	Neiguan (PC 6) 73, Zhongchong (PC 9) 4, Daling (PC 7) 4, Jianshi (PC 5) 3, Laogong (PC 8) 2
8	KI	83	5	Taixi (KI 3) 57, Dazhong (KI 4) 12, Yongquan (KI 1) 10, Zhaohai (KI 6) 8, Rangu (KI 2) 2
9	HT	67	3	Shenmen (HT 7) 62, Shaochong (HT 9) 3, Jiquan (HT 1) 2
10	LI	45	7	Hegu (LI 4) 20, Quchi (LI 11) 18, Shangyang (LI 1) 2, Shousanli (LI 10) 2, Binao (LI 14) 1, Jianyu (LI 15) 1, Yingxiang (LI 20) 1
11	CV	43	5	Qihai (CV 6) 11, Zhongwan (CV 12) 10, Guanyuan (CV 4) 9, Danzhong (CV 17) 9, Shenque (CV 8) 4
12	LV	40	2	Taichong (LV 3) 37, Dadun (LV 1) 3
13	TE	11	5	Waiguan (TE 5) 6, Guanchong (TE 1) 2, Sizhukong (TE 23) 1, Jiaosun (TE 20) 1, Sidu (TE 9) 1
14	LU	4	2	Shaoshang (LU 11) 3, Lieque (LU 7) 1
15	SI	3	2	Shaoze (SI 1) 2, Yanggu (SI 5) 1
16	Total	1,533	116	

GV, Governor Meridian; GB, Gallbladder Meridian of Foot Shaoyang; EX-HN, extraordinary acupoint; ST, Stomach Meridian of Foot Yangming; BL, Bladder Meridian of Foot Taiyang; SP, Spleen Meridian of Foot Taiyin; PC, Pericardium Meridian of Hand Jueyin; KI, Kidney Meridian of Foot Shaoyin; HT, Heart Meridian of Hand Shaoyin; CV, Conception Vessel; LI, Large Intestine Meridian of Hand Yangming; LV, Liver Meridian of Foot Jueyin; TE, Triple Energizer of Hand Shaoyang; LU, Lung Meridian of Hand Taiyin; SI, Small Intestine of Hand Taiyang. Frequencies of meridians refer to the total frequencies of acupoints on the same meridian. Number of acupoints refer to the total number of acupoints on the same meridian.

Table 2: Statistics of the 15 most frequently used acupoint combinations in the treatment of VaD.

Number	Acupoint combination	Frequency	Support (%)
1	Baihui (GV 20), Sishencong (EX-HN 1)	98	41.18
2	Baihui (GV 20), Fengchi (GB 20)	81	34.03
3	Baihui (GV 20), Shuigou (GV 26)	72	29.83
4	Baihui (GV 20), Shenting (GV 24)	70	29.41
5	Baihui (GV 20), Zusanli (ST 36)	62	26.05
6	Sishencong (EX-HN 1), Fengchi (GB 20)	60	25.21
7	Baihui (GV 20), Sanyinjiao (SP 6)	57	23.95
8	Baihui (GV 20), Neiguan (PC 6)	54	22.69
9	Sishencong (EX-HN 1), Shuigou (GV 26)	51	21.43
10	Sishencong (EX-HN 1), Baihui (GV 20), Fengchi (GB 20)	51	21.43
11	Baihui (GV 20), Shenmen (HT 7)	51	21.43
12	Sishencong (EX-HN 1), Shenting (GV 24)	49	20.59
13	Sishencong (EX-HN 1), Neiguan (PC 6)	46	19.33
14	Baihui (GV 20), Taixi (KI 3)	46	19.33
15	Sishencong (EX-HN 1), Baihui (GV 20), Shuigou (GV 26)	43	18.07

Support refers to the percentage of acupoint prescriptions containing the acupoint combination.

3.6. Community Structure. Community detection resulted in the division of the 116 acupoints into 5 communities. Nodes of the same color belong to the same community. The community structure is shown in Figure 5(a).

3.7. Acupoint K-Core Network. The largest k-core value was 19. At this value, there were 28 nodes, corresponding to Hegu (LI 4), Quchi (LI 11), Zusanli (ST 36), Fenglong (ST 40), Sanyinjiao (SP 6), Xuehai (SP 10), Shenmen (HT 7), Tianzhu (BL 10), Xinshu (BL 15), Ganshu (BL 18), Shenshu (BL 23), Taixi (KI 3), Dazhong (KI 4), Neiguan (PC 6), Benshen (GB 13), Fengchi (GB 20), Xuanzhong (GB 39), Taichong (LV 3), Mingmen (GV 4), Dazhui (GV 14), Yamen (GV 15), Fengfu (GV 16), Baihui (GV 20), Shenting (GV 24), Shuigou (GV 26), Guanyuan (CV 4), Sishencong (EX-HN 1), and Yintang (EX-HN 3), as shown in Figure 5(b).

3.8. Degree. The top 20 acupoints in degree are shown in Figure 6(a). Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), and Neiguan (PC 6) had the highest degrees, with values of 89, 76, 68, 67, and 65, respectively.

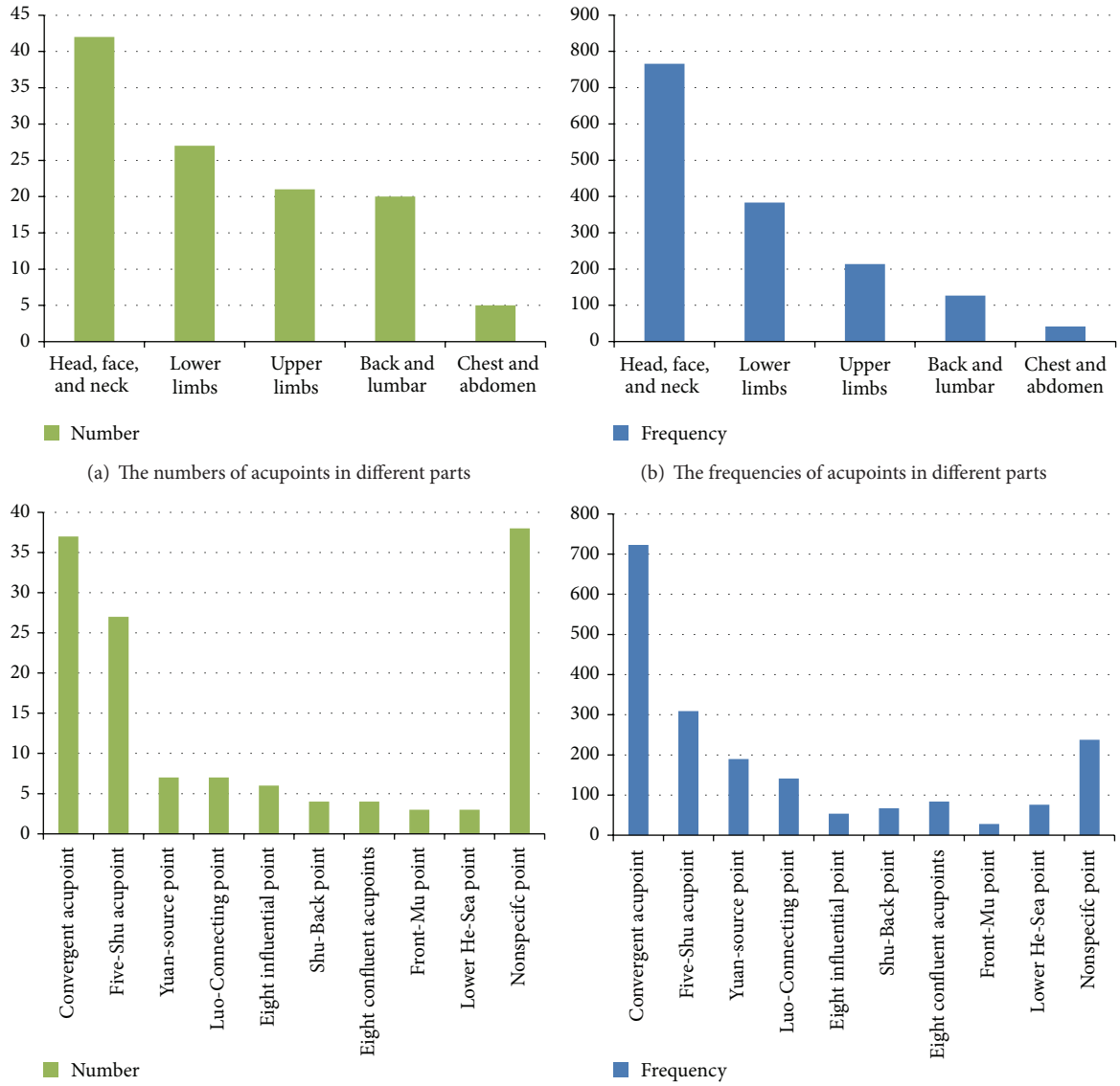
3.9. Betweenness Centrality. The top 20 acupoints in betweenness centrality are shown in Figure 6(b). Yongquan (KI 1), Baihui (GV 20), and Sishencong (EX-HN 1) had the highest betweenness centrality.

4. Discussion

4.1. Potential Acupoints and Combinations for VaD. In this study, acupoints and combinations with potential for treating VaD were discovered. These results may provide some reference for the selection of acupoints in treatment for VaD, which may promote the therapeutic effects in clinical practice. The results suggest that Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shuigou (GV 26), and Shenting (GV 24) are potential acupoints for treating VaD. In terms of meridian, acupoints on the Governor Meridian have potential for treating VaD. From the perspective of combinations, combinations between such acupoints as Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), Shuigou (GV 26), and Zusanli (ST 36) have potential for treating VaD. In addition, acupoints on the head, face, and neck have more potential for VaD than acupoints on other regions of the body. Specific acupoints have more potential than nonspecific acupoints. Specific acupoints, with specific names, are a group of acupoints on fourteen meridians with specific therapeutic effects. There are ten types of specific acupoints, Five-Shu acupoints, Yuan-Primary acupoints, Luo-Connecting acupoints, Xi-Cleft acupoints, Lower He-Sea acupoints, Back-Shu acupoints, Front-Mu acupoints, eight influential acupoints, eight confluent acupoints connecting the eight extra meridians, and convergent acupoints.

Community detection divided the acupoints into 5 communities. Acupoints within the same community have some characteristics in common. Blue nodes (Community A) were all Jing-Well acupoints. Yellow nodes (Community B) were all acupoints on the face and head. Most green nodes (Community C) were acupoints on the four limbs. Most purple nodes (Community D) were acupoints belonging to Governor Vessel. Most red nodes (Community E) were specific acupoints or acupoints with specific therapeutic effects, and only this community contained Bach-Shu acupoints and acupoints on the abdomen. Acupoints within the same community were more densely connected with each other compared with acupoints from different communities, indicating that an acupoint was more often used with acupoints within the same community compared with acupoints within other communities.

The 19-core network indicated that 28 acupoints, including Hegu (LI 4), Quchi (LI 11), Zusanli (ST 36), Fenglong (ST 40), Sanyinjiao (SP 6), Xuehai (SP 10), Shenmen (HT 7),



(a) The numbers of acupoints in different parts

(b) The frequencies of acupoints in different parts

(c) The numbers of different kinds of acupoints

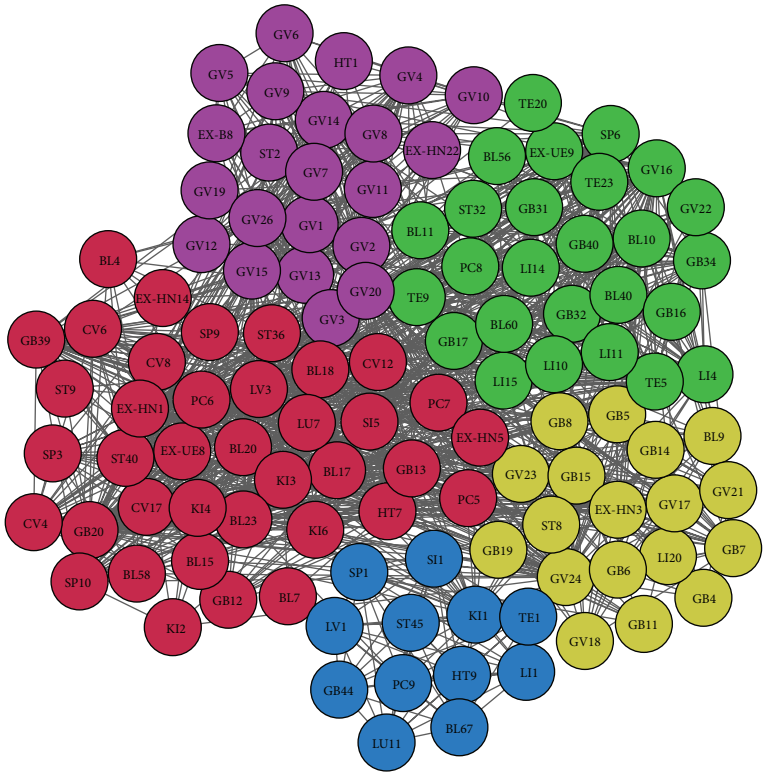
(d) The frequencies of different kinds of acupoints

Figure 4: The frequencies and numbers of acupoints in different body parts and different types of acupoints.

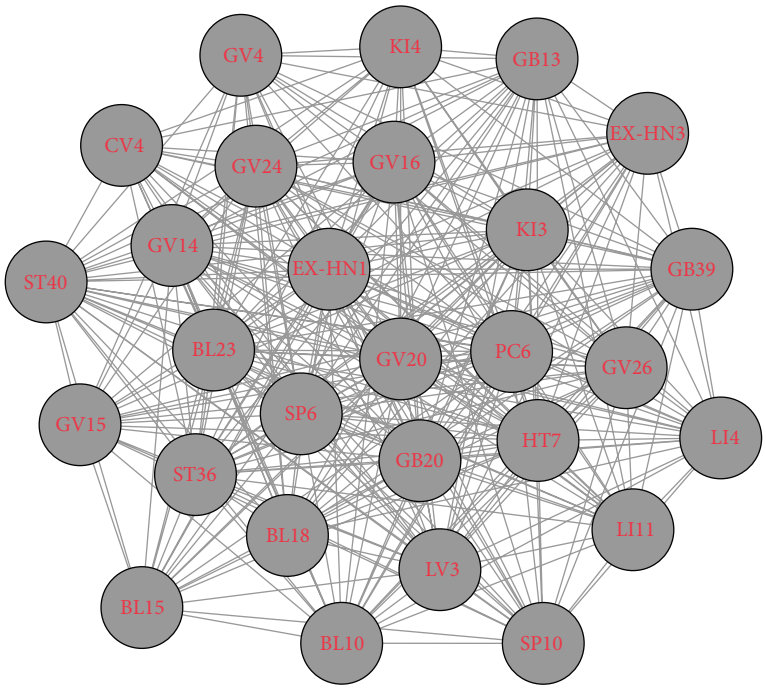
Tianzhu (BL 10), Xinshu (BL 15), Ganshu (BL 18), Shenshu (BL 23), Taixi (KI 3), Dazhong (KI 4), Neiguan (PC 6), Benshen (GB 13), Fengchi (GB 20), Xuanzhong (GB 39), Taichong (LV 3), Mingmen (GV 4), Dazhui (GV 14), Yamen (GV 15), Fengfu (GV 16), Baihui (GV 20), Shenting (GV 24), Shuigou (GV 26), Guanyuan (CV 4), Sishencong (EX-HN 1), and Yintang (EX-HN 3) are core acupoints in the network.

Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), and Neiguan (PC 6) had the highest degrees. This result indicates that these 5 acupoints have been combined with more acupoints than other acupoints. These acupoints have specific therapeutic effects on VaD. Therefore, these acupoints can be used together with other acupoints to enhance therapeutic effects.

Yongquan (KI 1), Baihui (GV 20), Sishencong (EX-HN 1), Neiguan (PC 6), and Shenting (GV 24), which belonged to 4 different communities, had higher betweenness centrality. Yongquan (KI 1), which had the highest betweenness centrality, did not have a relatively high degree. However, it connects Jing-Well acupoints with other types of acupoints, resulting in a high betweenness centrality. Acupoints with higher betweenness centrality play an important role in connecting different types of acupoints. Jing-Well acupoints, except Yongquan (KI 1), were often used with other Jing-Well acupoints only. Yongquan (KI 1) was not only used with other Jing-Well acupoints but also with other types of acupoints, such as other types of specific acupoints, nonspecific acupoints, and acupoints on other parts. The high betweenness centrality suggested that Yongquan (KI 1) may have multiple effects compared with other Jing-Well acupoints in treatment of VaD. From the perspective of TCM theory, Jing-Well acupoints can restore consciousness. Yongquan (KI 1) was

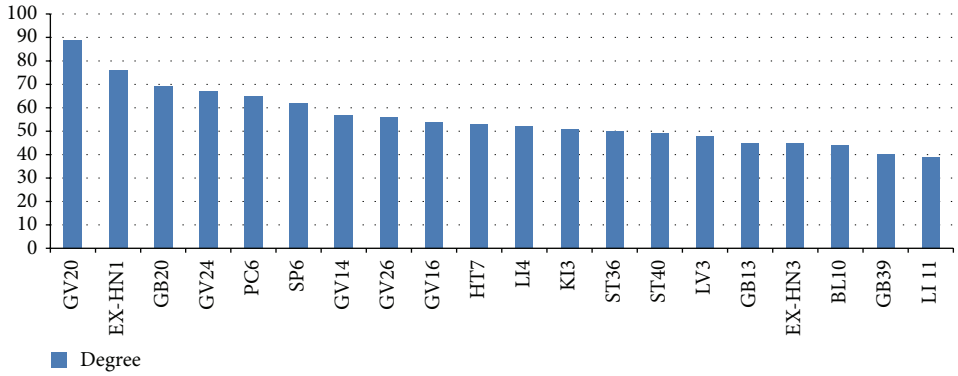


(a) Network structure of acupoints for the treatment of VaD

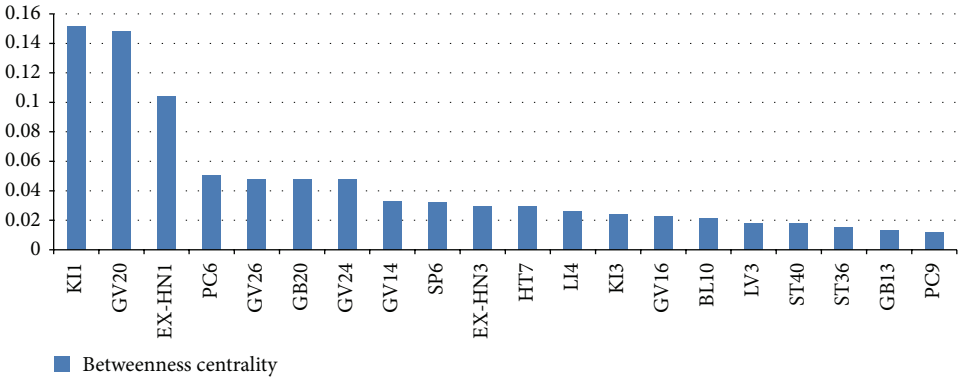


(b) 19-core network

Figure 5: (a) Network structure of acupoints for the treatment of VaD. Blue nodes (Community A) are all Jing-Well acupoints. Yellow nodes (Community B) are all acupoints on the face and head. Most green nodes (Community C) are acupoints on four limbs. Most purple nodes (Community D) are acupoints belonging to Governor Vessel. Most red nodes (Community E) are specific acupoints or acupoints with specific therapeutic effects, and only this community contains Bach-Shu acupoints and acupoints on the abdomen. Acupoints within the same community are more densely connected with each other than acupoints from different communities. (b) 19-core network. There are 28 acupoints in the 19-core network. They are core acupoints in the treatment of VaD.



(a) The 20 acupoints with highest degree



(b) The 20 acupoints with highest betweenness centrality

Figure 6: The 20 acupoints with highest degree and the 20 acupoints with highest betweenness centrality.

also used to tonify kidney in treatment of VaD. Therefore, Yongquan (KI 1) is used not only with other Jing-Well acupoints but also with other types of acupoints, such as other types of specific acupoints, nonspecific acupoints, and acupoints on other parts.

4.2. Underlying Molecular Mechanism of the Acupoint with the Most Potential to Treat VaD. According to our results, Baihui (GV 20), which had the highest frequency, has the most potential to treat VaD. A systematic review and meta-analysis also suggested that Baihui (GV 20) is a principal acupoint for acute intracerebral hemorrhage (ICH); in animal models of acute ICH, there was no difference in efficacy between Baihui (GV 20) alone and Baihui (GV 20) plus other acupoints [23].

Molecular biology studies have provided insights into the mechanisms underlying the effects of Baihui (GV 20) in VaD treatment, including antioxidant effects, antiapoptotic effects, neurotrophic effects, reduced blood-brain barrier (BBB) permeability, and regulation of the cholinergic and dopaminergic systems. Acupuncture at Baihui (GV 20) in combination with other acupoints decreases levels of 8-hydroxy-2′-deoxyguanosine, a product of oxidative damage to DNA induced by free radicals, suggesting that the benefit of acupuncture is partly due to antioxidant effects [11]. Acupuncture exerts therapeutic effects on VaD via antiapoptosis. The tumor suppressors p53 and Noxa are important in regulating apoptosis and mediate hypoxic cell death [24, 25]. Electroacupuncture at Baihui (GV 20), Dazhui (GV 14), and Shenshu (BL 23) blocks expression of p53 and Noxa in the hippocampal CA1 region of VaD rats [26]. Acupuncture at Baihui (GV 20) can improve neurogenesis via regulating brain-derived neurotrophic factor (BDNF) and cyclic AMP response element-binding protein (CREB). BDNF, which is essential for synaptic plasticity and is coupled to CREB activation [27], is important for long-term memory storage [28]. CREB is required for the proliferation, growth, survival, and differentiation of all types of cells. In the brain, the CREB and CRE-mediated system is involved in memory, learning, synaptic transmission, neuron survival, differentiation, and axon growth [29]. Acupuncture at Baihui (GV 20) significantly increases the levels of BDNF [15, 30], CREB proteins, CREB mRNA [30], and phosphorylated CREB, the active form of CREB [15]. The molecular mechanism underlying acupuncture at Baihui (GV 20) also involves cholinergic system regulation. Decreased cholinergic function in the brain can result in a decline in memory and cognitive function [31]. Acupuncture at Baihui (GV 20) significantly increases the levels of choline acetyltransferase (ChAT) and restores the expression of choline transporter 1 (CHT1) and vesicular acetylcholine transporter (VAChT) [30]. The dopaminergic system is also involved in the mechanism underlying the treatment of VaD with acupuncture at Baihui (GV 20). Dopamine is a key regulator in specific synaptic changes observed at certain stages of learning and memory

and of synaptic plasticity [32]. Acupuncture at Baihui (GV 20) increases dopamine levels in chronic cerebral hypoperfusion and ischemia-reperfusion injured rats [33]. In addition, acupuncture at Baihui (GV 20) and Zusanli (ST 36) preserves the integrity of the BBB, reducing BBB permeability. The BBB is constructed of tight conjunctions, including occludin and claudin-5, which form the endothelial barrier. Reduced expression of ZO-1, claudin-5, and occludin mRNA and protein contributes to BBB breakdown and edema in the ischemic brain [34]. Electroacupuncture at Baihui (GV 20) and Zusanli (ST 36) reduces brain damage and related behavioral deficits via upregulation of tight conjunction proteins, including ZO-1, claudin-5, and occludin [35]. These findings reveal parts of the molecular mechanism underlying acupuncture at Baihui (GV 20) to treat VaD.

4.3. Acupoints Selection in Treatment for VaD. The proper selection of acupoints is essential for the therapeutic effects of acupuncture because acupoints are specific with regard to morphological structure, biophysical properties, pathological response, and stimulating effects [36]. This specificity differentiates acupoints from nonacupoints as well as different acupoints from one another. The specific therapeutic effects of different acupoints have been reported for migraine [37], functional dyspepsia [38], ischemic stroke [39], and so forth.

The specificity of acupoints for the treatment of VaD has also been reported. Phosphorylated CREB levels were significantly increased after acupuncture therapy of needling Baihui (GV 20) and Zusanli (ST 36) compared to sham acupuncture therapy of needling nonacupoints [15]. Baihui (GV 20), Shuigou (GV 26), and Shenmen (HT 7) are all among the 10 acupoints with the most potential. A clinical trial demonstrated that needling Baihui (GV 20), Shuigou (GV 26), and Shenmen (HT 7) were all effective in improving the symptoms of VaD. However, their therapeutic effects differ. Needling Baihui (GV 20) improved calculation ability and short-term memory and corrected the personality changes of VaD patients, while needling Shuigou (GV 26) improved naming ability and short-term memory. The therapeutic effects of needling Baihui (GV 20) and Shuigou (GV 26) were superior to those of needling Shenmen (HT 7) [40]. A PET and SPECT study revealed that needling these three different acupoints in VaD patients affected different brain areas. Needling Baihui (GV 20) activated the inner temporal system, the thalamencephalon system, and the prefrontal cortical system. Needling Shuigou (GV 26) activated the prefrontal cortical system. Needling Shenmen (HT 7) generated an effect similar to but weaker than the effect generated by needling Shuigou (GV 26) [41]. These findings demonstrate that different acupoints have different therapeutic effects in acupuncture treatment for VaD. Consequently, the selection of acupoints, which directly influences the therapeutic effects of acupuncture, should be considered carefully. According to our results based on data mining, Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shuigou (GV 26), and Shenting (GV 24), which have higher frequencies in the modern literature, may have better therapeutic effects on VaD.

4.4. Single Acupoint or Acupoint Combination. Acupoint combinations also influence the therapeutic effects of acupuncture. An acupoint combination is considered to have a synergistic effect that enhances the therapeutic effect of acupuncture. For example, a lower prevalence of postoperative nausea and vomiting in patients treated with Neiguan (PC 6) plus Hegu (LI 4) was observed compared with those treated with Neiguan (PC 6) only [42]. In spite of extensive evidence suggesting a synergistic effect of acupoint combination and supporting its use, some studies have reported antagonistic effects [43–45]. An antagonistic effect occurs when one acupoint weakens the therapeutic effect of another acupoint [46]. For example, electroacupuncture can improve gastrointestinal movement in rats. The effect of needling Pishu (BL 20) alone was better than the effect of needling Pishu (BL 20) and Zusanli (ST 36) at the same time [44]. Therefore, whether the effect of acupoint combination is better than a single acupoint still remains a question and needs to be further studied.

Some studies have compared single acupoints and an acupoint combination for the treatment of VaD. The therapeutic effect of needling Baihui (GV 20), Shuigou (GV 26), and Shenmen (HT 7) in combination was better than the effects obtained by needling each alone [40]. In addition, needling Baihui (GV 20), Shuigou (GV 26), and Shenmen (HT 7) simultaneously activated more brain areas related to intellectual activities compared with needling each alone, generating a more extensive effect on the brain [41]. Antagonistic effects in acupuncture therapy for VaD have not been reported but may occur. Most acupuncture prescriptions for VaD contain acupoint combinations, and the use of acupoint combinations is supported. However, acupoint combinations should be selected carefully to avoid antagonistic effects. It is hard to tell whether a combination of acupoints will exert antagonistic effects with current knowledge or TCM theory. As abovementioned, acupoints with similar functions can exert antagonistic effects. There are many acupoints, and the number of acupoint combinations will grow geometrically. To test the antagonistic effects of each combination one by one is an exhausting job. To avoid antagonistic effects as possible, the acupoint prescriptions should be simplified as possible. The general principle is to select acupoints with relatively better therapeutic effects and acupoints with multiple indications and not to select many acupoints.

4.5. Limitations. This study has limitations as follows. First, other factors that influence acupuncture, such as manipulation and treatment duration, were not analyzed in this study. These data can be further mined in future studies. Second, largely due to the lack of treatment based on syndrome differentiation and different methods of syndrome differentiation in modern literature, potential acupoints and combinations for different syndromes of VaD were not analyzed. Although treatment based on syndrome differentiation is important and is often emphasized in TCM, treatment based on disease differentiation is equally important. Third, the real therapeutic effects of acupoints and combinations on VaD cannot be reflected by frequencies in the literature. However, these results suggest some potential acupoints and combinations to

be explored in future clinical trials to validate the effects of acupuncture on VaD. To optimize the acupoint prescription, data may be extracted not only from literature, but also from clinical practice. Further research can collect acupoint prescriptions and symptom improvements in clinical practice and optimize acupoint prescription with data mining. Mining clinical data has been practiced to optimize prescription of Chinese herbal medicine for unstable angina by Feng et al. [47]. In their study, five main symptoms of patients with unstable angina, the severity of each symptom, and the prescription of each patient were collected. The levels of average discounted reward (ADR) of different prescriptions were calculated to evaluate the clinical efficacy of different treatment options, with some optimized prescriptions achieved. Future studies can mine clinical data to optimize acupoint prescription.

5. Conclusions

In this study, data mining was used to discover potential acupoints and combinations for VaD. Potential acupoints include Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shuigou (GV 26), and Shenting (GV 24). In addition, combinations between Baihui (GV 20), Sishencong (EX-HN 1), Fengchi (GB 20), Shenting (GV 24), Shuigou (GV 26), and Zusanli (ST 36) were potential combinations. Based on our results, Baihui (GV 20) and Sishencong (EX-HN 1) should be selected with priority. Acupoints in head, compared to acupoints in other parts, should also be selected with priority.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Shuwei Feng and Yulan Ren contributed equally to this work.

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Research Article

Effects of Deep Electroacupuncture Stimulation at "Huantiao" (GB 30) on Expression of Apoptosis-Related Factors in Rats with Acute Sciatic Nerve Injury

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SD rats were randomly divided into normal control, model, deep EA, and shallow EA groups. The model was established by mechanical clamping of the sciatic nerve stem. For deep and shallow EA, the needles were inserted into "Huantiao" (GB 30) by about 16 mm and 7 mm, respectively, once daily for 14 days. The results showed that, compared with the normal control group, the nerve-muscle excitability of rat's hip muscle decreased and the nerve conduction velocity of sciatic nerve slowed down in the model group; meanwhile, the number of apoptotic cells and the expression level of Bax protein in the injured nerve increased significantly, and the expression level of Bcl-2 protein and the ratio of Bcl-2/Bax decreased considerably. Compared with the model group, the indices mentioned above were reversed in the two treatment groups, and the changes in the deep EA group were more significant than those in the shallow EA group. These results indicate that EA stimulation at GB 30 can improve the function of injured sciatic nerve, which is closely associated with its effects in upregulating the expression of apoptosis inhibitive factor Bcl-2 and downregulating apoptosis promotive factor Bax. Deep EA is relatively better.

1. Introduction

Peripheral nerve injury is one of the most common traumatic disorders. Slow recovery and prolonged loss of sensation or motor function may cause muscle atrophy, joint contracture, and deformity. If the injured nerve fibers are repaired, the continuity of nerves can be restored, providing favorable conditions for nerve regeneration.

Acupuncture has been proven to be an effective method for the treatment of peripheral nerve injury and is widely used to promote the recovery of nerve function [1, 2]. Research has shown that the nerve stump is ischemic in the early stage and that local blood circulation can be improved by acupuncture therapy [3]. With regard to the related mechanism, research has proved the positive effect of acupuncture on the repair of injured nerves from the perspectives of behavior, electrophysiology, and morphology [4–7]. Clinical practice confirmed that the sense of electric shock induced by deep acupuncture at Huantiao (GB 30) had a significant effect on the functional recovery of injured nerves [8, 9]. Experimental studies showed that acupuncture at GB 30 had a positive influence on motor recovery [4] and facilitated axonal regeneration in the injured peripheral nerves [10]. Previous work by our research group indicated that deep electroacupuncture (EA) stimulation at GB 30 improved the pathological changes and function of the injured sciatic nerve in rats, which was closely associated with its effects on the upregulation of nerve growth factor expression and downregulation of Fos expression in the damaged sciatic nerve. Deep EA was found to be better than shallow EA [11]. The present study aimed to further investigate the mechanism of repair of sciatic nerve injury following acupuncture at GB 30 and the difference between deep EA and shallow EA in terms of apoptosis.

2. Materials and Methods

2.1. Animals and Grouping. This experiment was conducted in accordance with *the Guide for Care and Use of Laboratory Animals* issued by the National Institutes of Health.

Forty-eight healthy pathogen-free Sprague Dawley (SD) rats (24 male and 24 female) with a body mass of 250 ± 20 g, were provided by the Experimental Animal Center, Liaoning University of Traditional Chinese Medicine, license number: SCXK (Liaoning) 2008-0005. The laboratory environment was as follows: temperature was 18–22°C, indoor light exposure was approximately 8 h, and relative humidity was about 45%. Free access to water and food was allowed, and males and females were kept separately with six rats per cage. According to the random number table, the rats were randomly divided into the normal control, model, deep EA, and shallow EA groups with 12 in each group.

2.2. Main Reagents and Instruments. The following reagents and instruments were used: cell apoptosis detection kit (Roche), Bcl-2 (B-cell lymphoma/leukemia-2), and Bax (Bcl-2 associated X protein) immunohistochemical detection kits (Shanghai BlueGene Biotech Co., Ltd.), Hwato acupuncture needles (Suzhou Acupuncture Supplies Factory), pulse electroacupuncture therapeutic apparatus (6805-A, Shantou Medical Equipment Factory), biophysiological experimental system (BL-420, Chengdu Taimeng Electronics Co., Ltd.), microtome (RM 2235, Leica), digital microscope (BX 41, Olympus), and MetaMorph microscopic image analysis system (UIC, Olympus).

2.3. Modeling. The acute sciatic nerve injury model was established by mechanical clamping of the sciatic nerve stem [12]. The rat was placed in the prone position on the operating table, and anesthesia was given by intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg). Routine skin preparation and sterilization were carried out, and a 1 cm vertical incision was made at the rear of the middle femoral shaft on the left to expose the bicep femoris muscle. The sciatic nerve was then dissociated by blunt dissection and then clamped with a 16 cm needle holder 0.5 cm below the femoral tubercle. The holder was released after squeezing for 10 s. This was repeated 3 times with an interval of 10 s until the sciatic nerve was seriously injured. The sciatic nerve with the injured trunk of about 3 mm was marked by a 9-0 noninvasive suture thread and then put back in place, and the skin was sutured. The above operation was performed by one person.

2.4. Processing Methods for Each Group

Normal Control Group. The rats were kept under the same conditions without modeling and treatment.

Model Group. The rats were kept under the same conditions after modeling without any treatment.

Deep EA Group. The sciatic nerve injury model was achieved by mechanical clamping of the sciatic nerve stem. After successful modeling, acupuncture was applied to the GB 30 (the depression in front of the femoral greater trochanter at the leading edge of the hip joint in the affected side) with the depth of about 16 mm to the extent that the muscle twitched instantly and the toes trembled. The electroacupuncture therapeutic apparatus was then connected, while the indifferent electrode was placed in the homolateral lower limb. Dilatational wave was applied with a frequency of 2 Hz/100 Hz and an intensity of about 2 mA and the affected limb was observed to twitch slightly. The treatment was given for 20 min each time, once daily for 14 days.

Shallow EA Group. The sciatic nerve injury model was achieved by mechanical clamping of the sciatic nerve stem. After successful modeling, acupuncture was applied to the GB 30 with the depth of about 7 mm without touching the nerve trunk. The electroacupuncture therapeutic apparatus was then connected, while the indifferent electrode was placed in the homolateral lower limb. Dilatational wave was applied with a frequency of 2 Hz/100 Hz and an intensity of about 2 mA and the affected limb was observed to twitch slightly. The treatment was given for 20 min each time, once daily for 14 days.

2.5. Criteria of Successful Modeling. Half an hour after modeling, 5 rats were randomly selected for EMG testing to observe the motor conduction velocity (MCV) of sciatic nerves. When the MCV dropped below 10 m/s, it was deemed that the sciatic neuraxon and myelin sheath had broken or were severely injured [13].

2.6. Observation Indices and Methods

General Status. The rats' mental state, limb activity, reaction, ingestion, water intake, and daily activities were assessed.

Determination of the Strength-Duration (S-D) Curve. The S-D curve was determined by applying the biophysiological experimental system to display the nerve-muscle excitability in the rat. The working electrode was placed on the buttock, and the auxiliary electrode was placed on the ankle joint of the homolateral posterior limb. First, the motor point of the muscle was detected by strong stimulation, and then the magnitude of the current was turned down when weak muscle twitches were observed with the naked eye. The stimulus threshold was measured with a pulse width of 0.1–1 ms, and the S-D curve was drawn using logarithmic coordinates.

Detection of Conduction Velocity of the Sciatic Nerve. The rat was placed in the prone position on the operating table, and anesthesia was given by intraperitoneal injection of 10% chloral hydrate (0.35 mg/100 g). The skin and muscles were cut using the modeling method to fully expose the sciatic nerve segment for surgery. The sciatic nerve was dissociated using a glass dissecting needle with two insulated bipolar acicular electrodes hooked at both ends of the nerve anastomosis, and the recording electrode was placed at the distal end of the stimulation electrode. The stimulus was then applied to determine the threshold causing evoked action potential.

This stimulus was repeated until the graph of action potential on the screen remained stable and the starting points of the artifact and action potential became clear. Screenshots were then obtained, and the amplitude of action potential of the nerve trunk was automatically displayed. If the amplitude of action potential of the nerve trunk was measured from the highest point to the lowest point, the measurement of the latent period lasted from the appearance of stimulus artifact to the initiation site of action potential. When the distance between the two stimulation electrodes was input, the nerve conduction velocity was automatically displayed. During the operation, the sciatic nerves were covered with saline-soaked gauze to ensure that the exposed nerves and muscles remained moist.

Detection of Cell Apoptosis by Terminal Deoxynucleotidyltransferase-Mediated dUTP-Biotin Nick-End Labeling (TUNEL). 8 rats randomly selected in each group were perfused with 4% paraformaldehyde and fixed on the operating table. The injured sciatic nerve tissues were removed and fixed for 24 h. Routine paraffin sections (5 μm) were prepared. After being dewaxed, the sections were treated according to the TUNEL kit instructions. Two sections were taken out from each rat. Eight nonoverlapped views were randomly selected for each section. The MetaMorph microscopic image analysis system was applied. Brown yellow particles in the nucleus were positive cells, and the number of apoptotic cells was measured.

Determination of the Expression of Bcl-2 and Bax Protein by Immunohistochemistry. After being dewaxed, the sections were treated with streptavidin-peroxidase (SP) immunohistochemistry. Three sections were taken out from each rat. Five no-overlapped views were randomly selected for each section. The MetaMorph microscopic image analysis system was used to measure the mean optical density of the positive products of Bcl-2 and Bax. The ratio of Bcl-2/Bax was then calculated.

2.7. Statistical Analysis. SPSS 17.0 statistical software was used to analyze the data, and the results are presented as mean ± standard deviation ($\overline{x} \pm s$). Variance was applied to evaluate integral differences, and equal variance was compared between the two groups by means of *LSD*. A nonparametric test was used in the case of nonconformity with normal distribution. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Observation of General Status. After modeling, the intake of food and water and defecation in the rats were normal. Infection and ulcers were not found on the distal limb. The rats walked by dragging toes or bouncing. Gait began to recover one week after treatment in the deep EA and shallow EA groups.

3.2. Changes in the S-D Curve in the Four Groups. As shown in Figure 1, the upper segment of the S-D curve (0.1–0.5 ms) in each group was steeper, indicating that stimulus intensity decreased with prolonged stimulus time; the lower segment (0.6–1 ms) appeared flat, indicating that stimulus intensity remained constant when stimulus time was long. Under a certain stimulus time, the voltage which aroused excitability of the sciatic nerve in the four groups was the greatest in the model group, followed by the shallow EA group, the deep EA group, and the normal control group. Compared with the normal control group, the voltage in the model group increased significantly ($P < 0.01$); compared with the model group, the voltage reduced significantly in the deep EA group ($P < 0.05$), which showed that after two weeks of treatment, the best recovery of injured sciatic nerve was achieved in the deep EA group. The second best recovery of injured sciatic nerve occurred in the shallow EA group.

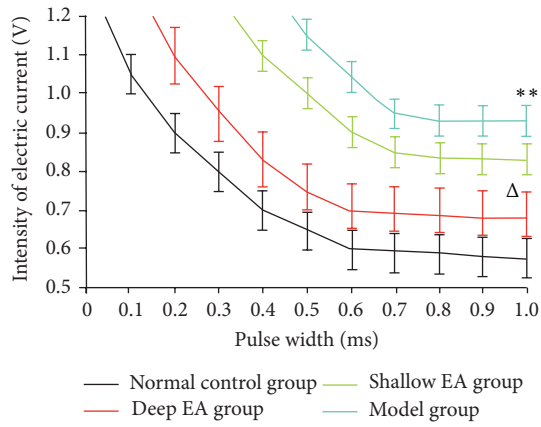

Figure 1: Effect of deep and shallow electroacupuncture (EA) stimulation of the ipsilateral "Huantiao" (GB 30) on the S-D curve in hip muscles of the rats with injured sciatic nerves ($\overline{x} \pm s$, 12 rats/group). $^{**}P < 0.01$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group.

3.3. Changes in Conduction Velocity of Sciatic Nerves in the Four Groups. As shown in Figure 2, the conduction velocity in the model group decreased ($P < 0.01$), suggesting segmental demyelination of most nerve fibers; compared with the model group, the conduction velocity in the deep and shallow EA groups increased significantly ($P < 0.05$); the conduction velocity in the shallow EA group was markedly lower than that in the deep EA group ($P < 0.05$).

3.4. Changes in the Number of Apoptotic Cells in the Injured Sciatic Nerves in the Four Groups. As shown in Figure 3, the number of apoptotic cells in the model group was increased compared with that in the normal control group ($P < 0.05$), while the numbers of apoptotic cells in the deep EA and shallow EA groups were significantly decreased compared with those in the model group ($P < 0.05$). The effect of deep EA was better than that of shallow EA ($P < 0.05$).

3.5. Changes in the Expression of Bcl-2 and Bax in Injured Sciatic Nerves in the Four Groups. As shown in Figures 4 and 5, the positive immunoreactivity of Bcl-2 and Bax proteins in the Schwann cell cytoplasm appeared brown. In the model

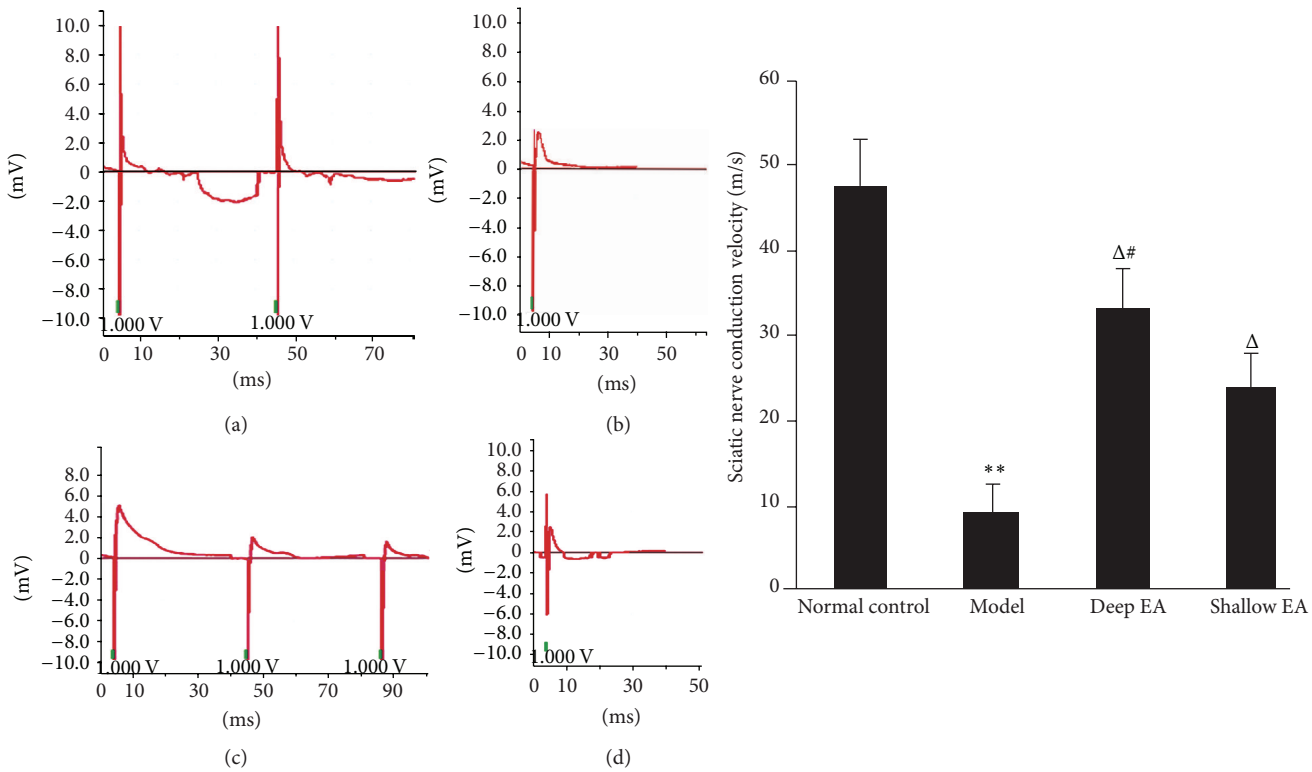

Figure 2: Effect of deep and shallow EA stimulation of ipsilateral GB 30 on the conduction velocity of the injured sciatic nerve in rats ($\bar{x} \pm s$, 12 rats/group). (a) Normal control group, (b) model group, (c) deep EA group, and (d) shallow EA group. $^{**}P < 0.01$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group; $^{\#}P < 0.05$ versus the shallow EA group.

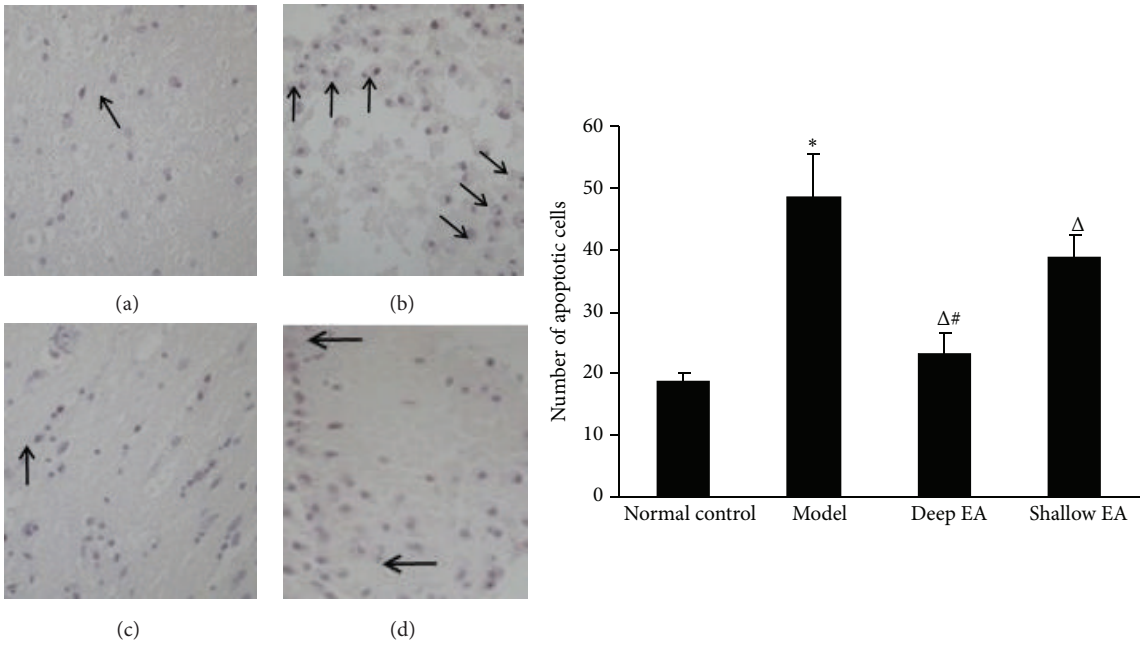

Figure 3: Effect of EA stimulation of ipsilateral GB 30 on number of apoptotic cells in the injured sciatic nerve in rats. Left panel: photos of TUNEL staining showing the number of apoptotic cells in the sciatic nerve (indicated by black arrowheads) in the normal control (a), model (b), deep EA (c), and shallow EA (d) groups (×200); right panel: bar graphs showing the number of apoptotic cells in the sciatic nerve in the four groups ($\bar{x} \pm s$, 8 rats/group). $^{*}P < 0.05$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group; $^{\#}P < 0.05$ versus the shallow EA group.

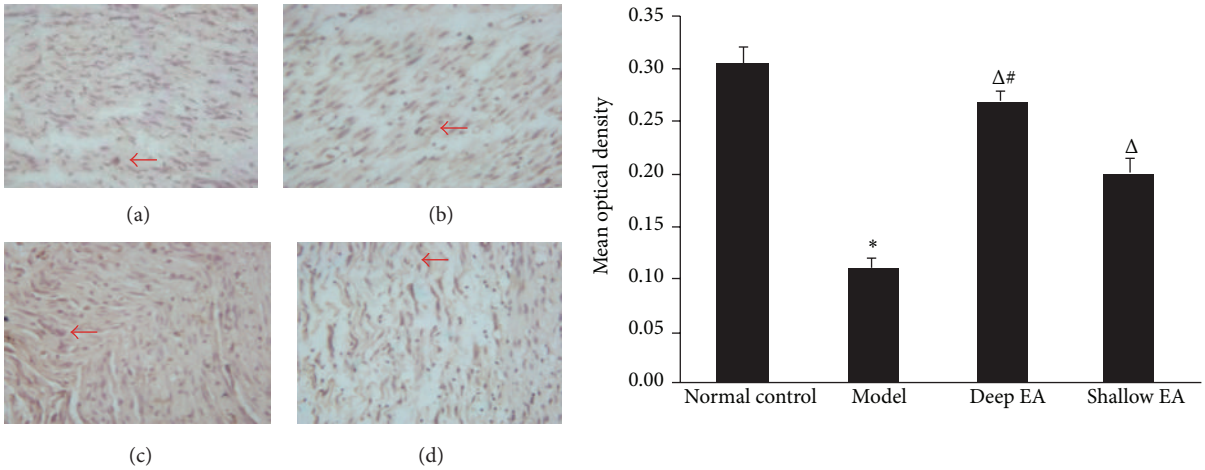

Figure 4: Effect of EA stimulation of ipsilateral GB 30 on Bcl-2 immunoreactivity in the injured sciatic nerve in rats. Left panel: photos of immunohistochemical staining showing the expression of Bcl-2 in the sciatic nerve (indicated by red arrowheads) in the normal control (a), model (b), deep EA (c), and shallow EA (d) groups (×200); right panel: bar graphs showing the expression levels (OD values) of Bcl-2 in the sciatic nerve in the four groups ($\bar{x} \pm s$, 8 rats/group). $^{*}P < 0.05$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group; $^{\#}P < 0.05$ versus the shallow EA group.

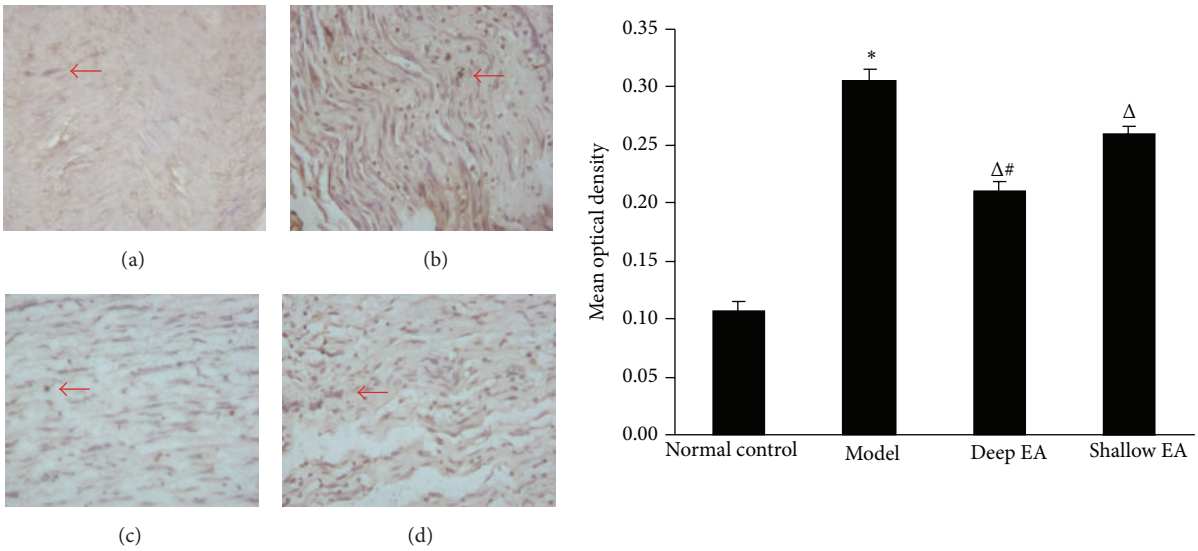

Figure 5: Effect of EA stimulation of ipsilateral GB 30 on Bax immunoreactivity in the injured sciatic nerve in rats. Left panel: photos of immunohistochemical staining showing the expression of Bax in the sciatic nerve (indicated by red arrowheads) in the normal control (a), model (b), deep EA (c), and shallow EA (d) groups (×200); right panel: bar graphs showing the expression levels (OD values) of Bax in the sciatic nerve in the four groups ($\bar{x} \pm s$, 8 rats/group). $^{*}P < 0.05$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group; $^{\#}P < 0.05$ versus the shallow EA group.

group, the expression of Bcl-2 in sciatic nerve was significantly lower than that in the normal control group, while the expression of Bax was significantly higher ($P < 0.05$); compared with the model group, the expression of Bcl-2 increased and that of Bax decreased in the deep EA and shallow EA groups ($P < 0.05$). The difference between the deep EA group and the shallow EA group was statistically significant ($P < 0.05$).

3.6. Changes in the Ratio of Bcl-2/Bax in the Injured Sciatic Nerves in the Four Groups. As shown in Figure 6, the ratio of Bcl-2/Bax in the model group was markedly lower than that in the normal control group ($P < 0.05$), while the ratios in the deep EA and shallow EA groups were significantly higher than those in the model group ($P < 0.05$). The ratio in the deep EA group was higher than that in the shallow EA group ($P < 0.05$).

4. Discussion

This study was designed to investigate the biological mechanism involved in the differences in repair of sciatic nerve

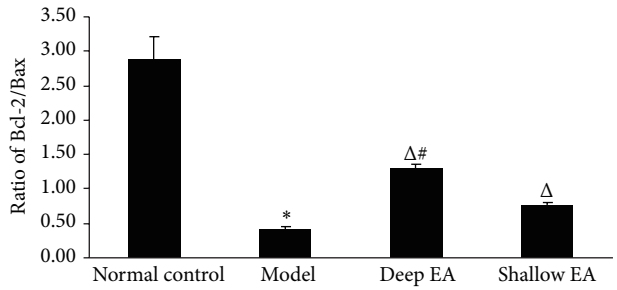

Figure 6: Effect of deep and shallow EA stimulation of ipsilateral GB 30 on the ratio of Bcl-2/Bax of the rats with injured sciatic nerves ($\overline{x} \pm s$, 8 rats/group). $^{*}P < 0.05$ versus the normal control group; $^{\Delta}P < 0.05$ versus the model group; $^{\#}P < 0.05$ versus the shallow EA group.

injury by deep EA and shallow EA at GB 30. The results showed that, compared with shallow EA, deep EA at GB 30, when the nerve trunk was reached, had a significantly better effect on the recovery of injured sciatic nerve. Deep EA improved the excitability of the nerve and promoted the recovery of motor nerve conduction, which may have been achieved by the increased expression of Bcl-2 and reduced expression of Bax, resulting in fewer apoptotic cells.

Bcl-2 gene families play important roles in apoptosis. According to their different roles, they are divided into two categories, one category can promote cell apoptosis; the other can inhibit cell apoptosis. Bcl-2 and Bax of the Bcl-2 gene families are two protein expression products closely related to apoptosis [14]. Bcl-2 is a type of membrane stabilizing protein related to organelles, especially mitochondria, and is mainly found in the outer membrane of mitochondria, endoplasmic reticulum membrane, and nuclear membrane. Inhibition of apoptosis can be achieved by inhibiting permeability of the mitochondrial membrane, maintaining the stability of the membrane, preventing the release of cytochrome C, and inhibiting the activation of Caspase by inhibition of free radical generation and intracellular calcium overload caused by Bcl-2. Cell damage can be reduced by the overexpression of Bcl-2 [15–17]. In contrast, the biological effect of Bax differs from that of Bcl-2 and can promote apoptosis [18, 19]. Bcl-2 and Bax may interact as a dimer. When Bcl-2 increases, the Bcl-2 homodimer is formed, and cells are protected; when Bax increases, the Bax/Bcl-2 heterodimer is formed, and cell apoptosis occurs [20–22]. Thus, the ratio of Bcl-2/Bax directly determines cell survival. The number of apoptotic cells is the balanced result of the two regulation factors, Bcl-2 and Bax [23]. It is generally recognized that the main form of neuronal cell death caused by peripheral nerve injury is apoptosis, a process of programmed cell death involving a variety of physiological and pathological factors and initiated by apoptosis-related genes [24–26]. Kotulska et al. [27] suggested that the expression of Bcl-2 and Bax is closely related to the recovery and viability of neurons after peripheral nerve injury as well as fiber regeneration and myelination. In this study, the function of the sciatic nerve in rats in each group varied due to changes in Bcl-2 and Bax at the injured site, which is consistent with previous results.

The S-D curve is used to evaluate the function of innervation by stimulating muscle with a square wave current of different pulse width. It is reflected by the curve of threshold of current strength when the muscle is excited. The lighter the nerve injury, the less denervation, and the lower the S-D curve, the lower the stimulus intensity to cause nerve-muscle excitation. The results of this study showed that in the deep EA group, the current intensity to induce nerve excitement was markedly lower than that in the model group, indicating better efficacy of deep EA in promoting the recovery of injured sciatic nerve. As a direct manifestation of nerve impulse conduction, the conduction velocity can reflect the regeneration conditions of nerve fibers to some extent. The nerve trunk cannot be completely destroyed by clamping sciatic nerves. Therefore, the rats, in which nerve conduction was completely blocked and the electric waves could not be displayed, were excluded from this study.

The treatment of flaccidity syndrome by acupuncture at GB 30 can be traced back to the *Internal Canon of Medicine*. The depth of acupuncture may determine its clinical efficacy [28, 29]. In recent years, the depth of acupuncture at GB 30 has been increased. It is proposed that 2-3 cun is appropriate to ensure that the nerve trunk is reached [30–32]. Studies have shown that deep acupuncture at GB 30 may have immediate and remarkable therapeutic and analgesic effects on sciatic nerve injury [33]. Research on the related mechanisms suggests that, for sciatic nerve injury in rabbits, acupuncture therapy can increase the content of acetylcholine esterase (AchE) in the intumescentia lumbalis of the spinal cord. By promoting the synthesis and release of AchE, nerve excitability was maintained thus promoting the recovery of nerve function [34]. EA can increase the content of 1L-1β in spinal cord tissue and stimulate the production of nerve growth factors (NGFs) [35], thus strengthening the transcription of NGF mRNA and exerting a positive effect on the repair of injured sciatic nerves [36]. The results of this study showed that, compared with shallow EA, deep EA at GB 30 better adjusted the substances related to the regulation of apoptosis, thus significantly improved the excitability and conduction of the sciatic nerve.

5. Conclusion

Better functional recovery of injured sciatic nerve may be achieved by acupuncture at GB 30 when the nerve trunk is reached. By stimulating the axis cylinder with continuous

and chronic demyelination, strong stimulation is generated, triggering strong nerve impulses to transfer nerve substances, which can inhibit the apoptosis of nerve cells in the injured area and promote tissue repair.

Conflict of Interests

All authors declare that they have no conflict of interests.

Authors' Contribution

The first two authors Lili Dai and Yanjing Han contributed equally to this work.

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Research Article

Function of Nucleus Ventralis Posterior Lateralis Thalami in Acupoint Sensitization Phenomena

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To observe the effect of electroacupuncture (EA) on nucleus ventralis posterior lateralis (VPL) thalami activated by visceral noxious stimulation and to explore the impact of EA on the mechanism of acupoint sensitization under a pathological state of the viscera, EA was applied at bilateral "Zusanli-Shangjuxu" acupoints. The discharge of VPL neurons was response to EA increased after colorectal distension (CRD). The stimulation at "Zusanli-Shangjuxu" acupoints enhanced discharge activity of VPL neurons under CRD-induced visceral pain. The frequency of neuronal discharge was associated with the pressure gradient of CRD which showed that visceral noxious stimulation may intensify the body's functional response to stimulation at acupoints.

1. Introduction

Acupoints are special locations on body surfaces where the Qi of meridians and internal organs is infused. They are also the key link underlying the interactions between meridians and internal organs. When internal organs are under a pathological state, acupoints become more sensitive [1–3]. The size and function of acupoints change accordingly with the change of visceral functions [4, 5]. Therefore in pathological conditions, the diagnostic and therapeutic effects of acupoints on visceral diseases are enhanced [6].

Spinothalamic tract is traditionally viewed as the major pathway of noxious inputs. Previous studies showed [7–10] that noxious inputs transmitted via spinothalamic tract can be affected by other noxious inputs. A current issue in neuroscience research is the mechanism underlying the peripheral and central sensitization caused by different noxious inputs [11].

This study evaluated the neuronal discharge of ventral posterior lateral nucleus (VPL; the most important brain center for somatovisceral relay) by noxious inputs from the body surface and colorectal distension (CRD). We also observed whether the effect of acupuncture on the receptive field (acupoint area) of VPL neurons on body surface was affected by visceral noxious inputs. The phenomena and mechanism of acupoint sensitization at the VPL level induced by visceral noxious inputs will be discussed.

2. Materials and Methods

2.1. Experimental Animals. Twenty-six healthy male Sprague-Dawley rats weighing 250–300 g were provided by the Laboratory Animal Center of Academy of Military Medical Sciences (animal certificate Lot Scxr (Beijing) 2009-0017). Before experiments, the animals were fasted for 12 hours but they were not deprived of water. Throughout the experiment, the animals' body temperature was maintained between 36 and 38°C by a temperature control device (Model: CL-8; Manufacturer: RWD/China). Animal experimental methods, experimental purposes, and the disposal of animals in experiment followed the *Guidelines on Proper Care of Experimental Animals* promulgated in 2006 by the Ministry of Science and Technology.

2.2. Experimental Methods

2.2.1. Recording Discharge of Thalamic VPL Neurons. Rats were anesthetized with an intraperitoneal injection of 10% urethane (1.0~1.2 g/kg, provided by Shanghai Sinopharm Chemical Reagent Co., Ltd.). The heads of rats were fixed on stereotaxic instruments. We incised the skin of the middle of skull and the suture was exposed by removing the resubcutaneous tissue and periosteum. Then we should adjust the frontal and back suture located in a horizontal plane. The three-dimensional location coordinates of VPL nuclei were determined according to Rat Brain Atlas [12] 3.0~4.0 mm behind the anterior fontanel, 3.0~3.5 mm next to skull sutures. Under observation with a surgical microscope, the tip of the glass microelectrode was inserted to VPL nuclei through the skull hole by the microelectrode manipulator (5000~5800 μm beneath the surface of the brain). Impedance at the tip of the glass microelectrode was set at 10–15 MΩ (filled with 2% pontamine sky blue). When the target neurons were identified, 2% agar was perfused onto the skull surface to protect the brain tissue from drying and reduce volatility caused by breathing.

For all recorded neurons, the responses to mechanical stimulations applied to their peripheral receptive field were checked to identify the distribution and size of the receptive fields (mechanical stimulations include touch and pressure by von Frey hairs (von Frey Model 2390; U.S. IITC Company), skin stimulation by tooth tweezers, and acupuncture stimulation). We also observed responses of these neurons to CRD. Only neurons that responded to both the mechanical stimulation on the skin receptive field and the 10 mmHg of CRD were included as the objects of observation (and were named as convergent neurons or CN).

2.2.2. Colorectal Distension. A 4 to 6 cm long balloon was made from a disposable condom tip and tied on a 4 mm diameter hose (Figure 1; BIOPAC Amplifier Module Model: MP150 System TSD104A; Manufacturer: BIOPAC Company, USA). The balloon was inserted through the anal orifice straight into the colon. The depth was approximately 4 cm. Three to five drops of the warmed paraffin oil were smeared on the balloon's surface before the balloon was placed into the colon to avoid direct damage to the inner wall of the colon and anus. The distance from balloon end to anus was about 0.5 cm. 20–80 mmHg CRD stimulus was given via a syringe, with the duration of about 30 s. The activation of convergent neurons was observed at different intensities of CRD stimulation. Pressure ≥ 40 mmHg was identified as visceral noxious stimulation [13]. The time interval between CRD stimulations was no less than 10 min to avoid colorectal sensitization caused by hyper stimulation.

2.2.3. EA. EA was applied at bilateral "Zusanli-Shangjuxu" points. The stimulation was set as a square wave pulse with a width of 5 ms and frequency of 20 Hz. The intensity was 1.5 times of the threshold of Aδ fiber [14] (the average threshold intensity of Aδ fiber reflex was 1.54 ± 0.50 mA) and the time for EA was 30 s. The discharge of VPL neurons to EA was observed before and after CRD.

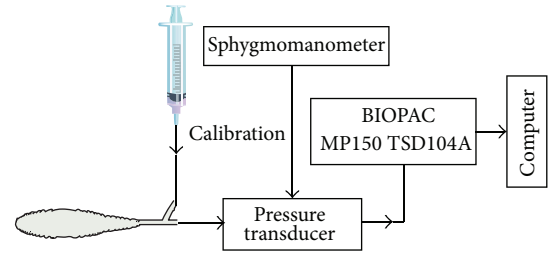

Figure 1: Experimental layout of colorectal distension.

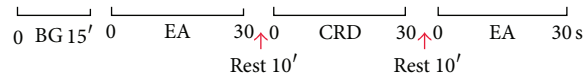

Figure 2: Experimental flow chart.

2.3. Experimental Procedure. (1) The background discharge of convergent neurons was recorded for 10–15 min using microelectrode amplifier (Model: MEZ 8201; Manufacturer: Nihon Kohden) and biological signal acquisition and analysis system (Model: MICRO 1401; Manufacturer: British CED Company). (2) EA was applied at bilateral "Zusanli-Shangjuxu" points for 30 seconds. (3) After an interval of 10 minutes, different intensities of CRD were given to rats for 30 seconds. The discharge of the convergent neurons to non-noxious stimuli (20 mmHg), noxious stimuli (40 mmHg), and strongly noxious stimuli (60, 80 mmHg) was recorded, respectively, to observe the activation of convergent neurons by different intensities of CRD. (4) After an interval of 10 minutes, EA was once again applied at bilateral "Zusanli-Shangjuxu" points for 30 seconds. The discharge of VPL neurons in response to EA stimulation before and after different intensities of CDR was observed to test the dose-effect relationship between stimulus intensity and response (Figure 2).

2.4. Statistical Analysis. The data was analyzed with Spike-II (the data analysis software of MICRO 1401 biological signal acquisition and analysis system) and SPSS 13.0 software. The number of neuronal discharge of VPL neurons in every 30 seconds and the activation/inhibition rate were counted and the mean and variance of neuronal discharge before and after the EA intervention were calculated. Comparison between groups was made with independent sample t-test. $P < 0.05$ was considered as statistically significant.

2.5. Histological Localization. When recording of neuronal discharge was completed, 20 μA of negative direct current was passed to the glass microelectrode via the microelectrode amplifier for 20–30 min. Pontamine sky blue in the glass microelectrode was imported into VPL nuclei to mark the position of recording electrode. Thereafter, the rats were euthanized and perfused through the heart with 4% of paraformaldehyde. Then the rats' brains were removed and fixed. After an interval of 72 hours, frozen sections of the brain were cut for H&E staining (Figure 3). Recording points that were not located in the VPL nuclei were removed from the study.

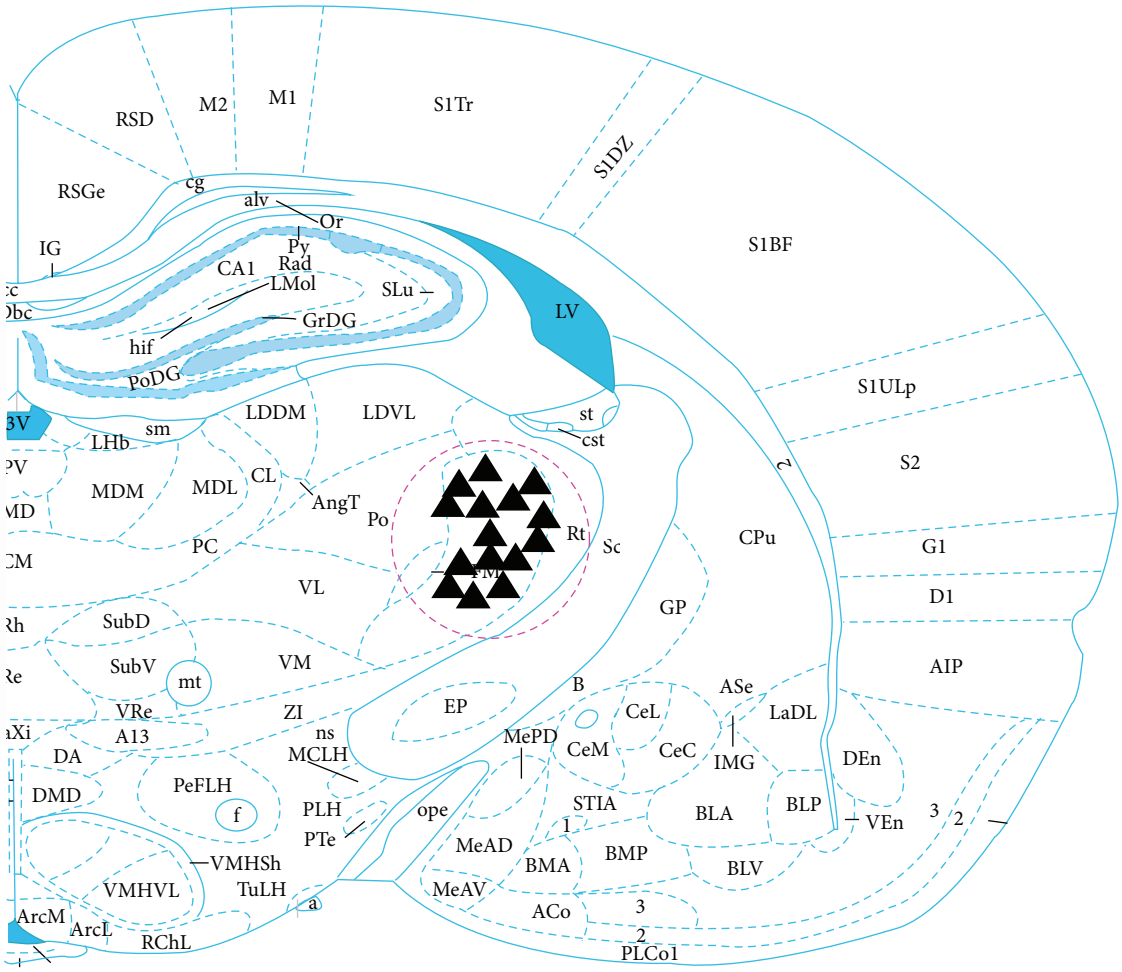

Figure 3: ▲ refers to the location of VPL neurons.

3. Results

3.1. General Characteristics of the Responses of VPL Neurons. A total of 126 VPL neurons that responded to mechanical stimulations from the body surface were identified in the 26 male SD rats, by referring to the Brain Atlas of the rat [14]. Figure 3 illustrates part of the pontamine sky blue positioning of VPL neurons. Their receptive fields were distributed at the poster lateral of the contralateral body, tail, hips, or hind legs. The receptive fields of most neurons were small but had clear boundaries. The receptive fields could be activated by gentle brushing or tapping by von Frey filaments (Figure 4).

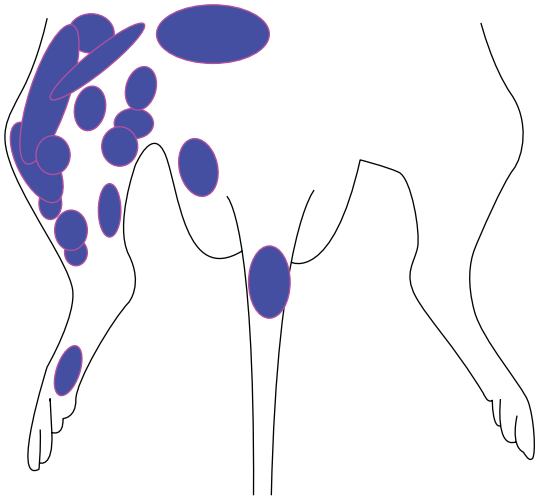

Figure 4: Distribution of the receptive fields of partial VPL neurons.

3.2. The Influence of Different Intensities of CRD on the Discharge of VPL Neurons. We isolated 54 convergent neurons from the 126 VPL neurons that responded to inputs of mechanical stimulation and systematically observed the discharge of 9 of the 54 convergent neurons caused by different intensities of CRD stimulation. The results showed that, after CRD stimulation ranging from 20 to 80 mmHg, the discharge frequency of VPL neurons significantly increased in rats more than before CRD stimulation ($P < 0.01$) (Figure 5).

3.3. The Influence of Different Intensities of CRD on the Discharge Frequency of VPL Neurons Induced by EA. The discharge of 45 convergent neurons was observed when rats were given different intensities of CRD. Among them, 12 convergent neurons were chosen from rats receiving 20 mmHg of nonnoxious CRD, 11 from rats receiving 40 mmHg of noxious

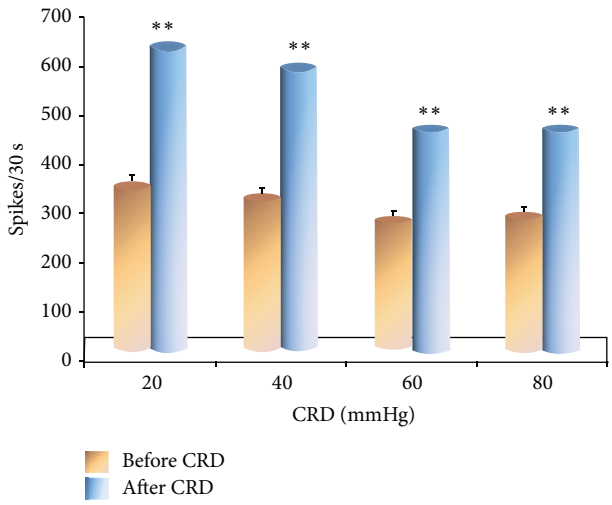

Figure 5: Discharge of VPL neurons before and after CRD.

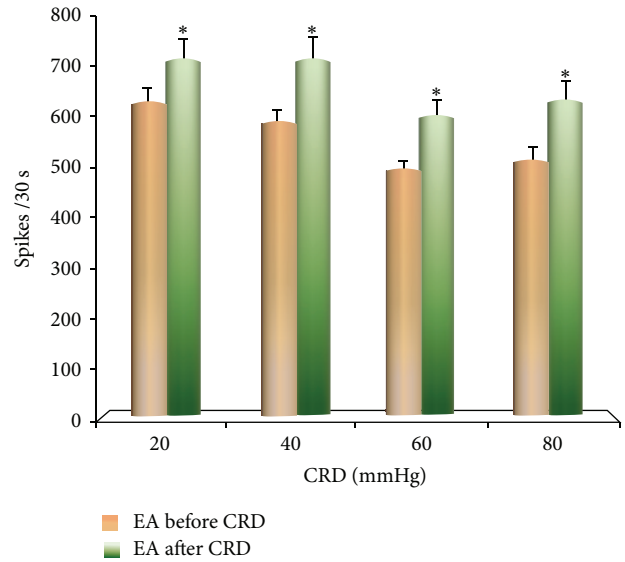

Figure 6: The influence of EA on the discharge of VPL neurons before and after CRD.

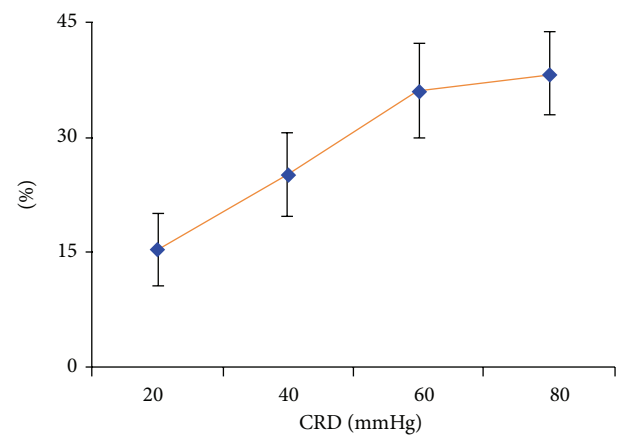

Figure 7: The influence of different intensities of CRD on VPL neuronal discharge induced by EA.

CRD, 12 from rats receiving 60 mmHg of strong nociceptive CRD, and 10 from rats that were given 80 mmHg of strong nociceptive CRD.

Equal intensities of EA were given to rats for 30 seconds before and after CRD. The results showed that the discharge frequency of VPL convergent neurons induced by EA increased significantly after CRD more than before CRD when rats were given different intensities of CRD ($P < 0.05$) (Figure 6).

3.4. The Influence of Different Intensities of CRD on the Discharge Number of VPL Neurons Induced by EA. After rats were given CRD, the discharge from VPL convergence neurons induced by EA increased over the discharge before CRD: 20 mmHg–15.38% ± 8.27; 40 mmHg–25.22% + 7.80; 60 mmHg–36.28 + 8. 18; 80 mmHg–38.40 + 8.32. Differences were statistically significant ($P < 0.05$).

As the intensity of CRD stimulation increased, there also was an increase in the percentage of the discharge number of VPL neurons from EA at acupoints. A certain dose-effect relationship could be observed between stimulation and response. It showed that acupoints on the body surface were sensitized after CRD. The effect of EA on acupoints was enhanced. The sensitization of acupoints increased as the intensity of visceral noxious stimulation increased (Figure 7).

The above results showed that noxious visceral stimulation facilitated the responses of VPL neurons to inputs of EA stimulation from acupoints on body surface.

4. Discussion

Our previous studies have shown that most neurons which responded to somatic afferent inputs also responded to inputs from CRD or skin vibrotactile stimulation. In most cases, the response was shown as sensitization of neurons. Responses of more than 50% of neurons to skin vibrotactile stimulation could be enhanced by CRD previously applied to experimental animals [15].

Results of this study showed that, within a certain intensity range, the discharge frequency of VPL convergent neurons increased as the intensity of CRD stimulation increased. Since CRD had an activation effect on spinal cord neurons, when EA was applied at acupoints after CRD, the discharge of VPL convergent neurons had a significant increase more than before CRD. This confirms that noxious visceral distension can sensitize VPL neurons making them respond more strongly to inputs from EA applied to acupoints on skin receptive fields. In other words, the neural facilitation of VPL neurons after noxious visceral stimulation led to dynamic changes of the response from the acupoint sensitized. As the intensity of visceral noxious stimulation increased, its effect on sensitization of acupoints on body surface also strengthened and showed a clear dose-response relationship. Our results show that VPL neurons are involved in the dynamic process of acupoint sensitization.

The thalamus is the most important brain structure to relay somatic and visceral afferent inputs to the cerebral cortex. There are three projection systems from the spinal cord to the bottom part of ventral thalamus: the spinothalamic tract, the cervical spinal column, and the postsynaptic dorsal column ascending fibers. A study by Yang et al. [16] on VPL of rats showed that 94% of VPL neurons could be activated by nonnoxious and noxious stimuli applied at peripheral receptive fields, whereas 6% of VPL neurons only responded to noxious stimulation. No VPL neurons responded only to nonnoxious stimulation. Nearly 60% of VPL neurons also responded to CRD, primarily with activation. VPL neurons, therefore, are involved not only in the transmission and processing of somatic sensory inputs, but also in the transmission and processing of visceral nociceptive inputs.

We observed in the rat thalamus VPL experiment that most neurons that responded to haptic inputs from contralateral body also responded to CRD and skin vibration tactile stimuli. The responses of more than half of the neurons to skin vibrotactile stimulation could be enhanced by CRD conditioned stimulation applied previously. In contrast, the responses of VPL neurons to CRD were not enhanced by skin tactile stimulation, if the order of conditioned stimulation was reversed; that is, the skin stimulation was given before CRD. Moreover, the effect was mainly shown as an inhibitory effect. A possible explanation for acupoint sensitization is that repeated CRD may cause the irritability of intestinal wall, which can be viewed as one type of visceral inflammation, and induces sensitization of afferent neurons [17]. Visceral noxious stimulation could also significantly enhance neuronal responses to skin tactile stimulation. The enhancement effect may be related with hyperalgesia caused by visceral disease [18, 19].

Many previous studies suggest that only noxious stimulation can significantly inhibit the afferent transmission of nociceptive inputs [18]. However, we observed that, at the single cell level, a gentle touch on skin could produce inhibitory effect on hypothalamic neurons' response to CRD, though this inhibitory effect was usually mild and transient. The conditioned stimulation of CRD significantly improved the responses of thalamic neurons to tactile inputs. The facilitation effect was related with the activity of excitatory intermediate neurons. That is, excitatory intermediate neurons could enhance the after-effects of excitatory responses caused by CRD and prolong the discharge duration of VPL neurons. If skin tactile stimulation was given after CRD, the discharge number of VPL neurons was higher than the discharge number when only CRD or skin tactile stimulation was given. The excessive sensitivity of central neurons to skin tactile stimulation may be related with hyperalgesia [18, 19]. Though only a few such sensitive points were found on skin receptive fields, the sensitization effect was lasting and was longer than the effect directly caused by skin stimulation. The effect also lasted significantly longer than the inhibitory effect of tactile stimulation on CRD response [17]. In this case, visceral nociceptive inputs had a stronger effect on the tactile inputs than the other way around, at least at the single cell level of thalamus VPL neurons. However, it should be emphasized that the perception of the visceral pain depends on the group response of neurons, which includes the interaction and feedback among nerve centers at cerebral cortex, thalamus, and other areas.

Our study showed that nociceptive stimulation of CRD could make VPL neurons more sensitive to EA stimulation applied at skin receptive fields. It indicates that viscera pathological condition can facilitate the afferent inputs from stimulation at the body surface. The interaction between somatic and visceral inputs occurs at the lumbosacral segments of the spinal cord. The segments (L1–L3) not only integrate information from the skin on lower abdomen and hind legs, but also are the location of afferent neurons for "Zusanli-Shangjuxu" points which were elected in our experiment and dominate the lower digestive tract. Many sensitive points on body surface are distributed at relevant acupoint zones that have a regulatory effect on digestive system functions. The phenomenon that visceral nociceptive inputs can facilitate the neural responses to afferent inputs from the body surface at corresponding spinal segments may be related with the mechanism underlying referred pain. It also provides a scientific explanation for the Chinese medical theory of "pain as acupoints" and "essence of acupuncture points."

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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Review Article

Analysis and Thoughts about the Negative Results of International Clinical Trials on Acupuncture

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An increasing number of randomized controlled trials (RCTs) of acupuncture have proved the clinical benefits of acupuncture; however, there are some results that have shown negative results or placebo effects. The paper carried out an in-depth analysis on 33 RCTs in the 2011 SCI database, the quality of the reports was judged according to Jadad scores, and the "Necessary Information Included in Reporting Interventions in Clinical Trials of Acupuncture (STRICTA 2010)" was taken as the standard to analyze the rationality of the therapeutic principle. The difference between the methodology (Jadad) scores of the two types of research reports did not constitute statistical significance ($P > 0.05$). The studies with negative results or placebo effects showed the following deficiencies with respect to intervention details: (1) incompletely rational acupoint selection; (2) inconsistent ability of acupuncturists; (3) negligible needling response to needling; (4) acupuncture treatment frequency too low in most studies; and (5) irrational setting of placebo control. Thus, the primary basis for the negative results or placebo effects of international clinical trials on acupuncture is not in the quality of the methodology, but in noncompliance with the essential requirements proposed by acupuncture theory in terms of clinical manipulation details.

1. Introduction

As an integral part to the Chinese medical and health care system, acupuncture therapy is widely applied in clinical applications, effective in treatment, economical, and safe and thereby generally accepted by Chinese people. Since the sixth century AD, acupuncture has successively spread to various countries of the world, making considerable contributions to relieving people from diseases worldwide. Along with the development of evidence-based medicine, international clinical trials on acupuncture have been increasing in number and raising greater controversies on whether or not acupuncture is effective. While the majority of international clinical trial reports on acupuncture have demonstrated that acupuncture therapy is indeed effective, some research has shown that acupuncture therapy benefits patients, but is equivalent to the placebo effect [1], and some people consider acupuncture therapy to be ineffective [2]. Currently, it is widely believed that such a result is a product of the higher-quality methodology for international randomized controlled trials (RCTs) of acupuncture. The purpose of the current study was to determine the basis for the negative results or placebo effects in published acupuncture RCTs from the perspective of methodology and interventions after comprehensively reading and analyzing the published acupuncture RCTs retrieved from the 2011 SCIE database, with the exception of research conducted in China.

2. Materials and Methods

2.1. Search Strategy. The computer retrieval was carried out in "Science Citation Index Expanded (SCIE)" and the retrieval type was "('acupuncture' [MeSH Terms] OR 'acupuncture' [All Fields] OR 'acupuncture therapy' [MeSH Terms] OR ('acupuncture' [All Fields] AND 'therapy' [All Fields]) OR 'acupuncture therapy' [All Fields] OR ('moxibustion' [MeSH Terms] OR 'moxibustion' [All Fields] AND '2011/1/1' [PDat]: '2011/12/31' [PDat] AND English [lang])."

2.2. Inclusion Criteria. The inclusion criteria were as follows: (1) randomized controlled acupuncture, acupressure, or

Table 1: Jadad score standard.

Items	Score standard		
	0	1	2
Randomization	Not randomized or inappropriate method of randomization	The study was described as randomized	The method of randomization was described and it was appropriate
Double blinding	Not blind or inappropriate method of blinding	The study was described as double blind	The method of double blinding was described and it was appropriate
Withdraws and drop outs	Not describing the follow-up	A description of withdraws and dropouts	—

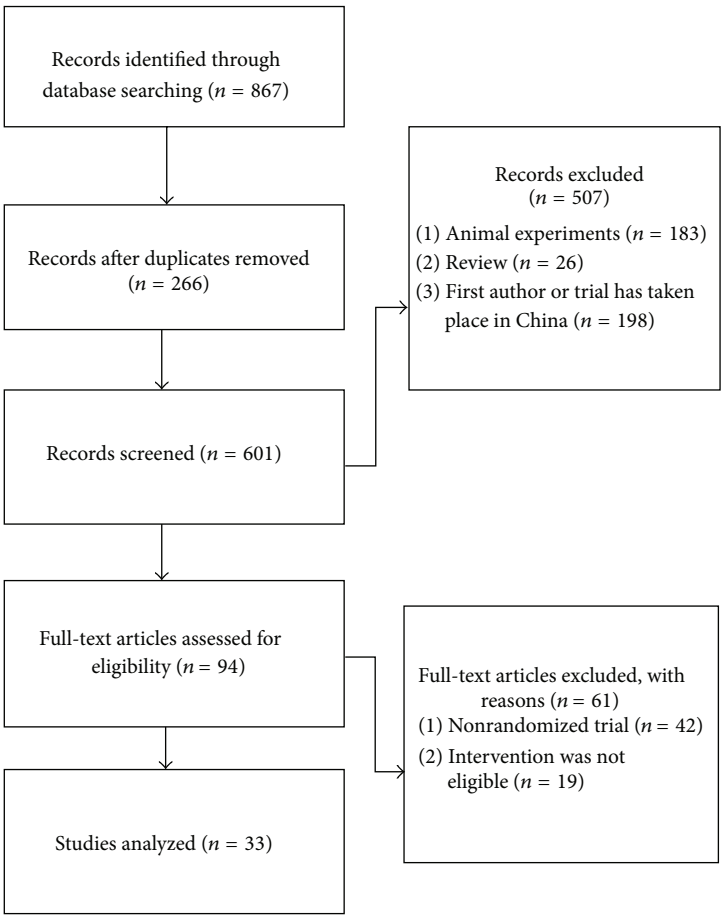

Figure 1: The flow diagram of the research.

moxibustion trials published in 2011 from the SCIE database; (2) patients underwent the trial regardless of age, gender, ethnicity, or course of disease; and (3) intervention of the observation or controlled group was based on the theory of meridians and collaterals and the patients were treated by acupuncture, acupressure, and/or moxibustion.

2.3. Exclusion Criteria. The exclusion criteria were as follows: (1) nonrandomized trials; (2) nonclinical trials; (3) the intervention did not conform to the objective of the research; (4) duplicated articles; and (5) the first author was from China or the trial was conducted in China.

2.4. Data Collection and Analysis. Evaluation was performed independently by two authors (Yang Hao and Wan-ning Liu). Relevant full articles were sorted and cross-examined. Any discrepancies were discussed or further evaluated by a 3rd author (Wei-hong Liu). Methodology was evaluated based on the Jadad score [3]. The specific evaluation standard is shown in Table 1.

3. Results

3.1. Articles Included. Of the 867 articles retrieved, 33 studies [2, 4–35] met the inclusion criteria for the current analysis (Figure 1).

3.2. Features of the Studies. Features of the included trials are detailed in Table 2.

3.3. Jadad Score of the Trials. According to the research results, the 33 reports were classified into two types (positive results and negative results or placebo effects). The 33 reports were read and the key points were extracted. According to the Jadad score, the lowest methodology quality was scored 0, while the highest methodology quality was scored 5. The clinical trial was considered low in quality if the score was ≤2 and was considered high in quality if the score was ≥3. The Jadad scores of the research report methodologies are shown in Table 3, and the Jadad score comparison of the acupuncture RCT methodologies is shown in Table 4.

By adoption of SPSS 13.0, data in Table 4 was subjected to a χ^2 test; the difference between the two groups was not statistically significant ($P = 1.0$). The quality of the clinical trial report methodology on acupuncture with positive results is similar to the clinical trial reports with negative results or placebo effects, which indicates that the difference in quality of the methodology is not the primary reason for the different clinical research results of acupuncture.

3.4. Analysis of Acupuncture RCTs Intervention Details with Positive Results, Negative Results, and Placebo Effects. The intervention details of the RCTs with positive results, negative results, and placebo effects were compared. Due to the small number of RCTs with negative results or placebo effects collected in 2011, the acupuncture RCTs from 2005 to 2010 in the SCIE database were retrieved. The retrieval terms were as follows: "acupuncture" [MeSH terms] OR "acupuncture" [all fields] OR "acupuncture therapy" [MeSH terms] OR "acupuncture" [all fields] AND "therapy" [all fields] OR "acupuncture therapy" [all fields] OR "moxibustion" [MeSH terms] OR "moxibustion" [all fields] AND "2005/1/1" [PDat]: "2010/12/31" [PDat] AND randomized controlled trial [PDat] AND English [lang]. Seven reports [36–42] with negative results or placebo effects were analyzed together with the 10 reports with negative results or placebo effects.

According to the "Necessary Information Included in Reporting Interventions in Clinical Trials of Acupuncture (STRICTA 2010)," the authors designed an intervention table for the RCTs and compared the intervention details of the RCTs with positive results, negative results, and placebo effects. The detailed information of the reports is shown in Tables 5 and 6.

It is known that the clinical treatment process of acupuncture involves not only the operation of acupuncture–moxibustion therapy, but also the rational selection of therapeutic principles and methods, rational application of acupoints, manipulation, and the correct setting of the therapy, and so on. It is apparent from the analysis of items displayed in Tables 5 and 6 that the intervention process in the negative results and placebo effects was replete with defects.

3.4.1. Interventions of Some Trials Are Improper. To select a proper therapeutic method is the key to assuring a curative effect; however, the authors showed that some interventions in the 17 reports were improper. For example, in item 001, moxibustion was adopted to treat constipation; in item 009, which involved the treatment of postmenopausal women suffering knee joint pain, women without medical knowledge performed acupressure by themselves at home; and in item 005, when treating the nausea and vomiting associated with labor and delivery, only a wrist band that slightly stimulates PC 6 was used. Despite certain therapeutic function, these measures are all not the most proper choice. For example, constipation is most often treated clinically with acupuncture; however, for excess syndrome or heat syndrome constipation, it is improper to use moxibustion. Similarly, it is doubtful that the wrist band which stimulates PC 6 is satisfactory to achieve a therapeutic effect like acupuncture. With respect to the studies with positive results, the applied methods were effective intervention, such as filiform needles, electroacupuncture, blunt needles, or auricular acupuncture. Clearly, effective intervention is an important factor for the results of the trial.

3.4.2. Acupoint Selection in Some Studies Is Not Completely Rational. Each acupoint has its therapeutic effect. Rich experiences in acupuncture have been accumulated through the inheritance for thousands of years in China. For example, the most effective acupoints for constipation treatment are ST25, ST36, ST28, ST29, and TE6, while the researcher for item 001 selected ST23 and ST27. Clinically, ST23 is often involved in the treatment of gastric diseases or mental diseases, such as vexation and manic-depressive psychosis, while ST27 is mainly used in the treatment of hypogastrium distention and fullness, difficult urination, hernia, spermatorrhoea, premature ejaculation, and other male diseases. The selection of improper acupoints results in a low clinical curative effect. In item 003, the researcher only selected LI4 to treat infantile colic. LI4 belongs to the large intestine meridian and plays a role in treating intestinal disease, but it is clinically known for its effect on head/face diseases, sweating disorders, and gynecologic diseases, including menstrual disorders, vaginal discharge, and parturition. If GV12 and ST36 are added in the prescription, the effect would be significantly improved. Thus, the researcher may select a single acupoint by reducing confounding factors in interventions as much as possible, but not considering that acupuncture therapy needs the compatibility of acupoints for enhancement of effect and improvement of curative effect in a practical environment. The acupoints selected in the studies with positive results were correct and most effective according to clinical experiences. The comparison indicated that the rational selection of acupoints is directly related to the validity of the trial.

3.4.3. Needling Response Is Neglected in Most Studies. The famous acupuncture work, *Biaoyoufu,* in ancient China says, "Quick needling response results in quick action, otherwise the late needling response causes treatment failure," which means that the patients' meridian-*qi* circulation should be considered when needling to realize the needling response. Of 17 reports with negative results or placebo effects, 65% (11/17) did not mention whether or not the needling response

Table 2: Features of the studies.

Number	Title	Disease	Intervention of the observation group	Intervention of the control group	Sample size	Outcome	Follow-up phase
001	"The Effectiveness of Moxibustion for the Treatment of Functional Constipation: A Randomized, Sham-Controlled, Patient Blinded, Pilot Clinical Trial"	Constipation	Moxibustion	Heat insulation moxa-moxibustion (place a heat insulator under the true moxa-moxibustion)	26	Invalid	2 weeks
002	"The efficacy of Acupressure at the Sanyinjiao Point in the Improvement of Women's General Health"	Woman health	Acupressure at SP 6	Sham acupuncture	86	Both valid, the real is the better	No mention
003	"Preliminary Research Article Electroacupuncture Is Not Effective in Chronic Painful Neuropathies"	Chronic neuralgia	Electroacupuncture for 12 weeks	Press the nonacupoint (not touch the tendo calcaneus in any meridian vessel on the back of leg)	16	Invalid	No mention
004	"Feeding, Stooling and Sleeping Pattern in Infants with Colic- A Randomized Controlled Trial of Minimal Acupuncture"	Infantile colic	Acupuncture	Blank	90	The symptoms improved, but no statistical difference	No mention
005	"The Effect of Acupuncture on Psychosocial Outcomes for Women Experiencing Infertility: A Pilot Randomized Controlled Trial"	Women experiencing infertility	Acupuncture	No intervention	32	Valid	No mention
006	"Effect of Acupuncture on Nausea and/or Vomiting during and after Cesarean Section in Comparison with Ondansetron"	Nausea and vomiting when delivery	Wrist band stimulative to acupoint	Wrist band nonstimulative to PC 6	450	Invalid	No mention
007	"Acupuncture in Children and Adolescents with Bronchial Asthma: A Randomised Controlled Study"	Bronchial asthma	Acupuncture	Conventional treatment of western medicine	93	Partial response	4 months
008	"True and Sham Acupuncture Produced Similar Frequency of Ovulation and Improved LH to FSH Ratios in Women with Polycystic Ovary Syndrome"	Polycystic ovarian syndrome	Electroacupuncture	Stick on the skin by sham needle tubing and adjust the current to 0	96	Both valid	3 months
009	"Effects of Auricular Acupuncture on Anthropometric, Lipid Profile, Inflammatory and Immunologic Markers: A Randomized Controlled Trial Study"	Obesity	Auricular acupuncture	Sham auricular acupuncture	204	Valid	No mention
010	"Acupuncture for "Frequent Attenders" with Medically Unexplained Symptoms: A Randomised Controlled Trial (CACTUS Study)"	Neurosis	Acupuncture	Conventional treatment	80	Valid	52 weeks
011	"The Effects of Acupuncture on the Inner Ear Originated Tinnitus"	Inner ear originated tinnitus	Acupuncture	Sham acupuncture (the Park sham acupuncture instrument)	63	Someone has valid in a short time	No mention

Table 2: Continued.

Number	Title	Disease	Intervention of the observation group	Intervention of the control group	Sample size	Outcome	Follow-up phase
012	"Evaluation of Wet-Cupping Therapy for Persistent Non-Specific Low Back Pain: A Randomised, Waiting-List Controlled, Open-Label, Parallel-Group Pilot Trial"	Persistent nonspecific low back pain	Wet-cupping + excise + analgesic-antipyretic	Excise + analgesic-antipyretic	32	Valid	2 weeks
013	"Acupuncture in Preterm Babies during Minor Painful Procedures"	Acupuncture	Acupuncture	Without any measure	10	Valid	No mention
014	"Moxibustion for Cephalic Version: A Feasibility Randomised Controlled Trial"	Malposition	Moxibustion	Without any measure	20	Invalid	No mention
015	"Patient Education Integrated with Acupuncture for Relief of Cancer-Related Fatigue Randomized Controlled Feasibility Study"	Cancer-related fatigue	Patient education integrated with acupuncture	Self-management	13	Valid	No mention
016	"Effectiveness of Acupressure and Acustimulation in Minimizing Driving Simulation Adaptation Syndrome"	Minimizing driving simulation adaptation syndrome	Acupressure and acustimulation	Sham wristband stimulation	25	Valid	No mention
017	"Comparative Effects of Acupressure at Local and Distal Acupuncture Points on Pain Conditions and Autonomic Function in Females with Chronic Neck Pain"	Chronic neck pain	Acupuncture at local points	Acupuncture at distal points and blank control	33	Valid and the local points intervention is better	No
018	"Electroacupuncture for Cervical Ripening Prior to Labor Induction: A Randomized Clinical Trial"	Cervical ripening prior to labor induction	Electroacupuncture	Moxibustion	72	Valid	No mention
019	"Effects of Acupuncture in Reducing Attrition and Mortality in HIV-Infected Men with Peripheral Neuropathy"	HIV-infected men with peripheral neuropathy	Acupuncture	Sham acupuncture	114	Valid in relieve of the pain and the mortality reduction, but the severe is not better than sham acupuncture	14 weeks
020	"Acupuncture Versus Paroxetine for the Treatment of Premature Ejaculation: A Randomized, Placebo-Controlled Clinical Trial"	Premature ejaculation	Acupuncture	Paroxetine	90	Valid	No
021	"Ear Acupuncture in the Treatment of Migraine Attacks: A Randomized Trial on the Efficacy of Appropriate Versus Inappropriate Acupoints"	Migraine attacks	Acupuncture on auricular point	Acupuncture on inappropriate auricular point	94	Valid	120 minutes
022	"Cost-Effectiveness of Acupuncture Care as an Adjunct to Exercise-Based Physical Therapy for Osteoarthritis of the Knee"	Osteoarthritis of the knee	Acupuncture care as an adjunct to exercise-based physical therapy	Education and excise	352	Valid	12 months

Table 2: Continued.

Number	Title	Disease	Intervention of the observation group	Intervention of the control group	Sample size	Outcome	Follow-up phase
023	"A Randomised, Double-Blinded, Placebo-Controlled Study of Acupressure Wristbands for the Prevention of Nausea and Vomiting During Labour and Delivery"	Nausea and vomiting when delivery	Wrist band stimulative to acupoint	Wrist band nonstimulative to PC 6	340	Invalid	No mention
024	"Effect of Acupuncture on Allergen-Induced Basophil Activation in Patients with Atopic Eczema: A Pilot Trial"	Atopic eczema	Acupuncture	Blank	10	Valid	No mention
025	"Acupuncture to Treat Primary Dysmenorrhea in Women: A Randomized Controlled Trial"	Primary dysmenorrhea	Acupuncture	Sham acupuncture (2–4 cm beside acupoints and Streitberger sham acupuncture)	92	Valid	1 year
026	"Getting the Grip on Nonspecific Treatment Effects: Emesis in Patients Randomized to Acupuncture or Sham Compared to Patients Receiving Standard Care"	Emesis	Acupuncture	Sham acupuncture (2 cm beside acupoints and park sham acupuncture)	277	Both better than conventional therapy	No mention
027	"Perioperative Acupuncture and Postoperative Acupressure Can Prevent Postoperative Vomiting following Paediatric Tonsillectomy or Adenoidectomy: A Pragmatic Randomised Controlled Trial"	Postoperative vomiting	Acupuncture + conventional therapy	Conventional therapy	154	Valid	Yes
028	"The Effect of Acupuncture on Postmenopausal Symptoms and Reproductive Hormones: A Sham Controlled Clinical Trial"	Postmenopausal symptoms and reproductive hormones	Acupuncture	Sham acupuncture (the Streitberger sham acupuncture instrument)	55	Valid	No
029	"Efficacy of Acupuncture in Preventing Atrial Fibrillation Recurrences after Electrical Cardioversion"	Atrial fibrillation	Acupuncture	Sham acupuncture	54	Valid	12 months
030	"Acupuncture for the Induction of Labour: A Double-Blind Randomised Controlled Study"	Accouching	Acupuncture	Sham acupuncture (the Park sham acupuncture instrument)	125	Invalid	Yes
031	"Comparison between the Effects of Trigger Point Mesotherapy Versus Acupuncture Points Mesotherapy in the Treatment of Chronic Low Back Pain: A Short Term Randomized Controlled Trial"	Lumbago and backache	Lidocaine injection at acupoint	Lidocaine injection at trigger point	62	The trigger point mesoderm has good effect	12 weeks
032	"Relaxation Acupressure Reduces Persistent Cancer-Related Fatigue"	Cancer-related fatigue	Relaxation acupressure	High frequency of acupuncture and low frequency of acupuncture	43	Relaxation acupressure valid	No mention
033	"Delayed Effect of Acupuncture Treatment in OA of the Knee: A Blinded, Randomized, Controlled Trial"	OA of the knee	Acupuncture and conventional therapy	Sham acupuncture and conventional therapy	55	Valid	One month

Table 3: Jadad scores of the research.

Research	Randomization	Double blinding	Withdraws and drop outs	Jadad score
Park et al. [4]	Computer generated random table in a 1 : 1 ratio with block size 4, and using a sealed envelope	Patient blinded	2	5
Kashefi et al. [2]	Randomized	Single blinded	10	3
Penza et al. [5]	Randomized	Patient and examiner blinded	Not mentioned	2
Landgren et al. [6]	Not mentioned	Nurse and parents blinded	5	2
Smith et al. [7]	Computer generated randomization schedule	Statistician blinded	2	4
El-Deeb and Ahmady [8]	Not mentioned	Double blinded	Not mentioned	3
Scheewe et al. [9]	Not mentioned	Not mentioned	27%	2
Pastore et al. [10]	Block randomization	double blinded	14	4
Hamid et al. [11]	Not mentioned	Not mentioned	35	1
Paterson et al. [12]	Simple randomization	Statistician blinded	3,1	4
Rogha et al. [13]	Not mentioned	Not mentioned	9	1
Kim et al. [14]	Block randomization	open	3	3
Ecevit et al. [15]	Not mentioned	Not mentioned	Not mentioned	0
Do et al. [16]	Computer generated randomization	Not mentioned	1	2
Johnston et al. [17]	Block randomization	Open	1	3
Cox et al. [18]	Not mentioned	Not mentioned	1	0
Matsubara et al. [19]	Not mentioned	Not mentioned	Not mentioned	0
Gribel et al. [20]	Block randomization	Open	0	2
Shiflett and Schwartz [21]	Randomized	Patient and assessor blinded	19	4
Sunay et al. [22]	Simple randomization	Single blinded	0	3
Allais et al. [23]	Simple randomization	Single blinded	1	2
Whitehurst et al. [24]	Not mentioned	Researcher blinded	49	0
Sinha et al. [25]	Simple randomization	Double blinded	11	4
Pfab et al. [26]	Block randomization	Researcher blinded	0	3
Smith et al. [27]	Block randomization	Patient and assessor blinded	2	3
Enblom et al. [28]	Not mentioned	Assessor and nurse blinded	32	2
Liodden et al. [29]	Block randomization	Double blinded	32	4
Sunay et al. [30]	Not mentioned	Single blinded	2	2
Lomuscio et al. [31]	Not mentioned	Patient, assessor, and statistician blinded	0	3
Modlock et al. [32]	Block randomization	Patient, assessor, and statistician blinded	19	4
di Cesare et al. [33]	Block randomization	Assessor blinded	2	3
Zick et al. [34]	Computer generated randomization	Patient blinded	8	3
Lev-Ari et al. [35]	Not mentioned	Patient and assessor blinded	14	2

Table 4: Jadad score comparison of the acupuncture RCT methodologies in the 2011 SCI database.

Result	Total number of reports	Number of reports scoring at 1-2	Proportion (%)	Number of reports scoring at 3–5	Proportion (%)
Positive	23	9	39.13%	14	60.87%
Negative/placebo	10	4	40.00%	6	60.00%

Table 5: Intervention of 17 research reports with negative results or placebo effects.

Number	Disease	Intervention	Acupoint	Treatment frequency	Needling response	Qualification of acupuncturist
1	Constipation	Moxibustion	ST 23, ST 27	3 times/week	Unmentioned	Experience of more than five years
2	Chronic neuralgia	Sham-electroacupuncture after electroacupuncture for 12 weeks	ST36, SP6, LR3, BL60	1 time/week	Unmentioned	Unmentioned
3	Infantile colic	Acupuncture	LI 4	2 times/week	Unmentioned	Certificated nurse practitioner
4	Malposition	Moxibustion	BL 67	2 times/day	Unmentioned	Operation by patients themselves who are trained
5	Nausea and vomiting when delivery	Wrist band stimulating to acupoint	PC 6	1 time/week	Unmentioned	Unmentioned
6	Aids to delivery	Acupuncture	BL 67, LI 4, SP 6 and DU 20	2 times/day	Unmentioned	Acupuncturists and midwives, who often implement acupuncture treatment
7	Lumbago and backache	Lidocaine acupoint injection	GB30, BL31, BL52, GV3, ā shì points, GB34, GB 41, BL60, KI4, TE5	1 time/week	Unmentioned	3-year training and 8-year clinical experience
8	Knee osteoarthritis	Physical therapy plus verum acupuncture	Selecting 6 to 10 acupoints from SP9, SP10, ST 34, ST35, ST36, EX-LE5, GB 34, ā shì points, remote end: LI 4, SP 6, LR 3, ST 44, KI 3, BL 60, and GB 41	2 times/week	Yes	67 physical therapists reaching the lowest acupuncture level required by Acupuncture Association.
9	Knee joint pain of postmenopause women	Routine nursing plus acupressure	EX-LE4, ST35, SP 10, ST 34, ST 36, SP 9, GB 34, and EX-LE2	1 time/day	Unmentioned	Operation by patients themselves
10	Emergency treatment	Routine treatment plus acupuncture	Operation according to traditional Chinese medical standards	1 time/day	Unmentioned	Eligible acupuncturists having the experience of 6–22 years
11	Irritable bowel syndrome	Acupuncture	8-16 acupoints (no specific acupoints are mentioned)	1 time/week	Yes	Members approved by British Acupuncture Association
12	Woman health	Acupressure at SP 6	SP 6	Every day during menstrual period	Unmentioned	Trained about finger force practice
13	Polycystic ovarian syndrome	Electroacupuncture	Electroacupuncture: BL23 on two sides, BL 28, SP 6, and SP 9	Two times/week within the first four weeks, and one time/week within later four weeks	Unmentioned	Acupuncturists with the experience of five years
14	Emesis	Acupuncture	PC 6	Three times/week to two times/week	Yes	Unmentioned
15	Irritable bowel syndrome	Acupuncture	CV10, ST25, LR 3, SP4, PC6, ST 37	Unmentioned	Yes	2000-hour training and experience of more than four years
16	Low back pain	Acupuncture	Unmentioned	Two times/week	Yes	Physicians from various majors, trained about acupuncture more than 140 hours
17	Smoking cessation	Acupuncture	HT 7, PC 7, HT 8, KI 3, and KI 6	Three times/week	Yes	Unmentioned

Table 6: Intervention of 23 research reports with positive results.

Author	Disease	Intervention	Acupoint	Treatment frequency	Needling response	Qualification of acupuncturist
Smith et al. [7]	Infertility	Acupuncture	PC 6, PC 5, HT 5, HT 7	1 time/week	Yes	Certificated acupuncturist, experience of more than 14 years
El-Deeb and Ahmady [8]	Nausea and vomiting after cesarean	Electroacupuncture	PC 6	Single time	Unmentioned	Unmentioned
Scheewe et al. [9]	Bronchial asthma	Acupuncture	BL 13, CV 17, LU 7 and acupoint selection according to syndrome differentiation	3 times/week	Yes	Experience of many years
Hamid et al. [11]	Obesity	Auricular acupuncture	HT 7, CO4, CO1, HX1, CO17	2 times/day	Unmentioned	Unmentioned
Paterson et al. [12]	Psychoneurosis	Acupuncture and moxibustion	Not mentioned	According to syndrome differentiation	Yes	Member of British Medical Acupuncture Society
Rogha et al. [13]	Endogenous tinnitus	Acupuncture	TE 17, GB 2, SI 19, and TE 21	3 times/day	Unmentioned	Unmentioned
Kim et al. [14]	Low back pain	Cupping	BL 23, BL 24, BL 25	3 times/week	Unmentioned	3-year training and 6-year clinical experience
Ecevit et al. [15]	Analgesia	Acupuncture	EX-HN3	1 time/week	Unmentioned	Qualified acupuncturist
Kim et al. [14]	Cancer fatigue	Acupuncture	LI 4, SP 6, ST 36, KI 3	Not mentioned	Yes	Phd of TCM in the US, with 20 years of clinical experience
Cox et al. [18]	Motion sickness	Auricular acupuncture	TF4	Single time	Unmentioned	Unmentioned
Matsubara et al. [19]	Neck pain	Acupressure	GB 21, SI 14, SI 15, LI 4, LI 10, LI 11	Single time	Unmentioned	Unmentioned
Gribel et al. [20]	Cervical dilatation	Electroacupuncture	LI 4, ST 36, LR 3, SP 6, BL 23, BL 32	3 times/day	Unmentioned	With 20 years of clinical experience
Shiflett and Schwartz [21]	Peripheral neuropathy in ADIS patients	Acupuncture	SP 6, SP 7, SP 9, and acupoint selection according to syndrome differentiation	2 times/week	Unmentioned	Has received standardized training
Sunay et al. [22]	Premature ejaculation	Acupuncture	LI 4, ST 36, KI 3, LR 3, EX-HN3, CV 3	2 times/week	Unmentioned	Certificated and experienced acupuncturist
Allais et al. [23]	Migraine	Blunt needle	AT4	Single time	With	Experienced acupuncturist
Whitehurst et al. [24]	Knee osteoarthritis	Acupuncture	Conventional acupoints used for knee osteoarthritis	Not mentioned	Yes	Member of British Medical Acupuncture Society, physiotherapist
Pfab et al. [26]	Eczema	Acupuncture	LI 11, LI 4, ST 36, SP 10	2 times/week	Unmentioned	Experienced acupuncturist
Smith et al. [27]	Dysmenorrhea	Acupuncture	SP 4, CV 3, ST 29, SP 6, BL 32, SP 8	3 times/week	Yes	Certificated acupuncturist of CMASA
Liodden et al. [29]	Postoperative nause	Acupuncture + acupressure	PC 6	For 24 hours	Unmentioned	Experienced acupuncturist
Sunay et al. [30]	Perimenopausal syndrome	Acupuncture	ST 36, LI 4, KI 3, LR 3, EX-HN3, CV 3	2 times/week	Yes	Certificated acupuncturist of 6 years

Table 6: Continued.

Author	Disease	Intervention	Acupoint	Treatment frequency	Needling response	Qualification of acupuncturist
Lomuscio et al. [31]	Atrial fibrillation	Acupuncture	PC 6, HT 7, BL 15	1 time/week	Unmentioned	Trained acupuncturist
Zick et al. [34]	Cancer fatigue	Acupressure	ST 36, SP 6, KI 3, LI 4, CV 6 GV 20, EX-HN3, HT 7, LR 3, SP 6	2 times a day, 3 times/week	Yes	Certificated acupuncturist with B.S. degree
Lev-Ari et al. [35]	Knee osteoarthritis	Acupuncture	GB 34, SP 5, ST 35, EX-LE5, LI 4, local points, ST 43, ST 34	2 times/week	Unmentioned	Unmentioned

was achieved. This is a problem that should be addressed by the research design and execution staff. The physicians of acupuncture and moxibustion in China mostly have such experiences that immediately after needle insertion they must observe the patient's response, earnestly feel the sense beneath the needle tip, and repeatedly operate the needle body so that the endurable feelings of sourness, numbness, swelling, heaviness, and pain can be felt by the patients. Meanwhile, the operator also feels heaviness and tightening beneath the needle tip, which is called the needling response. If such a feeling is generated, a good curative effect can be realized, whereas if it is not, the effect is slow or not apparent. As an intervention in the observation group, no needling response suggests no real or ineffective stimulation to the acupoint. In this way, the curative effect will be reduced greatly and a negative result may be more likely to occur. It is noteworthy that 65% (15/23) of the studies with positive results did not mention whether or not the needling response was achieved. As a complicated intervention, the effectiveness of acupuncture is influenced by multiple factors.

3.4.4. The Requirements of the Acupuncturist Is Neglected in Many Studies. As shown in Table 5, acupuncturists involved in the clinical trials had inconsistent qualifications. The proportion of excellent acupuncturists in the studies with negative results or placebo effects was 65% (11/17). Some of the acupuncturists work part-time and are actually nurse practitioners (003), some have achieved the lowest requirements (008), some are midwives without acupuncturist qualifications (006), and some ask the patients to operate on themselves at home (004, 009, and 012). Because of low-level acupuncturists and such simple treatments, it is really difficult to fully realize the curative effect of acupuncture. The proportion of excellent acupuncturists in the studies with positive results was 74% (17/23), which was significantly higher than the studies with negative results and placebo effects. Acupuncture is a therapeutic method that has high technical skill requirements. He [43] concluded that the proficiency and level of clinical acupuncture skill constitute decisive factors of a clinical curative effect, as well as the advantages of famous veteran physicians of traditional Chinese medicine. Inexperienced or unqualified acupuncturists undoubtedly lower the effectiveness and safety of acupuncture treatment, especially when patients are asked to treat themselves.

3.4.5. The Acupuncture Treatment Frequency Is Too Low in Most Studies. Among the 17 studies listed in Table 5, eight had a treatment frequency of 1-2 times/week (002, 003, 005, 007, 008, 011, 013, and 016), accounting for 47% of the studies; 53% of the studies had a treatment frequency ≥3 times/week (001, 014, and 017). Among the studies with positive results, eight had a treatment frequency of 1-2 times/week, accounting for 35%; 65% of studies with positive results had a treatment frequency ≥3 times/week. Indeed, the studies with positive results had a significantly higher treatment frequency. According to Cai and Ma [44], the influence of acupuncture at BL23 on urinary function peaks after the acupuncture is implemented for 1 hour and then slowly declines and recovers to the original level, with the effect lasting 2–6 hours. This finding is consistent with the metabolic principles in the human body. The curative effect of acupuncture is determined by the duration of the acupuncture effect remaining in the human body and the accumulation of multiple therapeutic effects. Therefore, the best treatment frequency of acupuncture is 1-2 times per day. In the event of one treatment per 2 days or an extended interval of time, it takes more time to accumulate the acupuncture effect, leading to a slower onset of effect. Moreover, different diseases require different treatment frequencies; for chronic diseases and permanent symptoms, the treatment frequency should be higher, and for chronic neuralgia (001), irritable bowel syndrome (011), and smoking cessation (017), it is evident that a good effect is difficult to realize if the frequency is one time per week.

In addition to all the factors above, based on the research demonstrating a clinical curative effect, the diseases which are best to be treated are selected. For example, for smoking cessation, a worldwide problem which is difficult to eradicate, if acupuncture is adopted at a frequency of one time per week, the effect is weak.

3.5. Reflections on Placebo Acupuncture Settings. The 17 studies with negative results or placebo effects are generated in comparison with other therapeutic measures. At the same time, the suitability of the control settings is also worthy of further analysis. The authors have analyzed the control setting list (Table 7) in these research reports and divided the control methods into the following three types: (1) no penetration into the skin (the Park sham needle) or heat insulation acupuncture; (2) slight penetration into the skin

Table 7: Control design in research report with negative or placebo result.

Number	Disease	Intervention of observation group	Intervention of control group
1	Constipation	Moxibustion	Heat insulation moxa-moxibustion (place a heat insulator under the true moxa-moxibustion)
2	Chronic neuralgia	Electroacupuncture for 12 weeks	Sham electroacupuncture (around the acupoint)
3	Infantile colic	Acupuncture	Without any measure
4	Malposition	Moxibustion	Without any measure
5	Nausea and vomiting when delivery	Wrist band stimulative to acupoint	Wrist band nonstimulative to PC 6
6	Accouching	Acupuncture	Sham acupuncture (the Park sham acupuncture instrument)
7	Lumbago and backache	Lidocaine injection at acupoint	Lidocaine injection at trigger point
8	Knee osteoarthritis	Physiotherapy and verum acupuncture	Physiotherapy and placebo acupuncture, or just physiotherapy
9	Knee joint pain of postmenopausal women	Usual care and acupressure	Usual care
10	Emergency treatment	Conventional treatment and acupuncture	Conventional therapy
11	Irritable bowel syndrome	Acupuncture	Sham acupuncture (unrelated to meridian points, without needling response)
12	Woman health	Acupressure at SP 6	Press the nonacupoint (not touch the tendo calcaneus in any meridian vessel on the back of leg)
13	Polycystic ovarian syndrome	Electroacupuncture	Stick on the skin by sham needle tubing, adjust the current to 0
14	Emesis	Acupuncture and moxibustion	Antinausea drug or sham acupuncture (two inches close to the acupoint by Park sham acupuncture instrument)
15	Irritable bowel syndrome	Acupuncture	Park sham needle, the nonacupoint close to acupoints
16	Low back pain	Acupuncture	Nonacupoint shallow penetration (1–3 mm)
17	Smoking cessation	Acupuncture	Nonacupoint shallow penetration (1–3 mm)

or press; and (3) stimulation of the nonacupoint parts. These three points will be analyzed one-by-one as follows.

3.5.1. No Penetration into the Skin as a Control. Park sham acupuncture instruments were used in items 006, 013, 014, and 015, which is the control that did not penetrate into the skin. The instrument incorporates a round and blunt needle head which can be retracted into the needle handle and does not penetrate into the skin when the needle is touching the skin. The outer surface of the needle is fixed by double-faced adhesive tape and equipped with a small pipe to prevent the patient from seeing the truth. Park et al. [45] reported that the needle head would inevitably stimulate the skin and have a vivid effect on the skin, which will result in a physiologic effect. The Park sham acupuncture changes the method and tools of stimulation; thus the control method can also generate some therapeutic effect, but the researcher considers it as the control measure that cannot generate an effect or only shows a placebo effect. Therefore, when the measures of the observation group indicate the same curative effect as that of the control group, the measure of the observation group is considered to be invalid or have placebo effect only. The measure of the control group has some therapeutic effect, so the result of the observation group is false-negative. The Park sham acupuncture method is similar to a pressing method. The acupressure is referred to as the "indicator" in acupuncture theory and exclusively used for infants, people afraid of acupuncture, nervous patients, or when the needle is lacking; acupressure is also a simple method with a treatment effect.

3.5.2. Slight Penetration into the Skin as a Control. The 016 and 017 studies carried out the control using the shallow stimulation method; however, in clinical acupuncture and moxibustion, shallow acupuncture itself is an effective therapeutic method. The *Miraculous Pivot* has recorded that the light stimulation just stimulates the skin, while the semipenetration involves the skin, but not the muscle. The *A-B Classic of Acu-moxibustion* has clearly described that 14 acupoints can be penetrated by one *fen* (approximately 3.3 mm) and 20 acupoints can be penetrated by 2 *fen* (approximately 6.6 mm) [46]. Another study [47] indicates that 42 patients with wrist myofascial pain were randomly distributed to the deep acupuncture group and the shallow acupuncture group with the same acupoints, and the acupuncture depth for the deep acupuncture group was 1.5 cm compared to 2 mm for the shallow acupuncture group. The McGill pain questionnaire was used as the evaluating indicator. The scores for the two

groups before the treatment were 35.4 ± 14.53 and 34.75 ± 11.43, respectively, compared to 14.54 ± 10.88 and 22.25 ± 16.08 after the treatment. The results indicate that the two groups can relieve the pain, and the curative effect was not statistically different. Therefore, with respect to pain-related disease and disease suitable for shallow acupuncture, shallow acupuncture is unsuitable for the acupuncture control without a curative effect because it will result in false-negative properties of the observation group.

3.5.3. Nonacupoint and Nonmeridian Acupoint as Controls. The researchers performing seven studies (002, 011, 012, 014, 015, 016, and 017) avoid or depart the known acupoints and meridians as the placebo control. The question involves how many acupoints the people have in their body. The WHO has approved that there are 361 meridian points and 48 extraordinary points; however, >2200 extraordinary acupoints have been collected [48] with formal names and main functions. Owing to all types of unfixed *a* shì points, it is easy to avoid the meridians, but hard to avoid the acupoints when designing the nonacupoint and nonmeridian controls. The parts avoiding the familiar meridians and acupoints are just defined as the nonmeridian and nonacupoint parts [49]. Furthermore, the area of the acupoints has not been measured until now, and the distance between the meridian or acupoint and the nonmeridian and nonacupoint part has not been determined. Therefore, the control with nonmeridian and nonacupoint parts is highly possible to apply the "point" with a therapeutic effect as the control, and the result of the observation group has a high possibility of a false-negative.

In summary, all of the three above-mentioned control methods showed a therapeutic effect; however, the researchers only think the therapeutic effect was from the placebo control and when the therapeutic effect of the observation group is similar to that of the control group, the conclusion is incorrect that the observation group therapy had no effect or was equal to the placebo. The other reason for the researcher to design the placebo control like this is possibly related to the "blind." In view of the particularity of acupuncture, it is impossible to identify the placebo therapy meeting the blind requirement and being similar to acupuncture. Many experts [50–52] have written articles to discuss the methods of setting the control group in acupuncture RCTs; however, Liu [53] suggests using modern medical methods as the standard control and aiming at the most effective and most advanced method in mainstream medicine to directly discover the advantage or disadvantage of acupuncture and give full attention to the medical development of acupuncture.

4. Discussion

By analyzing acupuncture RCTs in the SCI database, it is discovered that the methodologic quality of research with positive results is not different from that of research with negative results or placebo effects. The methodologic quality is not the primary reason contributing to the difference in research results; however, each study with negative results or placebo effects has disadvantages on the intervention side, such as incomplete rational acupoint selection, inconsistent ability of acupuncturists, negligence of the needling response to needling, low frequency of the acupuncture treatment, and irrational setting of placebo control. Those directly weaken the positive property of the results in the observation group, and the setting of the placebo acupuncture control is opposite to the theory of acupuncture. The placebo acupuncture method has certain therapeutic effects instead of purely a placebo effect, thereby causing the false-negative property of the results in the observation group. It was shown that the sham acupuncture (placebo acupuncture) in the current acupuncture RCTs and the placebo control method was not reached by consensus. The Society of Acupuncture and Moxibustion gradually found that the clinical trials under ideal conditions are not suitable for acupuncture and moxibustion. Seeking the clinical research methods in the practical world, practical clinical research may be able to break the limit of the placebo acupuncture control and find the advantage of acupuncture therapy.

We can see that the current clinical research for acupuncture and moxibustion still reflects many methodologic problems and is not mature in terms of theory and practices. It is necessary to establish a clinical research method for acupuncture and moxibustion to meet the requirements of the acupoint theory, practice features, and clinical trials so that the clinical trial results for acupuncture and moxibustion are scientific, comply with medical ethics, completely meet the treatment effect advantages of acupuncture, and promote acupuncture to mainstream medicine.

The limitations of the research are as follows: (1) the research report is of limited duration, thus this paper inevitably suggests selection bias; (2) a common phenomenon exists in the sector that the probability of publishing of a negative article is lower than for a positive article, which will cause bias to the research conclusion; and (3) the Jadad scale is used to evaluate the methodologic quality of the article. The greatest strength of the scale is directly evaluating the verified test features related to the bias in the test effect evaluation, which is simple and clear; however, the Jadad score will be too general and arbitrary if most of the research is not defined, whether or not they are random or double blind.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Wei-hong Liu conceived and designed the work; Yang Hao, Wan-ning Liu, Yan-jing Han, Xiao-hong Wang, and Chen Li performed the work; Wei-hong Liu and Yang Hao wrote the paper.

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Research Article

Efficacy of Acupuncture in Children with Nocturnal Enuresis: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background. Nocturnal enuresis (NE) is recognized as a widespread health problem in young children and adolescents. Clinical researches about acupuncture therapy for nocturnal enuresis are increasing, while systematic reviews assessing the efficacy of acupuncture therapy are still lacking. *Objective*. This study aims to assess the effectiveness of acupuncture therapy for nocturnal enuresis. *Materials and Methods*. A comprehensive literature search of 8 databases was performed up to June 2014; randomized controlled trials which compared acupuncture therapy and placebo treatment or pharmacological therapy were identified. A meta-analysis was conducted. *Results*. This review included 21 RCTs and a total of 1590 subjects. The overall methodological qualities were low. The results of meta-analysis showed that acupuncture therapy was more effective for clinical efficacy when compared with placebo or pharmacological treatment. Adverse events associated with acupuncture therapy were not documented. *Conclusion*. Based on the findings of this study, we cautiously suggest that acupuncture therapy could improve the clinical efficacy. However, the beneficial effect of acupuncture might be overstated due to low methodological qualities. Rigorous high quality RCTs are urgently needed.

1. Introduction

Nocturnal enuresis (NE) is a worldwide health problem frequently encountered in childhood and is defined as an involuntary voiding of urine during sleep with a frequency of at least twice a week in children, in the absence of congenital or acquired defects of the central nervous system [1]. It includes monosymptomatic nocturnal enuresis (MNE) with no daytime urinary symptoms and nonmonosymptomatic nocturnal enuresis (NMNE) that is accompanied by daytime urinary symptoms. Nocturnal enuresis affects 5%–10% of younger school-age children [2]. Enuretic children have a higher risk for psychosocial comorbidity and loss of self-esteem. Such feelings of humiliation, guilt, and shame are also a reasonable source of heartache to the children and their parents. The etiology and underlying physiological mechanisms of nocturnal enuresis are multifactorial; three commonly proposed mechanisms to bedwetting include excessive nocturnal urine production, bladder overactivity, and a failure to awaken in response to bladder sensations [3].

Current first-line nocturnal enuresis therapies center on the aforementioned mechanisms; generally accepted treatments are oral pharmacological therapies including desmopressin, tricyclics, or oxybutynin and behavioral therapies

[4]. Desmopressin has been widely used for several decades, and its reliable therapeutic effect has been proven to one-third of the unselected enuretic children. But the clinical effect cannot be maintained once the medication is stopped and the side effects associated with drugs may cause the patients to be reluctant to use them for long periods. The preferred behavioral treatment is bed alarm, which needs to be continuous and brings the enuretic children different degrees of sleep disorders at the same time [5].

Complementary and alternative medicine (CAM) is widely advocated to face the increasing demand for non-pharmacological approaches. As a mainstream CAM therapy, acupuncture treatment based on TCM theory has been commonly used to treat nocturnal enuresis in Chinese cultures. Compared to conventional care, its safety and cost effectiveness assure the maintenance of patients' compliance. Unfortunately, there is little published information to warrant acupuncture therapy as standard treatment of nocturnal enuresis. The aim of this review is to evaluate the efficacy of acupuncture therapy in the treatment of nocturnal enuresis when compared with placebo acupuncture or oral pharmacological treatment based on randomized controlled trials (RCTs).

2. Material and Methods

2.1. Literature Search Strategy. A comprehensive literature search of the Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Database of Systematic Review (CDSR), EMBASE, ISI Web of Science, and PubMed was conducted. We also searched Chinese databases, including China Knowledge Resource Integrated database (CNKI), WanFang Data, VIP, and Chinese Biomedical (CBM) Literature database. In addition, we searched databases that contained registered trials, such as ClinicalTrials.gov (http://www.clinicaltrials.gov). All databases were searched from their inception dates up to June 2014; languages were restricted to Chinese and English. The following medical subject headings or key words were used for English databases: enuresis, nocturnal enuresis, nighttime urinary incontinence, bedwetting, acupuncture, electroacupuncture, auricular acupuncture, ear acupuncture, scalp acupuncture, acupoint, moxibustion, acupressure, and acustimulation. For Chinese databases we used free text terms as "Zhen" or "Jiu" or "Xue Wei" and "Yi Niao." In addition, the bibliographies of relevant systematic reviews and clinical guidelines were manually searched. We also searched the gray literature that included dissertations, letters, government documents, research reports, conference proceedings, and abstracts when available. The reference section for each study was also searched.

2.2. Inclusion Criteria. Inclusion criteria are as follows: (1) research subjects: the enrolled patients had to be diagnosed with NE and no restrictions on race, age, or sex were imposed; (2) study design: the included studies were required to be RCTs in Chinese or English aiming to assess the efficacy of acupuncture therapy for NE; (3) experimental group interventions: experimental group mainly received acupuncture therapy (including needles, moxibustion, acupressure, electroacupuncture, and acupoint injection, among other techniques), either alone or in conjunction with another kind of acupuncture therapy, without differentiating different acupuncture therapy techniques, acupoints selection, or needle materials; (4) control group interventions: control interventions included placebo acupuncture or oral pharmacological treatment; (5) outcome measurements: the outcome measurement had to include overall clinical efficacy, number of wet nights per week, or maximum voided volume.

2.3. Exclusion Criteria. Exclusion criteria included the following: (1) articles regarding animal experiments, review articles, case reports, or expert experience reports; (2) nonrandomized studies; (3) studies that compared different acupuncture modalities or acupoints selection; (4) experimental groups that accepted complex therapy, while the contributing factor could not be distinguished; (5) studies that were duplicates for retrieving or publishing.

2.4. Data Extraction. Two reviewers (Zheng-tao Lv and Wen Song) reviewed each article independently and were blinded to the findings of the other reviewer. In accordance with the predetermined inclusion criteria, two reviewers independently performed a rigorous screening to identify qualified articles, and they extracted data independently from these articles using a standardized collection form, which includes first author, year of the study, sample size, nation or region, baseline characteristics, methodological features of the studies, quality of trial design, interventions, main outcome assessments, follow-up time, and withdrawal. If the required information was not available in the included studies, we contacted the original authors by email. Any discrepancies between reviewers were resolved through discussion until a consensus was reached. The third review author (Man Li) was consulted if a consensus could not be reached.

2.5. Quality of the Studies. The methodological quality of the included trials was evaluated using the Jadad quality scale [27]: (1) randomization (the study was described as randomized), (2) double blinding (participant masking and researcher masking), (3) reporting of the number of dropouts and reasons for withdrawal, (4) allocation concealment, and (5) generation of random numbers (by using computer, random numbers table, shuffled cards, or tossed coins). RCTs scored 1 point for each area addressed in the study design for a possible score between 0 and 5 (highest level of quality). The quality of all included studies was assessed by two authors (Zheng-tao Lv and Wen Song) and the articles were classified as high quality if their Jadad score ≥ 4 and low quality if their Jadad score ≤ 3. Disagreements regarding methodological quality were resolved with discussion between reviewers.

2.6. Data Synthesis and Analysis. The meta-analysis and statistical analysis were performed by using RevMan 5.1 analyses software of the Cochrane Collaboration. We extrapolated the odds ratio (OR) and the associated 95% confidence interval

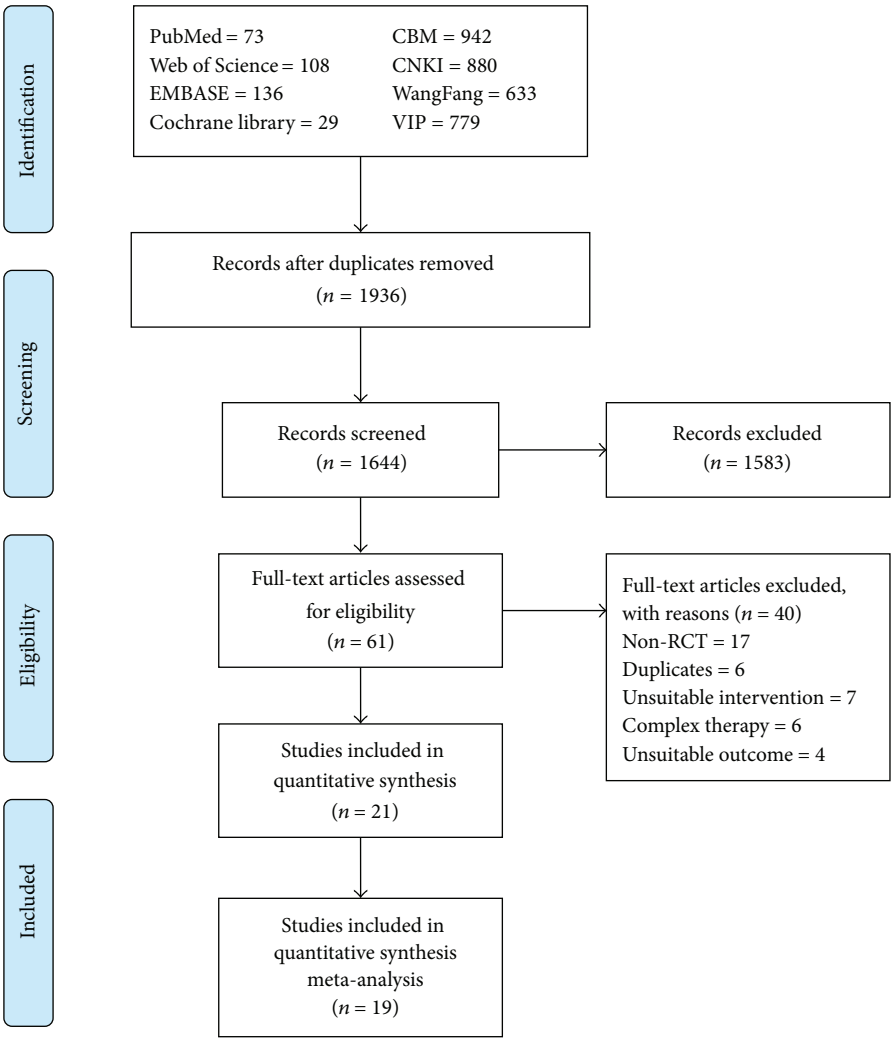

Figure 1: Flowchart of the literature search and study selection.

(CI) for treatment effect. The chi-squared test and the Higgins I^2 test were used to assess the heterogeneity of the data [28]. We pooled data across studies using random effect models if statistical heterogeneity exists; otherwise, a fixed effect model will be used. Publication bias was explored via a funnel-plot analysis. Begg's test and Egger's test were conducted when the number of included studies is equal to or greater than 5 (Stata Software, version 12.0). In case of heterogeneity, subgroup analysis was conducted.

3. Results

3.1. Literature Search Results. An initial search of RCTs yielded 3580 potential literature citations, including 346 English studies and 3134 Chinese studies, and 1936 duplicated articles were deleted. After screening titles and abstracts, 61 potentially relevant studies were selected and retrieved for a full-text assessment. Of the remaining 61 studies, 17 studies were excluded because they were not RCTs; 6 articles were duplicates; 7 studies took unsuitable intervention; 6 studies accept the complex therapy, for example, combination of two different kinds of acupuncture therapy; 4 studies did not report the suitable outcome. Finally, 21 studies meet our inclusion criteria [6–26]. Because only two of these studies [10, 17] compared acupuncture with placebo treatment (e.g., without active laser light but with or without skin contact), we just used them for systematic review (Figure 1).

3.2. The Characteristics and Methodological Quality of the Included Trials. The characteristics of the 21 trials are summarized in Table 1. These studies were published between 2001 and 2014. Sixteen studies were published in Chinese and five studies in English. The 21 RCTs included a total of 1590 patients with nocturnal enuresis: 826 patients in the acupuncture group (experimental group) and 731 patients in the control group. Age of the patients ranges from 3 to 21 years. Nineteen studies used 2-parallel-arm group designs [6, 8–12, 14–26] and two used a 3-parallel-arm group design [7, 13]. The experimental group mainly received acupuncture therapy (including needles, moxibustion, acupressure, electroacupuncture, and acupoint injection, among other techniques). Among the 21 studies, western medicine

Table 1: Characteristics and methodological quality of included studies.

Study	Study design	Sample size ($n1/n2$)	Nation/region	Age (mean or range)	Baseline	EC approval	Jadad score
Dong et al., 2012 [6]	RCT, parallel 2 arms	120 (60/60)	China	E: 8.61 (5~12) years C: 8.57 (5~13) years	Adequate	Not reported	1
Hong et al., 2011 [7]	RCT, parallel 3 arms	99 (33/33/33)	China	5~13 years	Adequate	Not reported	2
Hui et al., 2006 [8]	RCT, parallel 2 arms	67 (35/32)	China	E: 5~12 years C: 6~11 years	Adequate	Not reported	2
Liu, 2007 [9]	RCT, parallel 2 arms	60 (30/30)	China	5~12 years	Not reported	Not reported	1
Karaman et al., 2011 [10]	RCT, parallel 2 arms Prospective, randomized, placebo controlled, single-blind study	83 (57/26)	Turkey	E: 8.5 ± 3.2 years C: 8.9 ± 3.3 years	Adequate	Yes	3
Ding et al., 2007 [11]	RCT, parallel 2 arms	80 (42/38)	China	3~13 years	Adequate	Not reported	1
Tong and Zhan, 2009 [12]	RCT, parallel 2 arms	60 (30/30)	Guinea-Bissau	6~20 years	Adequate	Not reported	2
Moursy et al., 2014 [13]	RCT, parallel 3 arms	186 (62/62/62)	Egypt	15.7 years (range 10–21 years)	Adequate	Yes	3
Tian and Zhong, 2008 [14]	RCT, parallel 2 arms	228 (116/112)	China	E: 7.58 ± 2.16 years C: 8.26 ± 2.67 years	Adequate	Not reported	3
Ling and Chen, 2011 [15]	RCT, parallel 2 arms	60 (30/30)	China	E: 9.2 (5~16) years C: 9.1 (5~15) years	Adequate	Not reported	2
Radmayr et al., 2001 [16]	RCT, parallel 2 arms	40 (20/20)	Austria	E: 8.6 (5~16) years C: 8.0 (5~14) years	Adequate	Yes	2
Radvanska et al., 2011 [17]	RCT, parallel 2 arms Prospective, single-blind, randomized, placebo controlled design	29 (16/13)	Slovakia	E: 8.7 ± 1.4 years C: 8.6 ± 1.3 years	Adequate	Yes	4
Yang et al., 2012 [18]	RCT, parallel 2 arms	69 (35/34)	China	3~15 years	Adequate	Not reported	1
Luo, 2010 [19]	RCT, parallel 2 arms	40 (20/20)	China	E: 8.5 ± 0.1 years C: 8.4 ± 0.2 years	Adequate	Not reported	1
Tang et al., 2012 [20]	RCT, parallel 2 arms	48 (24/24)	China	E: 5~11 years C: 5~12 years	Adequate	Not reported	2
Qiu, 2008 [21]	RCT, parallel 2 arms	56 (31/25)	China	3~16 years	Not reported	Not reported	1
Zhu et al., 2003 [22]	RCT, parallel 2 arms	76 (41/35)	China	4~15 years	Adequate	Not reported	1
Zhang, 2010 [23]	RCT, parallel 2 arms	80 (40/40)	China	3~18 years	Adequate	Not reported	1
Chen and Gu, 2003 [24]	RCT, parallel 2 arms	72 (40/32)	China	5~14 years	Adequate	Not reported	1
Yuksek et al., 2003 [25]	RCT, parallel 2 arms	24 (12/12)	Turkey	E: 7.67 ± 2.34 years C: 7.41 ± 2.67 years	Adequate	No	1
Hong and Zhang, 2009 [26]	RCT, parallel 2 arms	30 (15/15)	China	8~21 years	Adequate	Not reported	1

therapy (e.g., desmopressin, Meclofenoxate) was used as the intervention method for the control group in 9 studies [6, 8, 9, 11, 13, 16, 22, 23, 25]; traditional Chinese medicine (TCD) was used in 10 studies [7, 12, 14, 15, 18–21, 24, 26]; and placebo treatment or sham-acupuncture was used in 2 studies [10, 17]. The main outcome indicators reported in the included studies were cure rate, improvement rate, and mean weekly number of wet nights; Two studies reported maximum voided volume (MVV) as outcome indicators [13, 17] (Table 2).

The mean Jadad score of these 21 studies was 1.7, ranging from 1 to 4 points (Table 1). Only 1 of 21 RCTs met the Jadad criteria for high quality [17]. All of the studies included suggested randomization, and 9 studies reported the method of random sequences generation [7, 8, 12–17, 20]. In that study, it was not feasible to blind the participant or the therapist. The outcome assessor was blinded in only two studies [10, 17]; we considered that the outcomes and their measurements are likely to be influenced by lack of blinding. Four studies

Table 2: Interventions and outcomes of included studies.

Study	Duration of treatment	Follow-up after treatment	Experimental treatment	Control treatment	Cure rate of intervention group	Cure rate of control group	Outcome measurement
Dong et al., 2012 [6]	5 weeks	6 months	Acupoint injection with scraping therapy ($n = 60$)	Western medicine: Meclofenoxate ($n = 60$)	46/60 (76.67%)	36/60 (60%)	Cure rate, improvement rate, follow-up at 1 and 6 months
Hong et al., 2011 [7]	1 month	Not reported	Moxibustion ($n = 33$) Acupuncture ($n = 33$)	Chinese patent medicine ($n = 33$)	20/33 (60.6%) 19/33 (57.6%)	8/33 (24.24%)	Cure rate, improvement rate
Hui et al., 2006 [8]	1 month	1 year	Heat-producing needling ($n = 35$)	Western medicine: imipramine hydrochloride ($n = 32$)	20/35 (57.2%)	14/32 (43.8%)	Cure rate, total effective rate, follow-up at 1 month
Liu, 2007 [9]	3 weeks	Not reported	Enuresis patch ($n = 30$)	Western medicine: Meclofenoxate ($n = 30$)	18/30 (60%)	9/30 (30%)	Cure rate, improvement rate
Karaman et al., 2011 [10]	4 weeks	6 months	Laser acupuncture ($n = 57$)	Placebo therapy: with a nonlaser light source ($n = 26$)	31/57 (54.4%)	3/26 (11.5%)	Complete improvement rate, partial improvement rate, mean number of weekly bedwetting episodes: the children were reevaluated 15, 30, 90, and 180 days after treatment
Ding et al., 2007 [11]	1 month	3 months	Enuresis patch ($n = 42$)	Western medicine: Meclofenoxate ($n = 38$)	25/42 (59.5%)	13/38 (34.2%)	Cure rate, improvement rate
Tong and Zhan, 2009 [12]	1 month	Not reported	Suspended moxibustion ($n = 30$)	Chinese patent medicine ($n = 30$)	17/30 (56.7%)	10/30 (33.3%)	Cure rate, improvement rate
Moursy et al., 2014 [13]	3 months	6 months	Laser acupuncture ($n = 62$)	Western medicine: desmopressin ($n = 62$) Combination therapy: acupuncture + desmopressin ($n = 62$)	33 /62 (53%)	35/62 (56.5%) 46/82 (74%)	Cure rate, improvement rate, mean weekly number of wet nights, MVV (maximum voided volume): the patients were evaluated once every 2 weeks for 3 months and once every 4 weeks for 6 months
Tian and Zhong, 2008 [14]	2 weeks	Not reported	Acupuncture ($n = 116$)	Chinese patent medicine ($n = 112$)	61/116 (52.59%)	47/112 (41.96%)	Cure rate, improvement rate
Ling and Chen, 2011 [15]	1 month	Not reported	Acupoint injection ($n = 30$)	Chinese patent medicine ($n = 30$)	18/30 (60%)	15/30 (50%)	Cure rate, improvement rate
Radmayr et al., 2001 [16]	6 months	Not reported	Laser Acupuncture ($n = 20$)	Western medicine: desmopressin ($n = 20$)	13/20 (65%)	15/20 (75%)	Response rate, partial response rate

Table 2: Continued.

Study	Duration of treatment	Follow-up after treatment	Experimental treatment	Control treatment	Cure rate of intervention group	Cure rate of control group	Outcome measurement
Radvanska et al., 2011 [17]	5 weeks	Not reported	Laser acupuncture ($n = 16$)	Placebo therapy: without active laser light but with or without skin contact ($n = 13$)	Not reported	not reported	Wet nights/wk, voiding frequency, nocturnal urine production on wet nights MVV (maximal voided volume), AVV (average voided volume)
Yang et al., 2012 [18]	1 month	Not reported	Ear point tapping with medicinal cake-separated moxibustion ($n = 35$)	Chinese patent medicine ($n = 34$)	21/35 (60%)	12/34 (35.3%)	Cure rate, improvement rate
Luo, 2010 [19]	3 months	Not reported	Acupuncture-massage ($n = 20$)	Chinese medicine ($n = 20$)	14/20 (70%)	4/20 (20%)	Cure rate, improvement rate
Tang et al., 2012 [20]	2 weeks	1 month	Massage ($n = 24$)	Chinese medicine ($n = 24$)	16/24 (66.7%)	11/24 (45.8%)	Cure rate, improvement rate
Qiu, 2008 [21]	1 month	Not reported	Ear point tapping ($n = 31$)	Chinese medicine ($n = 25$)	17/31 (54.8%)	15/25 (60%)	Cure rate, improvement rate
Zhu et al., 2003 [22]	3 weeks	3 months	Acupoint injection ($n = 41$)	Western medicine: Meclofenoxate ($n = 35$)	19/41 (46.5%)	6/35 (17.1%)	Cure rate, improvement rate
Zhang, 2010 [23]	1 month	Not reported	Medicinal cake-separated moxibustion with embedded needling ($n = 40$)	Western medicine: desmopressin ($n = 40$)	22/40 (55%)	6/40 (15%)	Cure rate, improvement rate
Chen and Gu, 2003 [24]	2 weeks	Not reported	Acupoint injection ($n = 40$)	Chinese medicine ($n = 32$)	36/40 (90%)	14/32 (43.7%)	Cure rate, improvement rate
Yuksek et al., 2003 [25]	6 months	Not reported	Acupressure ($n = 12$)	Western medicine: oxybutynin ($n = 12$)	10/12 (83.3%)	7/12 (58.3%)	Complete improvement rate, partial improvement rate, follow-up at 15 days and 1, 3, and 6 months
Hong and Zhang, 2009 [26]	1 month	Not reported	Needle warming moxibustion ($n = 15$)	Chinese medicine ($n = 15$)	13/15 (86.7%)	5/15 (33.3%)	Cure rate, improvement rate

reported complete follow-up of all subjects [10, 13, 14, 17]. All the studies presented selective reporting, characterized similarity of baseline. In general, the methodological and report qualities of the included studies were poor.

3.3. Meta-Analysis Results. The 21 included RCTs adopted in consistent interventions and different reported outcomes, with no unified efficacy standard. To reach a consistent understanding of the therapeutic effect of acupuncture therapy for nocturnal enuresis, intervention therapies for control group were further refined. We limited the control group methods to western or traditional Chinese medicine alone, as two studies used placebo treatment or sham-acupuncture as control group [10, 17] and one of these two studies did not report the cure rate as effective outcomes [17]. Furthermore, the definition of cure rate was consistent among the other included 19 studies; we conducted the meta-analysis to compare the overall cure rate determined in these studies.

Three studies reported mean weekly number of wet nights [10, 13, 17] and two studies reported maximum voided volume (MVV) [13, 17] as the effective outcomes, considering the lack of adequate numbers of studies; these results will be presented in the following part of our review.

The results of heterogeneity tests indicated that $I^2 > 50\%$ and $P < 0.1$ for the 19 included studies [6–9, 11–16, 18–26] and that the overall heterogeneity existed ($P = 0.002$, $I^2 = 54\%$). Therefore, a random effects model was used. The combined effects of 19 independent trial results showed that acupuncture therapy had further improved the cure rate in patients with nocturnal enuresis when compared with control group accepting medicine therapy (OR = 2.58; 95% CI, 1.84–3.61; $P < 0.0001$) (Figure 2).

3.3.1. Acupuncture versus Western Medicine. Our meta-analysis of ten studies [6, 8, 9, 11, 13, 16, 22, 23, 25], which compared acupuncture therapy with traditional Chinese medicine, yielded encouraging effects in favor of acupuncture therapy on nocturnal enuresis (OR = 2.16; 95% CI, 1.31–3.55; $P < 0.01$). Heterogeneity between studies existed ($P = 0.03$; $I^2 = 54\%$) (Figure 2).

3.3.2. Acupuncture versus Traditional Chinese Medicine. The same findings applied to other ten studies [7, 12, 14, 15, 18–21, 24, 26], which compared acupuncture therapy with western medicine, yielded encouraging effects in favor of acupuncture therapy on nocturnal enuresis (OR = 3.03; 95% CI, 1.88–4.88; $P < 0.01$). Heterogeneity between studies existed ($P = 0.01$; $I^2 = 56\%$) (Figure 2).

3.4. Subgroup Analyses. A subgroup analysis was conducted to further evaluate the clinical effect of acupuncture therapy and identify the heterogeneity within western medicine group. The western medicine group was divided into four groups according to the medication types. Four studies used Meclofenoxate as control intervention [6, 9, 11, 22], three studies used desmopressin as medicine control [13, 16, 23], and the remaining two studies [8, 25] treated nocturnal children with imipramine hydrochloride and oxybutynin, respectively. The pooled data showed significant difference between acupuncture therapy and Meclofenoxate (OR = 2.81; 95% CI, 1.62–3.96; $P < 0.0001$), with no obvious heterogeneity (Figure 3). The pooled effects of three independent trials suggested that there was no significant difference between desmopressin and acupuncture in treating NE (OR = 1.57; 95% CI, 0.38–6.57; $P = 0.54$) (Figure 4). Since only one trial utilized imipramine hydrochloride as medicine control and only one trial utilized oxybutynin, results from these two studies are presented as narrative description. There was no significant difference between imipramine hydrochloride and acupuncture therapy (OR = 1.71; 95% CI, 0.65–4.51; $P = 0.27$). Compared with oxybutynin, acupuncture could not further improve the clinical effect (OR = 3.57; 95% CI, 0.53–2; $P = 0.54$).

3.5. Acupuncture Therapy versus Placebo Treatment. Two studies used placebo treatment or sham-acupuncture as control group [10, 17]. However, results of these two studies were inconsistent. Radvanska et al. [17] compared the treatment efficacy of laser acupuncture therapy with sham-acupuncture; they found no significant effect of active laser acupuncture on maximal voided volume (first morning void excluded), maximal morning voided volume, voiding frequency, enuresis frequency before and after treatment, or nocturnal urine production among the patient groups, but it resulted in a significant increase in average daytime voided volume. There was no effect of skin contact during placebo laser acupuncture. Radvanska et al. [17] concluded that laser acupuncture had subtle effects on bladder reservoir function; however, it is an inefficient treatment for monosymptomatic nocturnal enuresis with reduced maximal voided volume. Karaman et al. [10] evaluated the effect of laser acupuncture therapy on patients with primary monosymptomatic nocturnal enuresis. The mean number of bedwetting episodes was 1.7 per week 6 months after laser therapy and 3.1 in the placebo group. Laser acupuncture therapy was significantly more beneficial compared to placebo in terms of complete dryness, partial improvement, and decrease in the mean number of weekly bedwetting episodes.

3.6. Other Outcomes

3.6.1. Mean Weekly Number of Wet Nights. Three studies reported mean weekly number of wet nights [10, 13, 17]. Moursy et al. [13] reported that the difference of reducing the mean weekly number of wet nights in laser acupuncture group, desmopressin group, and combination of laser acupuncture and desmopressin group had no statistical significance ($P > 0.05$). Radvanska et al. [17] found that the difference in the reduction of wet nights was not statistically significant between laser acupuncture group and placebo group. Karaman et al. [10] showed that laser acupuncture therapy was significantly more beneficial compared to placebo in terms of a decrease in the mean number of weekly bedwetting episodes as previously mentioned.

3.6.2. Maximum Voided Volume (MVV). Two studies reported maximum voided volume (MVV) [13, 17] as

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds ratio M-H, random, 95% CI	Year
1.1.1 Acupuncture therapy versus TCM							
Chen and Gu, 2003	36	40	14	32	4.2%	11.57 [3.33, 40.27]	2002
Tian and Zhong, 2008	61	120	47	120	8.1%	1.61 [0.96, 2.68]	2008
Qiu, 2008	17	31	15	25	5.0%	0.81 [0.28, 2.36]	2008
Tong and Zhan, 2009	17	30	10	30	5.1%	2.62 [0.92, 7.46]	2009
Hong and Zhang, 2009	13	15	5	15	2.5%	13.00 [2.07, 81.48]	2009
Luo, 2010	14	20	4	20	3.5%	9.33 [2.18, 39.96]	2010
Hong et al., 2011	19	33	8	33	5.1%	4.24 [1.48, 12.17]	2011
Ling and Chen, 2011	18	30	15	30	5.2%	1.50 [0.54, 4.17]	2011
Hong et al., 2011	20	33	8	33	5.0%	4.81 [1.67, 13.86]	2011
Yang et al., 2012	21	35	12	34	5.4%	2.75 [1.04, 7.30]	2012
Tang et al., 2012	16	24	11	24	4.5%	2.36 [0.73, 7.60]	2012
Subtotal (95% CI)		**411**		**396**	**53.8%**	**3.03 [1.88, 4.88]**	
Total events	252		149				
Heterogeneity: $\tau^2 = 0.34$; $\chi^2 = 22.66$, df = 10 ($P = 0.01$); $I^2 = 56\%$							
Test for overall effect: $Z = 4.55$ ($P < 0.00001$)							
1.1.2 Acupuncture therapy versus western medicine							
Radmayr et al., 2001	13	20	15	20	3.8%	0.62 [0.16, 2.43]	2001
Zhu et al., 2003	19	41	6	35	5.0%	4.17 [1.43, 12.20]	2003
Yuksek et al., 2003	10	12	7	12	2.4%	3.57 [0.53, 23.95]	2003
Hui et al., 2006	20	35	14	32	5.5%	1.71 [0.65, 4.51]	2006
Liu, 2007	18	30	9	30	5.0%	3.50 [1.20, 10.20]	2007
Ding et al., 2007	25	42	13	38	5.8%	2.83 [1.14, 7.03]	2007
Zhang, 2010	22	40	6	40	5.0%	6.93 [2.38, 20.16]	2010
Dong et al., 2012	33	60	26	60	6.9%	1.60 [0.78, 3.29]	2012
Moursy et al., 2014	33	62	35	62	6.9%	0.88 [0.43, 1.78]	2014
Subtotal (95% CI)		**342**		**329**	**46.2%**	**2.16 [1.31, 3.55]**	
Total events	193		131				
Heterogeneity: $\tau^2 = 0.30$; $\chi^2 = 17.47$, df = 8 ($P = 0.03$); $I^2 = 54\%$							
Test for overall effect: $Z = 3.03$ ($P = 0.002$)							
Total (95% CI)		**753**		**725**	**100.0%**	**2.58 [1.84, 3.61]**	
Total events	445		280				
Heterogeneity: $\tau^2 = 0.30$; $\chi^2 = 41.28$, df = 19 ($P = 0.002$); $I^2 = 54\%$							
Test for overall effect: $Z = 5.50$ ($P < 0.00001$)							
Test for subgroup differences: $\chi^2 = 0.93$, df = 1 ($P = 0.34$), $I^2 = 0\%$							

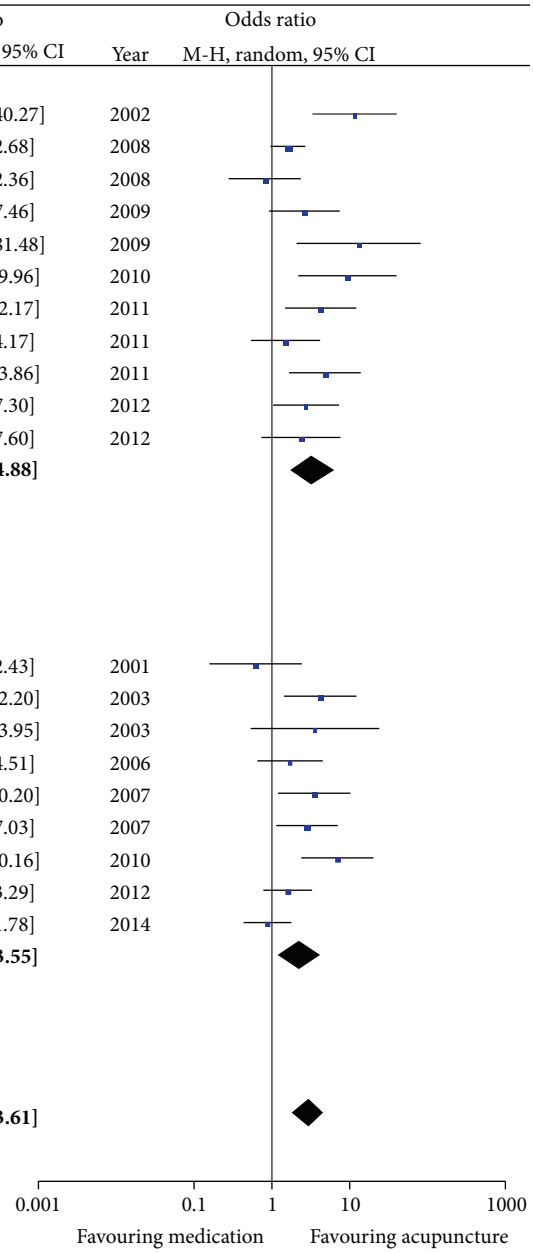

Figure 2: Forest plot of comparison: the clinical effective rate.

the effective outcomes. Moursy et al. [13] found that it significantly increased only in laser acupuncture group and combination of laser acupuncture and desmopressin group comparing with pretreatment values and desmopressin group, respectively. Thus, bladder capacity significantly increased only in patients receiving laser acupuncture treatment. However, Radvanska et al. [17] reported that the MVV had no difference between laser acupuncture group and placebo group.

3.7. Publication Bias Analysis. We conducted a funnel plot analysis of the aforementioned 19 studies [6–9, 11–16, 18–26]. P value associated with Begg's test was 0.009 and P value associated with Egger's test was 0.002. The resulting graph was asymmetrical, suggesting the possibility of publication bias, which was in line with results of Begg's test and Egger's test (Figure 5). In addition, language bias may exist because most of included studies were published in Chinese.

4. Discussion

4.1. Summary of Evidence. The present study analyzed data from 21 RCTs involving 1590 individuals that featured to assess the efficacy of acupuncture therapy to treat NE. Based on the findings in our systematic review and meta-analysis, acupuncture therapy can significantly improve the clinical

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds ratio M-H, fixed, 95% CI	Year
Zhu et al., 2003	19	41	6	35	14.3%	4.17 [1.43, 12.20]	2003
Liu, 2007	18	30	9	30	14.8%	3.50 [1.20, 10.20]	2007
Ding et al., 2007	25	42	13	38	22.7%	2.83 [1.14, 7.03]	2007
Dong et al., 2012	33	60	26	60	48.2%	1.60 [0.78, 3.29]	2012
Total (95% CI)		**173**		**163**	**100.0%**	**2.53 [1.62, 3.96]**	
Total events	95		54				

Heterogeneity: $\chi^2 = 2.81$, df = 3 ($P = 0.42$); $I^2 = 0\%$

Test for overall effect: $Z = 4.06$ ($P < 0.0001$)

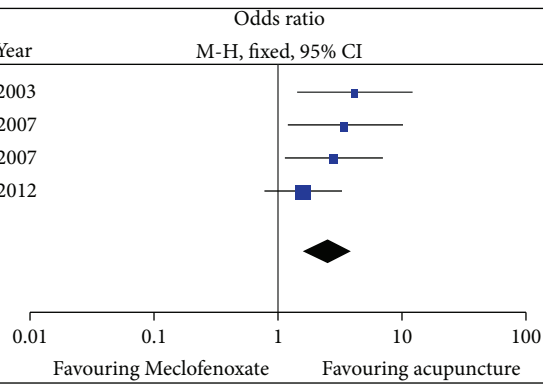

Figure 3: Subgroup analysis: acupuncture therapy versus Meclofenoxate.

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds ratio M-H, random, 95% CI	Year
Radmayr et al., 2001	13	20	15	20	29.7%	0.62 [0.16, 2.43]	2001
Zhang, 2010	22	40	6	40	33.2%	6.93 [2.38, 20.16]	2010
Moursy et al., 2014	33	62	35	62	37.1%	0.88 [0.43, 1.78]	2014
Total (95% CI)		**122**		**122**	**100.0%**	**1.57 [0.38, 6.57]**	
Total events	68		56				

Heterogeneity: $\tau^2 = 1.31$; $\chi^2 = 11.68$, df = 2 ($P = 0.003$); $I^2 = 83\%$

Test for overall effect: $Z = 0.62$ ($P = 0.54$)

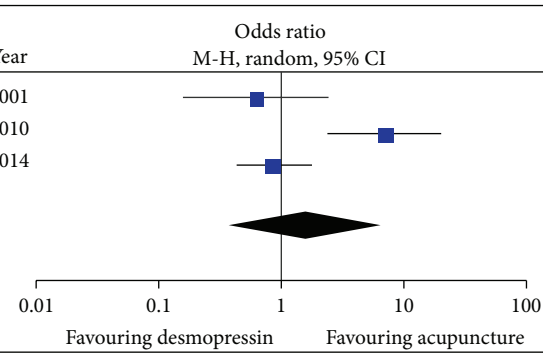

Figure 4: Subgroup analysis: acupuncture therapy versus desmopressin.

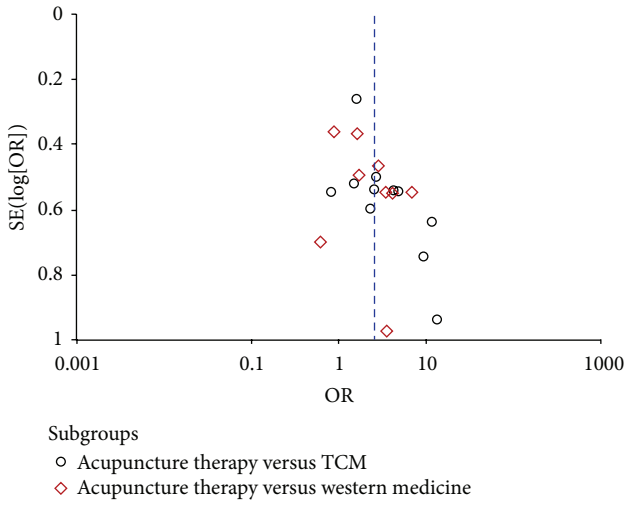

Figure 5: Funnel plot of randomized controlled trials.

efficacy in enuretic children when compared with placebo acupuncture or TCM. In contrast to western medicine, acupuncture therapy was more effective than Meclofenoxate. Conclusions regarding the safety of acupuncture therapy cannot be drawn due to the paucity of evidence provided by the included trials. However, the drawn conclusion should be interpreted cautiously owing to low methodological qualities of included studies.

4.2. Mechanism of Acupuncture Therapy. The pathogenesis of nocturnal enuresis is multifactorial; several factors such as psychosocial, developmental, hormonal, and genetic factors have been proven to be involved in nocturnal enuresis. Nocturnal polyuria, nocturnal detrusor overactivity, and high arousal thresholds are main pathogenesis of NE. To date, increasing evidence suggests that all three mechanisms can be attributed to brainstem disturbance. The locus coeruleus (LC) has axonal connections with the hypothalamic cells that produce vasopressin and also plays an important role in arousal from sleep [29, 30]. Pontine micturition center coordinates the micturition reflex and overlaps both functionally and anatomically with LC. A disturbance in this region of brainstem may cause a range of pathological changes which may result in the pathogenesis of NE.

Acupuncture points were selected in order to influence the spinal micturition centers as well as the parasympathetic innervation to the urinary tract [31]. With acupuncture stimulation, levels of enkephalins and endogenous opioids are increased in both plasma and central nervous system. An increased beta-endorphin level in human cerebrospinal fluid could be detected after acupuncture stimulation [32]. And beta-endorphin was found to be able to depress bladder contractions [33]. The therapeutic effects of acupuncture therapy can be achieved through the suppression of spinal and supraspinal reflexes which lead to bladder contraction. And the clinical efficacy of acupuncture was reflected in increase in maximum bladder capacity and suppression of

detrusor muscle activity; these functional changes might contribute to the improvement of NE.

In TCM theory, the generation and discharge of urine are associated with lung, kidney, spleen, and bladder. The pathogenesis of nocturnal enuresis is Qi deficiency of lung, spleen, and kidney; bladder is not controlled by Qi as well. Through different forms of stimulation on meridian points or specific parts of body, imbalance and instability between Zangfu organs are corrected to improve symptoms of NE and maintain the stability of inner state [34]. Based on the classical prescriptions of acupuncture, series of novel acupuncture modalities have been widely applied in clinic. In our systematic review, the specific interventions employed in these eligible trials included traditional fine needle acupuncture, moxibustion, electroacupuncture, auricular point sticking, acupoint catgut embedding, acupressure, transdermal drugs delivery systems, and acupoint injection. These techniques were considered as one type of therapy, without differentiating acupoint selection or acupuncture forms. Therefore, the findings in this review might indicate an overall efficacy trend, but definitive conclusions could not be drawn.

4.3. Comparison with Other Studies. In 2005, a systematic review reported that acupuncture in combination with another therapy could further significantly reduce the number of wet nights when compared to acupuncture therapy alone, and, regarding the comparison of acupuncture therapy with antidiuretic medication, the results showed that the outcome favored medication but was not significantly better than acupuncture therapy [35]. Our meta-analysis managed to summarize all published RCTs to compare the clinical efficacy of acupuncture therapy with pharmacological treatment or placebo treatment. The findings in our meta-analysis suggested that acupuncture therapy was more effective than both western diuretic medication and traditional Chinese medicine, which ran counter to the conclusion in aforementioned systematic review.

4.4. Limitations. Based on the studies included in our meta-analysis, the methodological qualities were judged to be generally poor, which might limit the value of conclusions about clinical efficacy of acupuncture therapy for treating NE. The vast majority of the included trials failed to describe detailed information about randomization and allocation concealment. Lack of blinding procedures in RCTs can also exaggerate the conclusions of these trials. Further assessment of acupuncture therapy needs to be taken by large-scale clinical studies which employ rigorous methodologies.

The diagnosis and therapeutic evaluation standards employed by studies, that are performed in China, are mainly in accordance with "Standards for Diagnosis of Syndromes or Diseases of TCM and Evaluation of the Therapeutic Effect" issued by the State Administration of TCM in 1994 [36]. In the studies published in English, the majority of recruited patients are diagnosed and evaluated according to the "Standardization and Definition of Lower Urinary Tract Dysfunction in Children" of the International Children's Continence Society (ICCS) [37]. To conduct a meta-analysis, the outcome measure adopted in included RCTs was clinical efficacy. Such terms, cure rate, complete improvement rate, and response rate, are synonyms; children having no bedwetting episodes on follow-ups were defined to be cured. The majority of our eligible studies failed to distinguish between NMNE and MNE, making it difficult to get a precise conclusion. To our knowledge, there is still no worldwide unified evaluation standard to assess the basic state and disease's progression of enuretic children. In addition, the duration of acupuncture sessions and follow-ups after treatments vary from studies to studies. Since acupuncture therapy has a long-lasting beneficial effect on enuretic children, the outcomes were supposed to be measured at the end of follow-ups after treatment.

The utilization of different acupuncture techniques by different investigator can greatly affect curative effect of acupuncture therapy [5]. Based on TCM theory, all acupuncture procedures need to be performed according to syndrome differentiation. A lack of understanding of TCM was reflected in the treatment models; treatment following the same pattern can reduce the therapeutic effect to some extent. Acupuncture sessions should be performed based upon strict diagnosis made by four basic diagnostic methods (inspection, auscultation, olfaction, and palpation) [38]. As various acupuncture modalities are difficult to master, practitioners and physicians are required to have a deep understanding of the mechanisms underlying NE so that acupuncture techniques could be applied appropriately. The investigators who lack universal knowledge of TCM theory should be encouraged to participate in the standardized training before the application of acupuncture.

In contrast to TCM, acupuncture therapy could further improve the clinical effect in treating nocturnal children; no subgroup analysis was made in this group because the acupuncture modalities and Chinese medicine types varied from studies to studies. The data extracted from these studies suggested an overall efficacy trend; the standardization of acupuncture techniques is one problem to be solved in need. In the subgroup analysis conducted in western medicine group, acupuncture therapy was more effective than Meclofenoxate while no significant difference could be detected between acupuncture and imipramine hydrochloride, desmopressin, or oxybutynin. Types and doses of administered drugs might affect the results of experiment to a certain extent. Given that the evidence from China occupies a large proportion, further rigorous experiments within western context are required. Considering all these above factors, the appearance of heterogeneity could be reasonably explained.

4.5. Suggestion for Future Research. The included studies in our systematic review comprise various methodological deficiencies, and the findings of the present review are somewhat limited due to low methodological qualities. Future randomized controlled trials should employ improved methodologies and reporting specifications as follows: (1) all clinical studies of acupuncture should be registered and comply with the revised standards for reporting interventions in clinical trials of acupuncture (STRICTA) [39]; (2) the sample sizes should be calculated; (3) the generation of

random allocation sequences and allocation concealment should be provided in detail; (4) these studies should be blinded and placebo controlled; (5) the standard of diagnosis should be unified and widely accepted; (6) the follow-ups after treatments are required to be at least 6 months so that patients could be revalued; (7) all adverse events associated with acupuncture should be reported and rigorously assessed.

5. Conclusion

In summary, the results of this study suggest that acupuncture therapy demonstrate better clinical efficacy than pharmacological treatment or placebo treatment in treating NE. Due to the low methodological qualities of included trials, the findings of current study should be interpreted with caution. Therefore, to further assess the potential beneficial effect of acupuncture therapy for NE, additional RCTs with rigorous experimental design, large-scale high quality methodological control, long follow-ups, and strict reporting specification are required.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Zheng-tao Lv and Wen Song contributed equally to this paper.

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Research Article

Regulation of Neurotrophin-3 and Interleukin-1β and Inhibition of Spinal Glial Activation Contribute to the Analgesic Effect of Electroacupuncture in Chronic Neuropathic Pain States of Rats

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Growing evidence indicates that neurotrophin-3, interleukin-1β, and spinal glia are involved in neuropathic pain derived from dorsal root ganglia to spinal cord. Electroacupuncture is widely accepted to treat chronic pain, but the precise mechanism underlying the analgesic effect of EA has not been fully demonstrated. In this study, the mechanical withdrawal threshold and thermal withdrawal latency were recorded. We used immunofluorescence and western blots methods to investigate the effect of EA on the expression of NT-3 and IL-1β in DRG and spinal cord of CCI rats; we also examined the expression of spinal GFAP and OX-42 in spinal cord. In present study, the MWT and TWL of CCI group rats were lower than those in the Sham CCI group rats, but EA treatment increased the pain thresholds. Furtherly, we found that EA upregulates the expression of NT-3 in DRG and spinal cord of CCI rats, while EA downregulates the expression of IL-1β. Additionally, immunofluorescence exhibited that CCI-induced activation of microglia and astrocytes was inhibited significantly by EA treatment. These results demonstrated that the analgesic effect of EA may be achieved through promoting the neural protection of NT-3 as well as the inhibition of IL-1β production and spinal glial activity.

1. Introduction

Neuropathic pain, characterized by spontaneous pain, hyperalgesia, and allodynia, is often caused by peripheral nerve injury [1, 2]. Such pain is often persistent and poorly treated by existing therapies [3]. Now we know that neuroinflammation [4], purinergic signaling [5] and some other pain signaling molecules play key roles in the development of neuropathic pain. However, the neural protection and inhibition of spinal glia mechanism in treating neuropathic pain need be given more attention to.

Neurotrophin-3 (NT-3), a member of the neurotrophins, is a target-derived neurotrophic factor that regulates sensory neuronal survival and growth [6]. NT-3 is a potent negative modulator of the neuropathic pain state associated with chronic constriction injury (CCI) of the sciatic nerve [7], which can prevent the development and maintenance of thermal hyperalgesia [8]. Additionally, NT-3 delivered by exogenous administration has been reported to alleviate the mechanical hyperalgesia induced by intramuscular acid injection in transgenic mice [9]. Moreover, NT-3 can be produced by astrocytes [10] and microglia [11]. All these studies demonstrate the benefit of NT-3 on painful states.

Interleukin-1β (IL-1β), a polypeptide proinflammatory cytokine, plays an important role in modulating neuronal excitability in the peripheral nervous systems [12]. It is released under conditions associated with persistent pain including inflammatory pain and neuropathic pain [12]. Recent studies suggest that spinal IL-1β may be produced by glial cells (microglia and astrocytes) in different chronic pain states [13, 14]. Furthermore, there is growing recognition that

spinal glia contributes to the development and maintenance of central sensitization in chronic pain [15].

Acupuncture has been used for more than 3000 years in traditional Chinese medicine [16]. Electroacupuncture (EA) is a procedure in which fine needles are inserted into an individual at discrete points, followed by electrical stimulation to relieve pain [17, 18]. In traditional Chinese medicine, Zusanli (ST-36) and Yanglingquan (GB34) are commonly used in acupuncture to treat neuropathic pain in the waist and lower extremities. ST36 is located 5 mm beneath the capitulum fibulae and lateral-posterior to the knee joint and GB34 is about 5 mm superior-lateral to ST36. ST36 and GB34 acupoints distribute near the common peroneal nerve and the superficial and deep peroneal nerves. Additionally, some studies [2, 19] have demonstrated that EA might alleviate neuropathic pain behavior of CCI rats and we have reported that EA could increase pain thresholds of rats with CCI [20]. When injury occurred, the nociceptive signals would form and come into the DRG neurons and then spinal dorsal horn following the corresponding nerves. Therefore, the analgesic effect of EA at ST36 and GB34 acupoints may be achieved by the regulation of NT-3, IL-1β, and spinal glia in DRG neurons or spinal dorsal horn.

The aim of the present study was to investigate whether the analgesic effect of EA was associated to following mechanism: (1) promote the neural protection of NT-3; (2) the anti-inflammatory effect by decreasing IL-1β; (3) inhibiting the activation of spinal glia.

2. Materials and Methods

2.1. Animals. The Institutional Animal Care and Use Committee of Wenzhou Medical University approved all experiments performed in accordance with the guidelines of the International Association for the Study of Pain. Male Sprague Dawley rats (200–250 g) were used for this study. The rats were randomly divided into 3 groups: Sham CCI group, CCI group, and CCI plus EA group. All animals were housed in plastic boxes at 22–24°C and provided free access to food and water under a 12/12 h reversed light-dark cycle.

2.2. Chronic Constriction Injury Model. The CCI model of neuropathic pain was chosen based on a previous description [21]. Briefly, after all rats were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and the right sciatic nerve was exposed at the mid-thigh level, proximal to the sciatic trifurcation, four ligature knots (4-0 chromic gut) were loosely tied with 1 mm intervals. In the Sham CCI group, the right sciatic nerve was exposed for 2-3 minutes but not ligated.

2.3. Mechanical Withdrawal Threshold (MWT). In order to evaluate mechanical allodynia, the 2392 Electronic von Frey Anesthesiometer (IITC Life Science, USA) was applied to estimate the MWT. All rats were placed individually inside a wire mesh-bottom cages (20 cm × 14 cm × 16 cm) and given 20 min of adaptation. The probe was positioned below the plantar surface of the paw with von Frey filaments at a range of 0.1–70 g, with increasing force until the rat paw twitches. At the time of paw withdrawal, the maximum force was recorded. Each rat was tested alternately in 5 min intervals, and each rat was tested 6 times. Excluding the maximum and minimum forces, the average value was used as the MWT.

2.4. Thermal Withdrawal Latency (TWL). In order to evaluate thermal hyperalgesia, the 37370 Plantar Test Apparatus (Ugo-Basile, Milan, Italy) was used to test the TWL. The rats were placed in a transparent acrylic chamber (17 cm × 11.5 cm × 14 cm) and given 20 min of adaptation. The radiant heat was set at 50°C and placed to the plantar surface of the hind paw. The withdrawal of the paw, indicating the sensation of pain in the rat, caused the infrared source stop and the reaction time was recorded. The hind paw was tested alternately at 10 min intervals and the cut-off time for heat stimulation was 40 s. Each rat was tested six times over the course of the experiment. Excluding the maximum and minimum times, the average value was expressed as the TWL.

2.5. Electroacupuncture (EA) Treatment. In the EA group, EA was started on day 7 after the CCI injury [22] and then given daily for the following 7 days; all EA was given between 9:00 and 11:00 a.m every day. The rats were maintained without anesthesia in an immobilization apparatus designed by our laboratory (patent application number: 201110021482.5, State Intellectual Property Office), a system convenient for acupuncture research and helpful to reduce stress for experimental rats. At ipsilateral ST-36 and GB-34, two needles were inserted to a depth of approximately 2-3 mm and connected to the output terminals of an EA apparatus (HANS-200E, Jisheng Medical Instruments). The frequency of stimulation was alternately applied as a square wave at 2/100 Hz, and the intensity of the stimulation was applied for 30 min at 2 mA.

2.6. Immunofluorescence. Half of all experimental animals were taken randomly for immunofluorescence study (n = 6 in each group). On day 14, the rats were deeply anesthetized using 5% chloral hydrate and perfused with 200 mL normal saline into the aorta, followed by 250 mL of 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.2–7.4). Subsequently, the ipsilateral L4-6 DRGs and whole L4-L5 lumbar spinal cords were removed, postfixed, and replaced with 30% sucrose. Transverse spinal sections (free-floating, 30 mm) and DRG sections (10 mm) were cut in a cryostat (Leica) and processed for immunofluorescence [23]. To ensure that immunohistochemical data were comparable between groups, free-floating sections were carefully processed by immunohistochemistry under the same conditions (such as the washing times, the incubating time, and the temperature). Followed by a PBS wash for 5 min, five times, all sections were sequentially blocked with 10% goat serum albumin, for one hour, in PBS + T (0.3% Triton-X 100) at room temperature and were incubated overnight at 4°C with different primary antibodies: rabbit polyclonal anti-NT-3 (1 : 200, Santa Cruz, USA), rabbit polyclonal anti-IL-1β (1 : 200, Santa Cruz, USA), mouse monoclonal anti-GFAP (astrocyte marker, 1 : 1000, Calbiochem, USA), and mouse monoclonal anti-OX-42 (microglia marker, 1 : 1000,

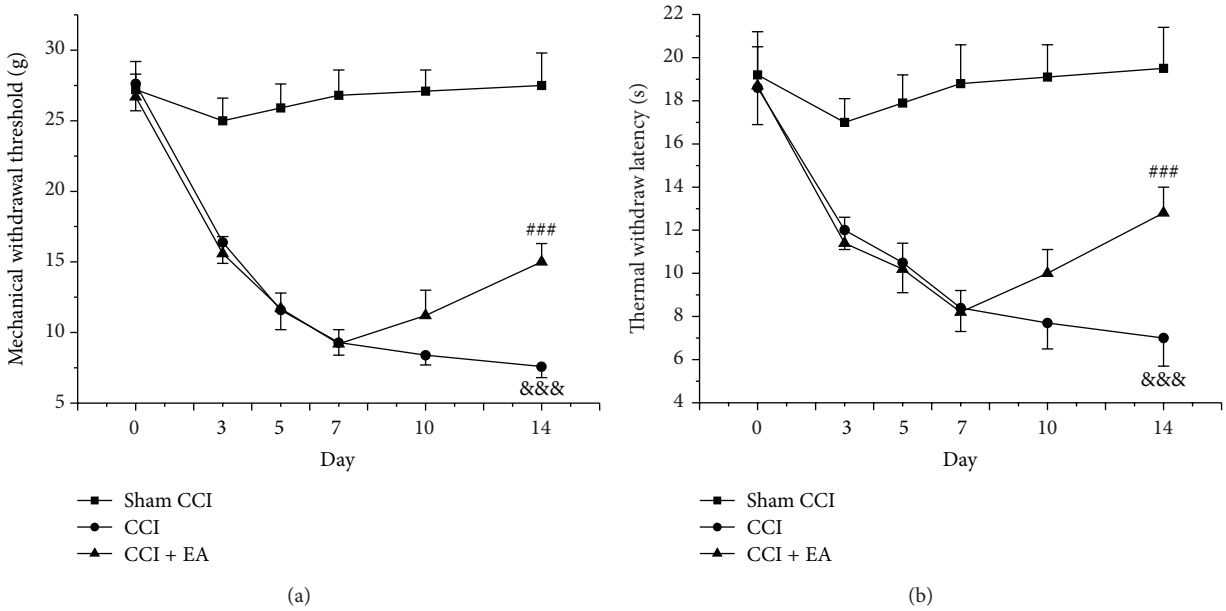


Figure 1: Analgesic effects of EA treatment on mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) induced by chronic constrictive injury. On day 14, the MWT (a) and TWL (b) in each group were recorded and compared with each other. $^{\&\&\&}P < 0.001$, versus the Sham group; $^{\#\#\#}P < 0.001$, versus the CCI group.

ABD Serotec, USA). After the primary antibody incubation, the sections were then incubated for 1 h at room temperature with secondary antibodies (1 : 400, DyLight 488-labeled goat anti-rabbit IgG or DyLight 594 AffiniPure goat anti-mouse IgG, EarthOx, USA). Finally, sections were washed with PBS and the cover-slips were mounted onto slides using antifade mounting medium (Beyotime, China). The stained sections were examined with a fluorescence microscope (Olympus, Japan).

The quantitative analysis was performed on each animal from five randomly selected sections per animal. The immunofluorescence brightness and density of the staining were tested by Image Pro Plus software: the immunofluorescence density was used for examining the positive cell of NT-3 in DRGs; the immunofluorescence bright area was used for examining the expression of IL-1β, GFAP, and OX-42 in spinal cord.

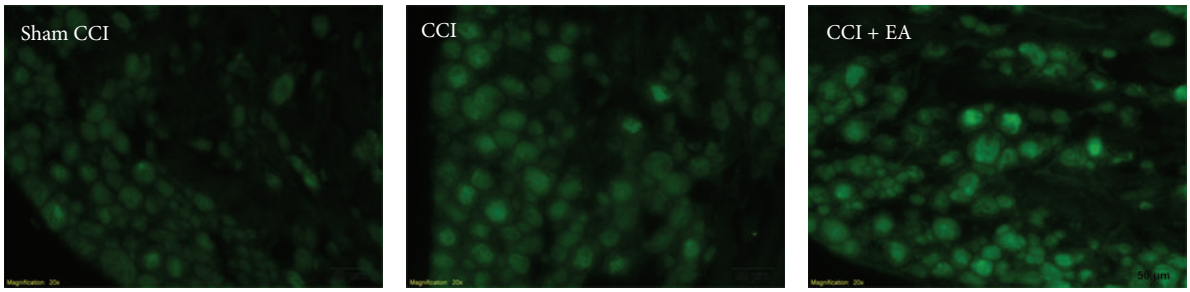
2.7. Western Blots. The remainder of experimental animals were used for western blots ($n = 6$ in each group). On day 14 after the CCI operation, the rats were deeply anesthetized and the L4-L5 spinal cord segments were isolated immediately and flushed with ice-cold PBS. The segments were lysed and microfuged at 12,000 rpm for 5 min at 4°C, and subsequently the supernatant was collected. Protein samples (30 μg) were loaded on a 10% Tris–HCl SDS-PAGE gel (Bio-Rad, Hercules, CA) for 30 min at 70 V and 55 min at 120 V. After electrophoresis, the proteins were electrotransferred to a polyvinylidene fluoride (PVDF) membrane for 50 min at 300 mA. The membranes were blocked with Tris-buffered saline (TBS), containing 0.1% Tween-20, 5% skim milk, and 0.2% BSA for 2 h at room temperature and incubated over night at 4°C with primary antibodies: anti-NT-3 (1 : 300, Santa Cruz, USA) and anti-IL-1β (1 : 250, Santa Cruz, USA). The membranes were washed four times with TBST and incubated (1.5 h, room temperature) with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG 1 : 5000, Chemicon, USA) in blocking buffer. After being washed, the labeled proteins were visualized using the enhanced chemiluminescence (ECL) kit (Beyotime, China). The immune complex was collected on Kodak light film and the quantity of band intensity was detected by a DNR Micro Chemi Chemiluminescence gel imaging system. The band densities were normalized to each glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.8. Statistical Analysis. All results were expressed as the means ± standard deviation (SD). The statistical differences were analyzed using one-way ANOVA with Tukey or Dunnett's post hoc tests for multiple comparisons. A P value < 0.05 was considered statistically significant.

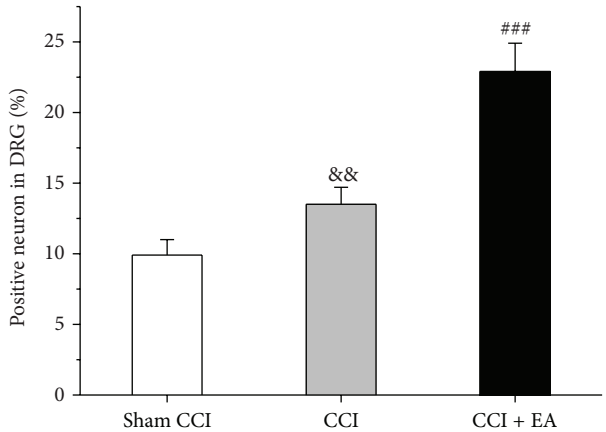
3. Results

3.1. Effect of EA on Mechanical and Thermal Hyperalgesia of CCI Rats. Before and on days 3, 5, 7, 10, and 14 after the CCI operation, the MWT and TWL were measured. On day 14, the MWT and TWL in the CCI group were significant lower than those in the Sham CCI group ($F_{(2,21)} = 458.4$, $P < 0.001$; $F_{(2,21)} = 144.6$, $P < 0.001$). However, the MWT and TWL in the EA group were higher than those in CCI group ($P < 0.001$, $P < 0.001$), implying EA treatment could increase the mechanical and thermal threshold in the rats suffering from neuropathic pain after CCI operation (Figures 1(a) and 1(b)).

3.2. Effect of EA on the Immunoreactive Changes of NT-3 in DRGs. The expression of NT-3 in DRG was observed through immunofluorescence. The immunofluorescence



(a)



(b)

Figure 2: Effect of EA on CCI-induced increase of the immunoreactive changes of NT-3 in DRG. (a) NT-3 immunopositive neurons in ipsilateral DRG of each group. (b) Quantification of positive neurons showing that EA treatment promoted the expression of NT-3. $^{\&\&}P < 0.01$, versus the Sham group; $^{\#\#\#}P < 0.001$, versus the CCI group.

density >12 was used for examining positive cells. In the CCI group, the number of positive neurons was more than that in the Sham CCI ($F_{(2,15)} = 61.1$, $P < 0.001$). However, after EA treatment, the expression of NT-3 increased further ($P < 0.001$; versus the CCI group) (Figures 2(a) and 2(b)).

3.3. Effect of EA on the Quantitative Changes of NT-3 Protein in Spinal Cord. The NT-3 expression at the protein level in spinal cord was analyzed using western blotting. The relative optical density (ROD) value for the NT-3 protein expression in the CCI group was significantly higher than that in Sham CCI group ($F_{(2,15)} = 96.2$, $P < 0.001$), and EA treatment increased that ROD in the EA group. Similar to the result of immunohistochemistry, the level of NT-3 protein significantly increased after EA treatment ($P < 0.001$) (Figures 3(a) and 3(b)).

3.4. Effect of EA on the Immunoreactive and Quantitative Changes of IL-1β in Spinal Cord. The expression of IL-1β in spinal cord was observed through immunofluorescence and western blotting. CCI injury increased the expression of IL-1β in spinal cord ($F_{(2,15)} = 50.2$, $P < 0.001$; $F_{(2,15)} = 144.6$, $P < 0.001$), compared with the Sham CCI group (Figures 4 and 5). However, in the CCI + EA group, the immunoreactivity and quantitativeness of protein of IL-1β were lower than those in the CCI group ($P < 0.001$, $P < 0.001$), implying EA could inhibit the expression of IL-1β in spinal cord of CCI rats.

3.5. Inhibitory Effect of EA on Spinal Glial Activation. By means of immunohistochemistry, we used GFAP and OX-42 to label astrocyte and microglia in the spinal cord, respectively. Expression of GFAP and OX-42 was obviously upregulated in the spinal dorsal horn on day 14 after CCI injury ($F_{(2,15)} = 44.4$, $P < 0.001$ and $F_{(2,15)} = 67.48$, $P < 0.001$, versus Sham CCI group). The expression of GFAP and OX-42 in CCI + EA group was lower than that in CCI group ($P = 0.002$ and $P = 0.003$). This result indicated that EA could inhibit the activation of astrocyte and microglia induced by CCI (Figures 6(a) and 6(b)).

4. Discussion

NT-3 is a target-derived neurotrophic factor that regulates sensory neuronal survival and growth [6]. It has been reported NT-3 could prevent the development and maintenance of thermal hyperalgesia with CCI of the sciatic nerve [8]. So, NT-3 may release the neural plasticity caused by CCI and decrease the neurocells sensibility to stimulation. Here we have shown that the pain hypersensitivity of CCI rats was released and NT-3 protein was upregulated in DRG and spinal cord after EA treatment. These findings suggest that NT-3 could promote the analgesia effect of EA in neuropathic pain.

A growing body of evidence implicates that spinal glia was involved in the modulation of chronic pain [24] and

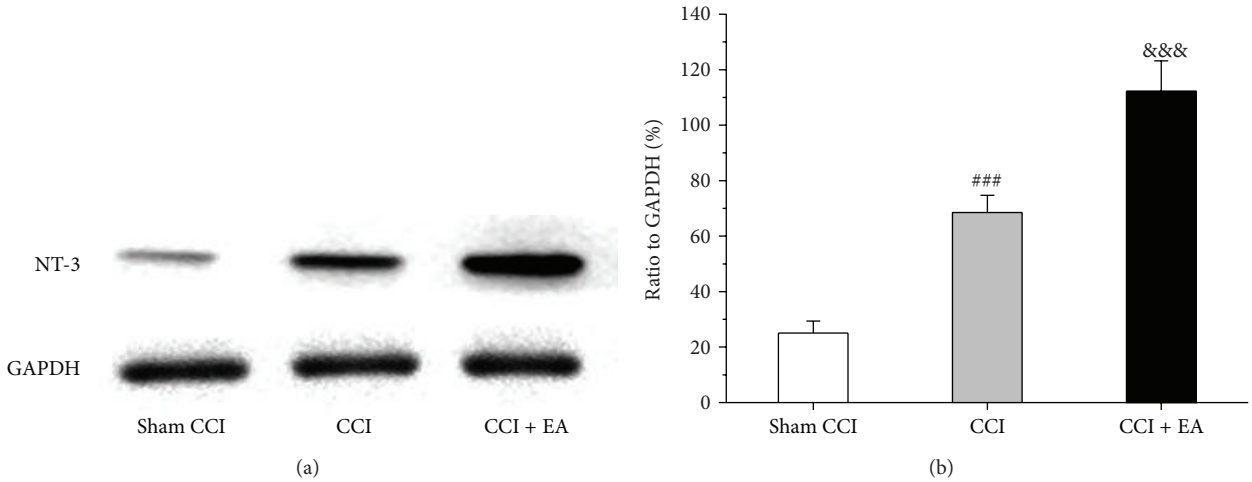


Figure 3: Effect of EA on CCI-induced increase of the quantitative changes of NT-3 protein in spinal cord. (a) Changes in the relative content of NT-3 protein in spinal cord of every group. (b) Quantification of bands showing that EA treatment promoted the expression of NT-3. $^{###}P < 0.001$, versus the Sham group; $^{\&\&\&}P < 0.001$, versus the CCI group.

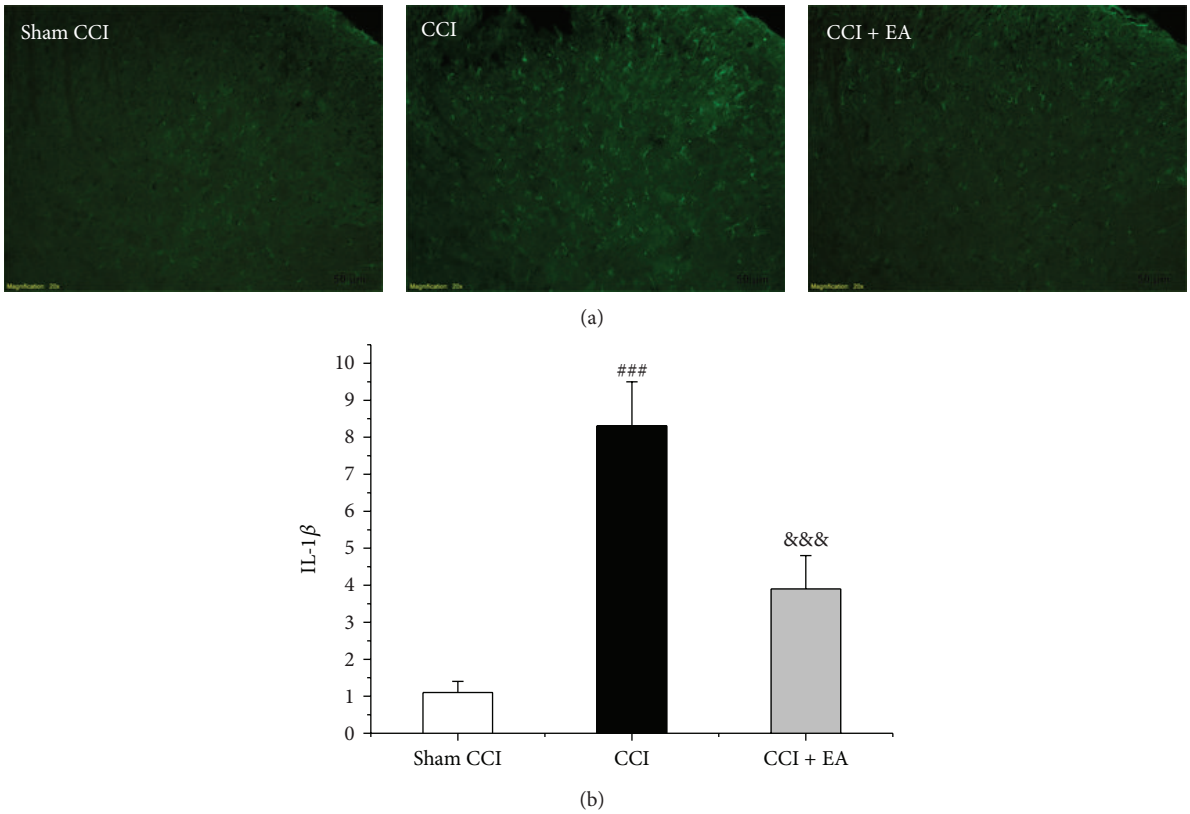


Figure 4: Effect of EA on CCI-induced increase of the immunoreactive of IL-1β in spinal cord. (a) Results of IL-1β immunofluorescence in ipsilateral spinal dorsal horn. (b) Changes of the positive area showing that EA treatment suppressed the expression of IL-1β. $^{###}P < 0.01$, versus the Sham group; $^{\&\&\&}P = 0.001$, versus the CCI group.

EA analgesia [15, 25]. Painful syndromes are associated with different glial activation states: glial reaction (i.e., upregulation of glial markers such as glial fibrillary acidic protein (GFAP) and OX-42 and/or morphological changes, including hypertrophy and proliferation) [26]. In parallel with these reports, the present study showed that CCI promotes the glial reaction, whereas EA inhibits the reaction. Furthermore, it has been demonstrated that knockdown of NT-3 markedly increased the expression of GFAP, OX-42 in the spinal dorsal horn during inflammatory pain [15]. This finding suggests that the antihyperalgesic role of NT-3 in neuropathic pain may be mediated through the inhibition of glial activity.

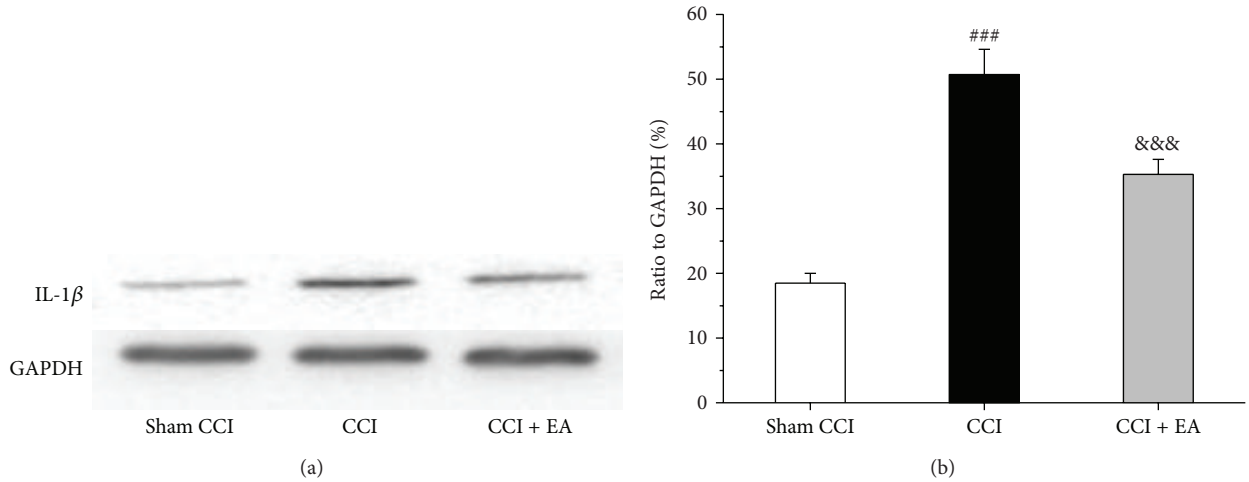


Figure 5: Effect of EA on the increase of IL-1β protein induced by CCI in spinal cord. (a) Results of western blot from spinal cord in each group. (b) Changes in the relative content of IL-1β protein from picture (a). $^{\#\#\#}P < 0.01$, compared with the Sham group; $^{\&\&\&}P < 0.001$, compared with the CCI group.

Spontaneous and evoked pain after nerve injury are thought to derive from hyperexcitability of primary and/or secondary afferent neurons generated by neurotrophins and proinflammatory cytokines released from activated inflammatory cells including microglia [27, 28] that become activated in the dorsal horn, astrocytes [29], and macrophages that invade the lesion site [30]. IL-1β is a cytokine released from spinal glial cells in response to pathophysiological changes that occur during different disease states, such as neuropathic pain and inflammatory [12, 31]. Initial reports suggested that IL-1β is an extremely potent hyperalgesic agent when injected systemically, intraperitoneally, or intraplantarly in rats [32]. In addition, the hypothalamic and ventral midbrains mRNA levels of IL-1β raised by inflammation could be reversed to normal levels by acupuncture stimulation [33]. Now that glia plays an important role in nociceptive transmission in neuropathic pain [34] and glial activity would be inhibited by NT-3; the synthesis and secretion of proinflammatory cytokines from glial cells would be decreased too. Corresponding with this conclusion, we also found the upregulation of IL-1β in spinal cord in CCI rats was suppressed after EA treatment.

In recent years, we have changed it as follow: more and more attention given to the possible roles of neurotrophins and cytokines in the therapeutic effects of acupuncture. The cross talk of neurotrophins and cytokines from peripheral nervous system (PNS) to central nervous system (CNS) is involved in the pathophysiology of many human diseases and may contribute to the effects of acupuncture [35]. Therefore, the relationship between NT-3 and IL-1β may be an important contributor to chronic pain mechanism. It has been reported that IL-1β could act on sensory neurons to increase their susceptibility for injury [36, 37], while NT-3 might release the neural plasticity caused by nerve injury and decrease the neurocells sensibility to stimulation. Interestingly, it has been demonstrated that IL-1β could upregulate the expression of NT-3 [38]. Based on these findings, we thought that IL-1β might increase the expression of NT-3 at the start of neuropathic pain, while NT-3 might depress the expression of IL-1β at the following stage. Indeed, EA treatment was given on day 7 after CCI injury in this study. So, EA may have an anti-inflammatory effect through upregulating the expression of NT-3. This conclusion is in accordance with the study that demonstrated that NT-3 might serve as an anti-inflammatory factor to suppress neuropathic pain [39].

The relationship between NT-3, IL-1β, and spinal glial cell is very important to explain the analgesic effect of EA in neuropathic pain states of rats now. Recently, it was reported that antisense oligodeoxynucleotides specifically against NT-3 intrathecally administered could suppress expression of spinal GFAP, OX-42, and proinflammatory cytokines stimulated with arthritis [15]. In addition, the inhibition of proinflammatory mediators by NT-3 pretreatment in primary microglia with LPS stimulation was corroborated [40]. Based on these published reports and the result of our study, we thought NT-3 may be involved in the analgesic effect of EA on neuropathic pain states of rats mediated through the inhibition IL-1β production and spinal glial activity.

The perception of pain requires the activation of multiple neurons across the pain system and the interactions between the thalamus, cortex, and limbic system [41]. When injury occurs, the peripheric receptor is activated and nociceptive signals are carried from the periphery to the dorsal horn of the spinal cord mostly by two populations of small diameter primary afferents, the peptidergic and the nonpeptidergic [42]. The peptidergic population expresses neuropeptides, such as substance P and calcitonin gene-related peptide, while the nonpeptidergic fibers are devoid of neuropeptides, express the purinergic receptor $P2X_3$, and bind the isolectin B4 (IB4) [42]. Considerable studies have demonstrated that chronic constriction injury of the sciatic nerve induces persistent pain behaviors in rats [43, 44] and purinergic signaling has been proved to be implicated in neuropathic

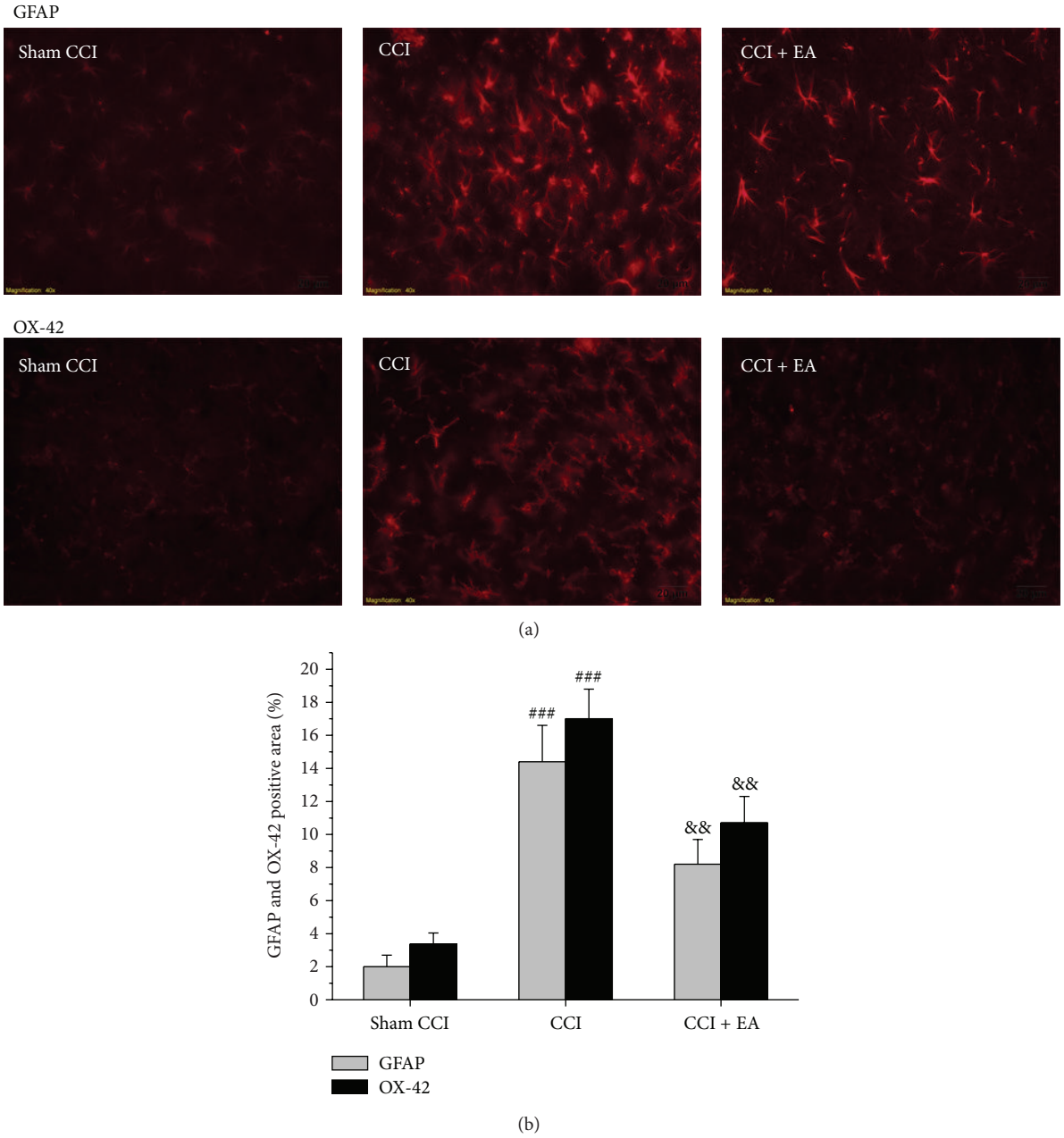


Figure 6: Effect of EA on CCI-induced increase of the immunoreactivity of GFAP and OX-42 in the spinal dorsal horn. (a) Results of GFAP and OX-42 immunofluorescence in ipsilateral spinal dorsal horn. (b) Changes of the positive area from picture (a) showing that EA treatment suppressed the expression of GFAP and OX-42 in spinal cord. $^{###}P < 0.001$ and $^{###}P < 0.001$, versus the Sham group; $^{\&\&}P = 0.002$ and $^{\&\&}P = 0.003$, versus the CCI group.

pain [43, 45, 46]. Additionally, our previous study [20] has proved that EA might increase the pain thresholds through downregulating the expression of $P2X_3$ receptor. It is a truth that some purinergic receptors (e.g., $P2X_4$ and $P2X_7$) are coexpressed with spinal glial. So, following the inhibition of glial activity, purinergic signaling would be inhibited too. A previous study has also shown that peripheral P2X receptors are involved in mediating the peripheral excitation of C- and Aδ-fiber [47]. Importantly, the ability of NT-3 to prevent and reverse thermal hyperalgesia, believed signaled by C-fibers, is a novel finding with respect to modulation of neuropathic pain [48]. In addition, the involvement of C-type afferents in EA analgesia has been proved [17]. Therefore, we speculated that NT-3 may depress purinergic signals in the analgesic effect of EA, which needs further study.

Based on previous [20] and the present study, we thought that the neuroprotective effect of NT-3 plays a key role in the analgesic effect of EA. The incremental NT-3 inhibited the activation states of spinal glia and downregulated the expression of IL-1β. All these changes promote the stability of

neurocells and decrease the generation of pain signals which is transmitted through C-fiber by purinergic signaling.

In conclusion, the present study provided new evidences that EA may exert analgesic effect by inhibiting the sprouting of nociceptive signals. Further, this effect may be achieved through the neural protection of NT-3, which decreases the expression of IL-1β and the activation of spinal glia. These results of this study provide a new and promising understanding about the mechanism underlyingthe analgesic effect of EA.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Wenzhan Tu and Wansheng Wang contributed equally to this work.

Acknowledgments

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Research Article

Brain Network Response to Acupuncture Stimuli in Experimental Acute Low Back Pain: An fMRI Study

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Most neuroimaging studies have demonstrated that acupuncture can significantly modulate brain activation patterns in healthy subjects, while only a few studies have examined clinical pain. In the current study, we combined an experimental acute low back pain (ALBP) model and functional magnetic resonance imaging (fMRI) to explore the neural mechanisms of acupuncture analgesia. All ALBP subjects first underwent two resting state fMRI scans at baseline and during a painful episode and then underwent two additional fMRI scans, once during acupuncture stimulation (ACUP) and once during tactile stimulation (SHAM) pseudorandomly, at the BL40 acupoint. Our results showed that, compared with the baseline, the pain state had higher regional homogeneity (ReHo) values in the pain matrix, limbic system, and default mode network (DMN) and lower ReHo values in frontal gyrus and temporal gyrus; compared with the OFF status, ACUP yielded broad deactivation in subjects, including nearly all of the limbic system, pain status, and DMN, and also evoked numerous activations in the attentional and somatosensory systems; compared with SHAM, we found that ACUP induced more deactivations and fewer activations in the subjects. Multiple brain networks play crucial roles in acupuncture analgesia, suggesting that ACUP exceeds a somatosensory-guided mind-body therapy for ALBP.

1. Introduction

Low back pain (LBP) is one of the most common clinical syndromes and affects 80–85% of people at some point in their life. This disorder typically causes serious socioeconomic problems, including health and economic issues, and even medications abuse [1, 2]. Most LBP does not have a definitive cause, and it has been shown that traditional Chinese medical acupuncture has beneficial effects on this intractable pain [3, 4].

Previous brain imaging studies have found that external stimuli, including acute experimental pain, could evoke deactivations in the default mode network (DMN), a network believed to be involved in the areas of memory and social affective and self-referential cognition [5, 6]. Pain stimulus could also induce extensive activations in the limbic system [anterior cingulated cortex (ACC), periaqueductal gray (PAG), prefrontal cortex] and somatosensory system (thalamus, primary somatosensory cortex (S1), secondary somatosensory cortex (S2), posterior parietal cortices, insula, supplementary motor area, striatum, and cerebellum) areas [7, 8], as well as the pain matrix (S1, S2, insular, frontal lobe and parietal lobe). The pain matrix showed a strong relationship with pain, which plays an important role in the conduction and communication of pain [9]. Moreover, research on acupuncture analgesia has recently become increasingly popular. Some researchers have found that acupuncture yields activations in the attentional- and emotional-related regions (DMN, dorsomedial, and dorsolateral prefrontal cortex (dmPFC and dlPFC)), and deactivations in the somatosensory system (left anterior insula, bilateral S1, and S2) compared with cutaneous stimuli. Therefore, some researchers considered that acupuncture could function as a somatosensory-guided mind-body therapy [10], while others

held the belief that the effect of acupuncture may not be limited to DMN or the somatosensory system. These researchers found that acupuncture evoked more deactivations of the limbic-paralimbic-neocortical network, which was thought to be centered on the limbic system, but fewer activations in the somatosensory and attentional systems compared with cutaneous stimuli [11]. Taken together, these fMRI studies, with or without experimental heat pain on limbs, have greatly contributed to our understanding of the analgesic mechanism of acupuncture; however, only a few have examined clinical pain, with the least focus on acute low back pain (ALBP). Hence, it is interesting to explore how acupuncture modulates the brain networks in ALBP subjects using fMRI.

There are two major barriers preventing fMRI studies on clinical ALBP. Firstly, it is hard to distinguish ALBP qualitatively and quantitatively from the multiple potential etiologies and their various degrees. Moreover, it is difficult to conduct experiments because ALBP is characterized by sudden onset and aggravation. In addition, using experimental heat pain to simulate ALBP is problematic, because it is difficult to expose the volunteers' back when they are lying down in the MRI scanner.

In this block design fMRI study, we introduced a simple and quantitative ALBP model induced by hypertonic saline injection in healthy volunteers to investigate the neural mechanism of acupuncture analgesia. For each experimental ALBP subject, we delivered comparable therapeutic stimuli, including ACUP and SHAM at the BL40 acupoint (Weizhong) on the right lower limb. Moreover, before and after the application of therapeutic stimuli, we collected data on subjective pretreatment and posttreatment LBP and their corresponding acupuncture sensations.

Therefore, experimental ALBP could not only act as a clinical LBP but also as evaluation criteria for the therapeutic stimuli.

2. Materials and Methods

2.1. Subjects and Acupoint. Twenty-eight healthy, right-handed subjects (11 women; age range: 22–30 years) participated in this study. All participants had some knowledge of acupuncture due to previous cultural exposure; had never received acupuncture treatment; had a body mass index within the standard range (±10%); had no psychiatric or medical illnesses (i.e., multiple sclerosis and epilepsy); and had no painful episodes (including dysmenorrhea) or did not take any drugs (i.e., antipyretics and sleeping pills) within the last month. The study was conducted with the understanding and written consent of each subject. All experiments and protocols were approved by the Ethics Committee of Zhujiang Hospital Affiliated to Southern Medical University, China.

In traditional Chinese medicine, BL40 (Weizhong acupoint) is considered as one of the four most important acupoints and proven to have unique efficacy in the treatment of ALBP. For instance, A Complete Collection of Acupuncture and Moxibustion, written by Xufeng who is an acupuncture-moxibustion expert in the Ming Dynasty, states that lumbar-back problems could be treated by puncturing this acupoint [12]. BL40 is anatomically located at the midpoint of the transverse crease of the popliteal fossa (Figure 1); therefore, each subject's keen was leaned on mattress to keep lower limbs in a valgus position for therapeutic stimuli (Figure 2).

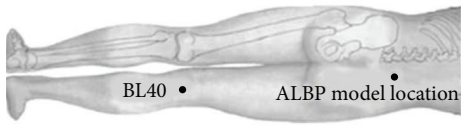

Figure 1: The location of the BL40 and ALBP model.

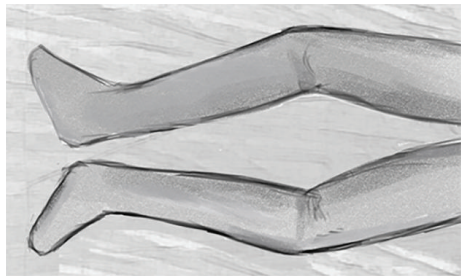

Figure 2: The posture of the subjects when inserting the needle at the point.

2.2. Experimental Procedures. Anatomical scans of the brain and functional images of sensory control stimulation were collected prior to stimulation imaging. Initially, the subjects were subjected to a baseline (the normal time) resting state (rs) MRI scan for 6 min. A preliminary ALBP model was induced in the right lower back muscle of each subject using a method modified from previous studies [13, 14]. In the experimental ALBP model, we could control the levels of pain, which gave the subjects a similar level of pain in the experiment, resulting in a smaller margin of error. The variety of clinical LBP cannot meet this requirement.

After the baseline scan, we located an injection point 2 cm lateral to the spinous process of the fourth lumbar vertebra for the ALBP model. Thereafter, we filled an indwelling needle (24 gauge) with sterile hypertonic saline (10 mL, 5%) and attached it via a long connecting tube to a computer-controlled power injector (Spectris Solaris EP; Medrad, Inc., Warrendale, PA, USA), before vertically inserting it into the above-described location at a depth of 1.5 cm (Figures 1 and 3). After 1 min, the hypertonic saline was injected intramuscularly from the above-mentioned computer-controlled power injector into the ALBP subject. This injection included a bolus injection (0.1 mL within 5 s) and subsequent continuous injection (0.15 mL/min) to produce persistent ALBP. During the first 6 min of ALBP stimulation, we performed an rs-fMRI scan to evaluate the pain status. After the pain rsfMRI scan, we obtained two functional scans for each ALBP subject: one scan during acupuncture stimulation (ACUP) and one scan during tactile stimulation (SHAM) pseudorandomly, with ALBP occurring continually throughout the scanning process. The ACUP or SHAM run comprised a block design with six 30 s blocks of

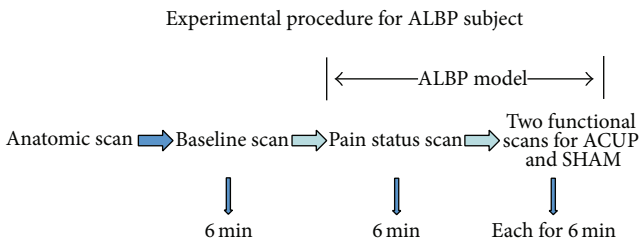

Figure 3: The experimental paradigm for the ALBP subjects included five steps.

rest time (OFF block) interspersed between six 30 s blocks of stimulation (ON block); ACUP (or SHAM) was administered at BL40 by the same experienced acupuncturist during the six ON blocks of each functional scan. Each functional scan lasted for 6 min, and the time interval between the two functional scans (ACUP and SHAM) was set at 20 min to maximize washout of the sustained effects induced by the former therapeutic stimulation (Figure 4). All MRI scans were performed with each subject laying still in a Philips 3.0 T Achieva scanner (Royal Philips Electronics, Eindhoven, Netherlands) with their eyes and ears covered.

Notably, we had examined the effects of different injection speeds (0.1 mL/min, 0.15 mL/min, 0.2 mL/min) of the hypertonic saline after a bolus injection (0.1 mL within 5 s), in the preliminary experiment. We found that 0.15 mL/min was most suitable one for our experiment, because it induced a persistent moderate-high pain in ALBP subject.

2.3. Acupuncture Modulation. ACUP was administered by inserting a nonmagnetic (pure silver), 0.4 mm-diameter, 60 mm-long acupuncture needle (Beijing Zhongyan Taihe Medicine Co., Ltd, Beijing, China) vertically into BL40 at a depth of approximately 2 cm (Figure 1). To obtain a subjective acupuncture sensation, namely, *de qi* sensation [15], the needle was manually twirled (±180°) at 1 Hz with "even reinforcing and reducing" needle manipulation in traditional Chinese medicine, while SHAM was delivered with a von Frey monofilament. The acupuncturist poked this monofilament through a needle-guide tube and tapped it gently over the skin of the BL40 with the same amplitude and rate as that used during ACUP [16].

Considering that SHAM may cause subjective bias towards the stimulation, all subjects were asked to keep their eyes and ears closed in order to prevent them from discriminating the therapeutic stimulation. Moreover, all subjects were purposely misguided that they would receive two different forms of acupuncture and needed to concentrate on the degree of acupuncture sensations of BL40. Therefore, SHAM aimed to control for not only the superficial and cutaneous somatosensory effects around BL40 but also the cognitive processing induced by the subject's expectation of "ACUP" [17].

2.4. Psychophysical Data Collection and Analysis. After each MRI scan, each subject was asked to quantify the *de qi* sensations at BL40 using a 10-point scale (0 = none, 1–3 = mild, 4–6 = moderate, 7–9 = strong, and 10 = unbearable) [16]. Moreover, each ALBP subject was asked to rate the intensity of LBP before and after each MRI scan using a 10-point visual analog scale (0 = none, 1–3 = mild, 4–6 = moderate, 7–9 = strong, and 10 = unbearable). Correspondingly, the scores of the *de qi* sensations were compared between ACUP and SHAM in the ALBP group [18] and pre- and posttreatment LBP between ACUP and SHAM in the ALBP group, using the Wilcoxon signed-rank test; P values < 0.05 were considered to be statistically significant (SPSS 13.0, IBM Corporation, NY, USA).

2.5. Imaging Data Collection and Analysis. Structural and functional scans were acquired with a 3.0 T Philips Achieva MRI System (Royal Philips Electronics, Eindhoven, Netherlands) with an 8-channel head array coil equipped for echo planar imaging. The images were axial and parallel to the anterior commissure-posterior commissure line, which covered the whole brain. Structural images were collected prior to functional imaging using a T1-weighted fast spin echo sequence (repetition time/echo time = 500/14 ms, flip angle = 90°, 0.859 mm × 0.859 mm in-plane resolution, slice thickness = 1 mm). Blood oxygenation level-dependent functional imaging was acquired using a T2*-weighted, single-shot, gradient-recalled echo planar imaging sequence (repetition time/echo time = 2000/40 ms, flip angle = 90°, 3.4 mm × 3.4 mm in-plane resolution, 180 time points for a total of 360 seconds). In addition, fMRI image collection was preceded by 5 dummy scans to minimize gradient distortion.

2.5.1. Preprocessing of Experimental MRI Data. Data analysis was performed with SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/). Preprocessing includes motion correction, slice-timing correction, normalization to the Montreal Neurological Institute standard brain (MNI152), and spatial smoothing with a Gaussian kernel of full width at half maximum of 8 mm. For motion correction, the subject's data was excluded if translation or rotation of the subject's head movements was more than 1.5 mm or 1.5°.

(1) Rs-fMRI Data Analysis. The preprocessing data were then processed to produce regional homogeneity (ReHo) map image files. The ReHo analysis was performed according to previous reports [18] and calculated using Kendall's coefficient to measure ReHo or the similarity of a ranked time series from a given voxel with that of its nearest 26 neighboring voxels in a voxelwise manner. Kendall's coefficient value was calculated for this voxel, and an individual Kendall's coefficient map was obtained for each subject. Each ReHo map was divided by its own mean ReHo within the mask for standardization purposes [18]. The ReHo value differences between the pain status and baseline were calculated using two-tailed, paired t-tests ($P < 0.05$) and corrected for multiple comparisons false discovery rate (FDR). The results were displayed using BrainNet viewer software (http://www.nitrc.org/projects/bnv/).

(2) Task fMRI Data Analysis. In the first-level analysis, the preprocessing task functional data were modeled using a

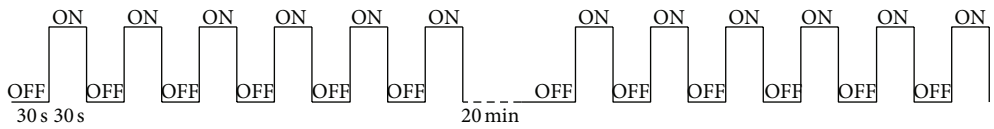

Figure 4: Each functional scan lasted for 6 min, including six OFF-ON blocks; the time interval between the two functional scans was 20 min. During the six ON blocks of each functional scan, ACUP or SHAM was applied at BL40.

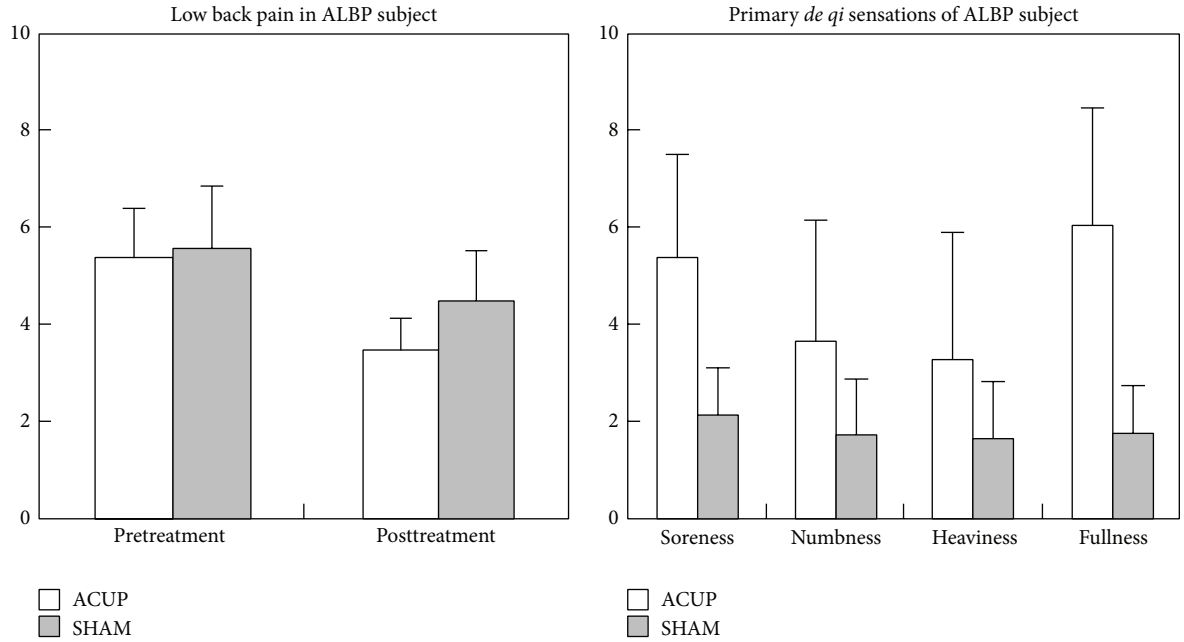

Figure 5: Results of psychophysical analysis in ACUP and SHAM. For ALBP subjects, there were significant differences between the ACUP and SHAM in the mean value of posttreatment pain ($P = 0.043$), soreness ($P = 0.014$), and fullness ($P = 0.001$).

general linear model. Explanatory variables, including the stimulation task (ACUP or SHAM, ON status) and the OFF status, were modeled using a boxcar function that convolved with the canonical hemodynamic response function in SPM8. Subsequently, parameter estimates were assessed using least-square regression analyses. Next, statistical parametric maps of the stimulation task (ACUP or SHAM) minus the OFF status contrast were collected at each voxel for each subject. In the second-level analysis, a one-sample t-test was applied to ACUP (or SHAM) minus the OFF status to assess the main effect of the stimulation, and a paired t-test was applied to ACUP minus SHAM to assess differences between the ACUP and SHAM conditions in the ALBP subjects. The threshold was set ($P < 0.05$) and corrected for multiple comparisons (FDR: <0.05). The resulting images were displayed using rest software (http://restfmri.net/forum/rest).

3. Result

3.1. Psychophysical Responses. The intensity of the lower back pain and *de qi* sensations are expressed below as mean ± standard deviation. Soreness, numbness, fullness, and heaviness were the primary *de qi* sensations in the current study. In the ALBP group, the mean values of pretreatment LBP were 5.40 (S.D. = 0.98) and 5.60 (S.D. = 1.24) and those of posttreatment LBP were 3.47 (S.D. = 0.75) and 4.51 (S.D. = 1.06) for ACUP and SHAM, respectively. There were significant differences in the score for the soreness and fullness between ACUP and SHAM for ALBP subjects (Figure 5).

3.2. fMRI Results. Compared with baseline (the normal time), the pain status showed higher ReHo values in the right medial prefrontal cortex (mPFC), right middle frontal gyrus, right insula, right precuneus (PCN), right parahippocampus (PHP), and right posterior lobe-cerebellar tonsil. However, the pain status showed lower ReHo values in the right superior temporal gyrus, left middle temporal gyrus, left S1, left ACC, left PHP, and right inferior parietal lobule ($P < 0.05$, FDR < 0.05, Table 1, Figure 6).

Compared with the OFF status, ACUP significantly affected the activations and deactivations; deactivations were found in the somatosensory system (left primary motor cortex (M1), S2, and frontal eye field), limbic system (left insula and mammillary body, right hippocampus (HP), bilateral dmPFC, pregenual ACC (pACC), PAG, and PHP), pain matrix (left S1, left insular, temporal lobe, and frontal lobe), DMN (right angular gyrus, supramarginal gyrus, lateral temporal cortex, HP, bilateral dmPFC, and PHP), and bilateral

Table 1: Resting state regional homogeneity alterations corresponding to pain status (pain status compared with baseline) paired t-test analysis $P < 0.05$, FDR < 0.05.

	BA	Cluster sizes	Peak Z-score	Peak MNI coordinate		
				X	Y	Z
R mPFC	8	32	3.02	20	27	60
R middle frontal gyrus	9	211	5.87	3	50	22
L middle temporal gyrus	21	54	−3.49	−70	−54	5
R superior temporal gyrus	38	32	−3.59	55	12	−30
L S1	2	59	−2.88	−60	−20	42
R inferior parietal lobule	40	20	−5.94	66	−36	20
L PHP	—	31	−2.62	−10	−3	−21
R PHP	35	29	4.51	30	−7	−21
L anterior cingulate cortex	32	42	−2.74	−6	25	39
R precuneus	7	54	2.70	19	−66	33
R insula	13	60	2.47	39	0	22
R cerebellar tonsil	—	53	2.42	11	−60	−48

FDR: false discovery rate; MNI: Montreal Neurological Institute; mPMC: medial prefrontal cortex; PHP: parahippocampus; S1: primary somatosensory cortex.

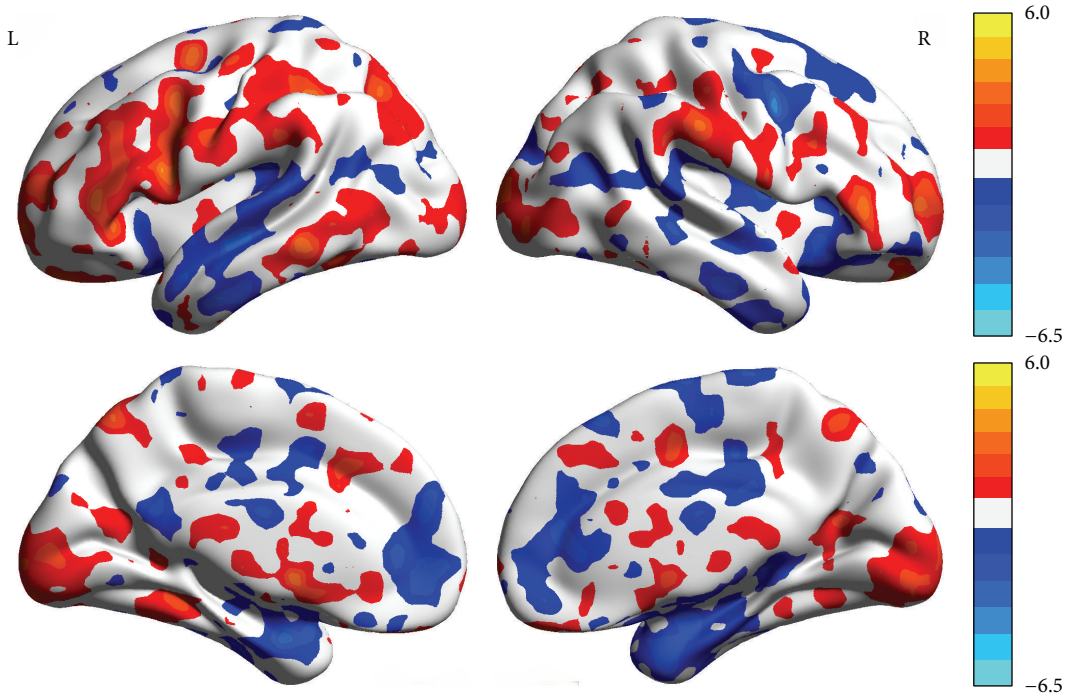

Figure 6: The brain network change in the pain status (pain status and baseline paired t-test).

thalamus. The activations, including the right M1, S1, and bilateral supplementary motor areas, right insula, and pMCC, were limited ($P < 0.05$, FDR < 0.05, Table 2, Figures 7 and 8).

Compared with the OFF status, SHAM only produced limited deactivations, such as those in the left insula, left frontal operculum, and left M1, while widespread activations included the somatosensory system (right frontal eye field and bilateral supplementary motor area), attentional system (bilateral dlPFC), limbic system (right frontopolar area, bilateral orbitofrontal cortex, PCN, PHP, HP, temporal pole, amygdala, mammillary body, and PAG), DMN (bilateral angular gyrus, supramarginal gyrus, PCN, PHP, HP, and temporal pole), bilateral thalamus, cerebellum anterior lobe, and lateral occipital gyrus ($P < 0.05$, FDR < 0.05, Table 3, Figures 7 and 8).

Compared with SHAM, ACUP only produced limited activations, including those in the right insula and right M1. In contrast, widespread deactivations were observed, including those in the somatosensory system (left supplementary motor area, bilateral frontal eye field), attentional system (right dlPFC), limbic system (left PAG, bilateral pACC, dmPFC, PHP, HP, and mammillary body), DMN

Table 2: fMRI signal changes evoked by ACUP (ACUP (ON status compared with OFF status)) one-sample t-test analysis $P < 0.05$, FDR < 0.05.

	BA	Cluster sizes	Peak Z-score	Peak MNI coordinate		
				X	Y	Z
Left insula	13	52	−3.51	−42	−15	15
Left M1	6	48	−2.56	−36	10	10
Left S2	43	36	−3.01	−37	−12	2
Left frontal eye field	8	66	−3.26	−15	35	53
Left dlPFC	46	97	3.81	−39	36	18
Right M1	6	348	6.16	51	6	12
Left PAG		44	−3.26	−3	−30	−3
Left PHP		35	−3.02	0	−24	0
Left thalamus		26	−2.56	4	−30	−5
Right dmPFC	8	43	−3.98	15	33	45
Right supramarginal gyrus	40	55	4.87	63	−27	33
Right S1	2	40	3.97	55	−20	30
Right supramarginal gyrus	40	48	−3.28	54	−60	39
Right angular gyrus	39	40	−3.00	50	−60	30
Right lateral temporal cortex	21	34	−2.56	49	−55	26
Right pMCC	31	101	3.98	18	−24	39
Bilateral SMA		79	2.54	12	0	60
Right PHP	35	46	−4.00	24	−27	−18
Right HP		40	−3.89	25	−20	−20
Bilateral pACC	32	34	−3.42	0	33	21
Left dmPFC	24	30	−3.23	15	20	20

FDR: false discovery rate; MNI: Montreal Neurological Institute; M1: primary motor cortex; S2: secondary somatosensory cortex; dlPFC: dorsolateral prefrontal cortex, periaqueductal grey (PAG); PHP: parahippocampus; dmPFC: dorsomedial prefrontal cortex; S1: primary somatosensory cortex; pMCC: posterior mid-cingulate cortex; SMA: supplementary motor area; HP: hippocampus; pACC: pregenual anterior cingulate cortex.

(right supramarginal gyrus, angular gyrus, bilateral dmPFC, PHP, and HP), bilateral thalamus, cerebellar anterior lobe, and lateral occipital gyrus ($P < 0.05$, FDR < 0.05, Table 4, Figures 7 and 8).

4. Discussion

To the best of our knowledge, this is the first fMRI study to investigate how acupuncture modulates the brain networks in experimental ALBP subjects. Behaviorally, we delivered similar pain to every subject in accordance with the ALBP model, whereas, compared with SHAM, ACUP showed stronger acupuncture sensations and weaker pain sensations, suggesting that acupuncture alleviated ALBP. As previously found, our fMRI analysis showed that ACUP induced more deactivations but less activations compared with pain status and SHAM. Furthermore, these deactivations in the ALBP subjects were mostly in the regions of the limbic system and DMN, including the antinociceptive and affective (pACC, PAG, aMCC, mammillary body, and dmPFC) and memory (DMN and mammillary body) related brain regions [16]. In contrast, the activations in the ALBP subjects were found in the attentional (dmPFC, dlPFC, pMCC, and right insula) and somatosensory system (right S1, M1, and insula) related regions compared with baseline [10]. Therefore, our results showed that multiple brain networks play important roles in modulating ALBP.

4.1. The Network Change in the Pain Status. Similar to other pain stimulation research, the results indicated higher ReHo values in some areas of the brain network. The right mPFC, right middle frontal gyrus, right insula, and right PCN are included in the pain matrix, which has a strong relationship with pain. Different parts of the matrix play different roles in the generation and transmission of pain; for example, S1 and S2 are associated with algesthesia, while the insular cortex and anterior cingulate are associated with the emotional component of pain [9]. The higher ReHo values in the pain matrix represented the pain state via the ALBP model. The mPFC is associated with the processing of emotional information and mediates the functional interactions among the brain regions that participate in pain processing [19, 20], whereas PCN is likely involved in the shifting of attention between different spatial locations [21]. Therefore, changes in ReHo may reflect pain accompanied by the processing of emotionally intense information.

ACC participates in pain perception and integration of the sensory, attentional, and cognitive components of pain [22, 23]. The decrease of ReHo in ACC suggests a reduction in efficient pain processing or compensatory damage in

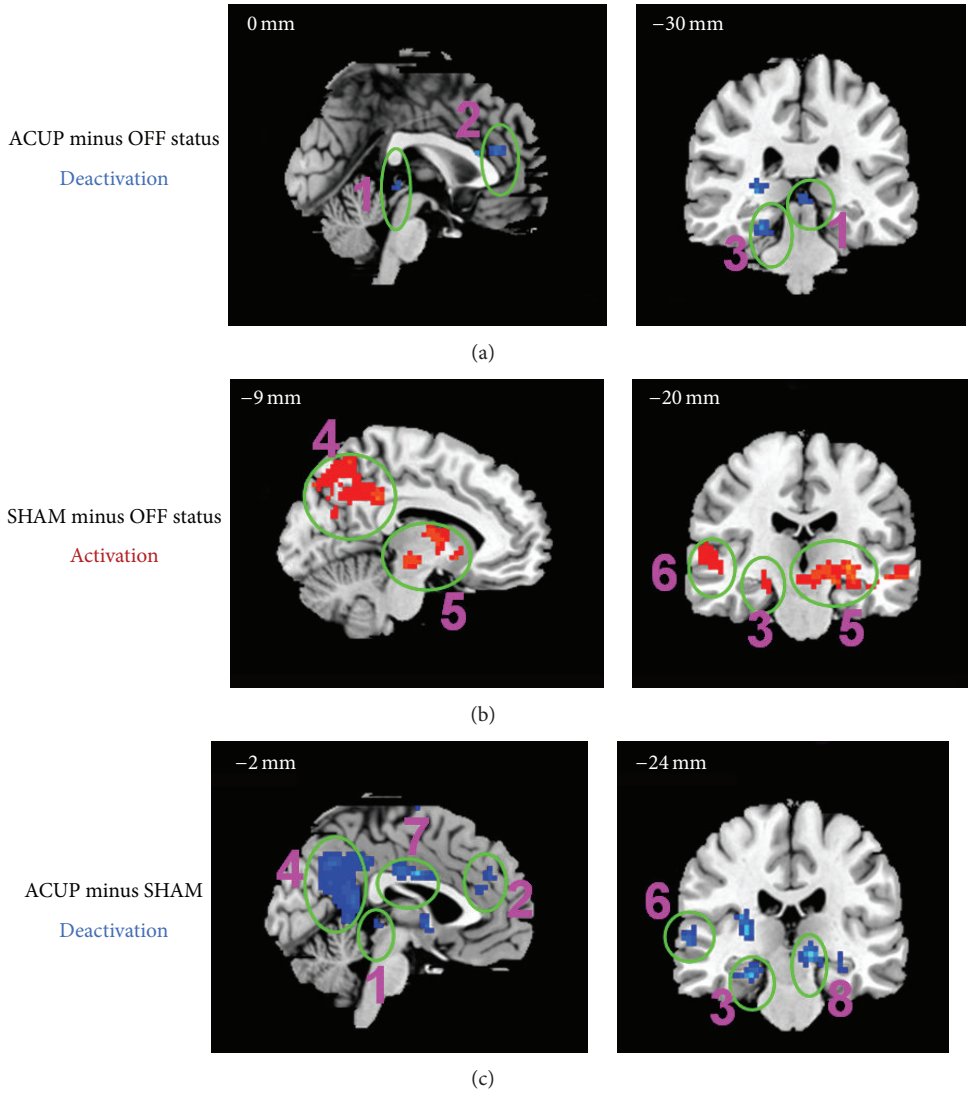

Figure 7: The fMRI signal increases and decreases in cortical and subcortical brain structures, (1) PAG; (2) pACC, aMCC, and anterior dmPFC; (3) PHP and HP; (4) PCN, PCC, and RSC; (5) striatum, thalamus, red nucleus, and substantia nigra; (6) lateral temporal cortex; (7) pMCC; (8) mammillary body.

functionally relevant regions such as the prefrontal cortex and caudate [24]. Pain is well documented to potentially interrupt cognition and sustained attention to a direct action toward a painful stimulus or threat [25]. The insula is an important component of the pain system, and its functions involve judgment about potential dangers [26]. The results showed higher ReHo values in the right insula, possibly indicating an increase in the judgment function and evasive actions during the pain state; because the insula also participates in learning and memory regarding pain [27], the higher ReHo values in the insula indicate increased function. The ACC and insula exhibited higher ReHo values in the pain matrix during experimental LBP. The negative correlations between ACC and the insula were enhanced, suggesting that the anterior insula reduces the response to peripheral nociceptive stimuli via a self-control function.

Furthermore, the brain regions with decreased ReHo values were concentrated in the left hemisphere, which verifies Naqvi's conclusion that this hemisphere corresponds to the affective consequences of pain, whereas the right hemisphere corresponds mainly to homeostatic and autonomic control [28].

4.2. The Effect of Acupuncture in the Brain Network

4.2.1. Limbic System. Interest in the role of PAG and ACC for pain modulation has a long history [7, 16, 29–32]. Anatomically, nociceptive signals can ascend to PAG and the posterolateral thalamus, for which the signals project to S1, S2, and ACC [21]; moreover, they could directly project through the midline and intralaminar thalamic nuclei to other limbic areas, including PAG, ACC, and amygdala [32]. Functionally, investigators reported that PAG demonstrated coherence with ACC (rostral and pregenual) in the resting state and formed a core intrinsic functional ACC-PAG-RVM network for pain modulation [31, 33]. Furthermore, Hui et al.

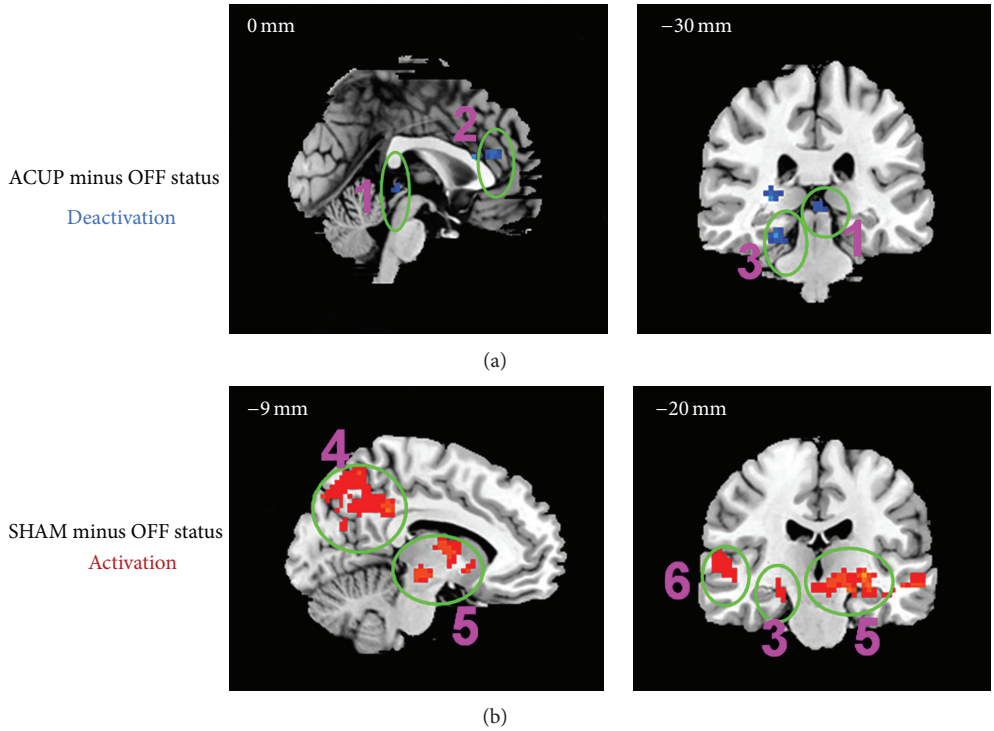

Figure 8: The fMRI signal increases evoked by ACUP and SHAM, (1) right insula and frontal operculum cortex; (2) dlPFC; (3) supramarginal gyrus/angular gyrus; (4) orbitofrontal cortex; (5) lateral temporal cortex and temporal pole.

summarized a series of their studies conducted over the last decade, and found that acupuncture analgesia was mainly relevant in terms of the deactivations in the limbic-paralimbic-neocortical network (LPNN), including PAG and ACC [16]. Consistent with these findings, our results may suggest that acupuncture therapy reverses the activation of limbic structures evoked by pain, resulting in an analgesic effect.

In 1968, Melzack and Casey described pain in terms of its three dimensions: "sensory-discriminative," "affective-motivational," and "cognitive-evaluative" [34]. Many studies have proved that the pACC and PAG are involved in not only acute pain but also emotion [31, 32]. The pACC has been found to be closely related to the affective network and could be specifically evoked by positive events [35], while PAG has been found to be significantly connected with its surrounding areas and is important for the control of emotions, for example, fear and the affective aspect of pain [31]. Furthermore, the aMCC and mammillary body may also be activated by negative emotion [36, 37]. Based on above analysis, we speculated that the unpleasantness of acute pain could induce dysfunction in these emotion-processing regions, and acupuncture may be beneficial for treating this dysfunction.

4.2.2. Default Mode Network. The DMN generally shows specific spontaneous activations when a person is left undisturbed, for example, lying peacefully in an MRI or positron emission tomography scanner. Interestingly, these activations transform into coordinated deactivations during attention-demanding tasks such as pain or acupuncture stimuli [5, 6]. Anatomically, DMN comprises the regions along the anterior and posterior midline, the lateral parietal cortex, the prefrontal cortex, and the medial temporal lobe (MTL); it therefore overlaps with the limbic system to a certain degree. The precise function of DMN remains debatable; however, analysis of its intrinsic activity has revealed that its function might be divided into the MTL and dmPFC subsystems, with a midline core (PCC and anterior mPFC) [6].

Consistent with this finding, ACUP in the ALBP subjects yielded widespread deactivations in the PCC and MTL subsystems, including the HP/PHP, pMCC/PCN/PCC/retrosplenial cortex, and the angular gyrus, which play key roles in recalling the past or imagination of the future [6, 38]. In addition to DMN, the deactivated mammillary body, which usually acts as a relay for impulses coming from the amygdala and HP through the mamillothalamic tract to the thalamus, is part of the larger Papez circuit and is involved in storing memory [39]. Furthermore, investigators demonstrated that acupuncture could modulate memory encoding and retrieving in patients with mild cognitive impairment [40]. Consequently, we propose that acupuncture reduced spontaneous memory-related cognition, which might provide psychological relief from pain.

Besides the MTL subsystem, ACUP, also deactivated the dmPFC subsystem, including the supramarginal gyrus and the dmPFC. Prior studies have demonstrated that the dmPFC is linked with lower levels of autonomic outflow regions, including PAG and the hypothalamus in monkeys and rats, respectively [41, 42], and with the pACC in humans [43]. Clinical studies further found that acupuncture with the *de qi*

Table 3: fMRI signal changes evoked by SHAM (SHAM (ON status compared with OFF status)) one-sample t-test analysis $P < 0.05$, FDR < 0.05.

	BA	Cluster sizes	Peak Z-score	Peak MNI coordinate		
				X	Y	Z
Left insular	13	99	−3.60	−39	−12	15
Left frontal operculum	6	78	−3.46	−40	0	20
Left M1	44	60	−3.01	−23	−10	14
Left SMA	6	271	4.19	−27	3	57
Right dlPFC	46	80	4.01	−48	39	21
Right SMA	6	210	3.69	30	42	24
Left dlPFC	8	189	3.44	37	43	20
Right frontopolar area	9	154	3.23	30	25	30
Left mammillary body		456	5.67	−15	−3	3
Right thalamus		356	4.57	−12	0	0
Right mammillary body		234	3.58	19	23	8
Left thalamus		315	4.43	8	3	3
Amygdala		56	3.01	3	2	7
Left Hp	23	43	4.88	−27	−36	−6
Right PHP	36	32	3.45	−20	−23	0
Orbitofrontal cortex	37	34	3.03	−19	34	3
Right temporal pole	42	35	3.23	−24	−35	0
Left PCN	7	78	4.75	6	−57	36
Right pACC	23	56	3.89	5	−50	42
Left ACC	24	57	3.76	−4	−66	22
Left angular gyrus	19	49	3.35	27	−42	−27
Right angular gyrus	19	54	3.45	−28	−44	−34
Left cerebellum anterior lobe	—	46	3.25	34	−22	−20
Right cerebellum anterior lobe	—	45	3.24	−32	−10	20

FDR: false discovery rate; MNI: Montreal Neurological Institute; M1: primary motor cortex; SMA: supplementary motor area; dlPFC: dorsolateral prefrontal cortex; HP: hippocampus; PHP: parahippocampus, PCN: precuneus; pACC: pregenual anterior cingulate cortex; ACC: anterior cingulate cortex.

sensation could inhibit the dmPFC for treating various psychological problems, such as schizophrenia and anxiety disorders [44, 45]. Moreover, acupuncture could decrease sympathetic activity and increase parasympathetic activity by inhibiting the dmPFC [46].

4.2.3. Contact between the LPNN and DMN. Regulation of negative LPNN activity was a notable result of acupuncture. This network is thought to have significant relationships with pain conduction and changes in the brain function network involved with acupuncture regulation [16]; DMN of the brain overlaps with LPNN that is deactivated by acupuncture. Research has shown that, in terms of brain function, DMN interacted with LPNN, with broad activation. We confirmed Fang et al.'s conclusion [11] that this intrinsic organization may be a core function of LPNN network in response to ACUP.

4.3. Activation Network in Acupuncture Studies. Some researchers believe that ACUP serves as a somatosensory-guided mind-body therapy, which effectively combines peripheral sensory stimuli and cognitive ratings [10]. This view suggests that (1) active cognitive ratings during acupuncture evoked stronger *de qi* sensations than passive sensory stimuli [47]; (2) stronger de qi sensations evoked by acupuncture enhance more cognition than tactile stimulation [10]; and (3) paying attention to the pain can upregulate pain, while distraction can downregulate pain [48]. In addition, acupuncture stimuli could act as a placebo, and de qi sensation ratings may promote this placebo effect [49]. Broadly consistent with these views, our result in the ALBP subjects showed that both ACUP and SHAM, along with sensory stimuli and cognitive rating, evoke prominent activations in dlPFC and pMCC (Figures 7 and 8). Furthermore, the right insula and frontal operculum cortex activated by ACUP was another important attention-related area [50]. As previously observed, these four regions were thought to provide a higher level role in attentional control, including continuous monitoring of the external world, searching behavior for active solution derivation, and regulating the skeletomotor system in the presence of interfering stimuli [10, 32, 50–52]. Unlike the findings from a previous study [10], we found that ACUP evoked stronger *de qi* sensations in ALBP subjects, inducing weaker activations in both dlPFC and pMCC compared with

Table 4: fMRJ signal changes in the comparison of ACUP minus SHAM (ACUP compared with SHAM) paired t-test analysis $P < 0.05$, FDR < 0.05.

	BA	Cluster sizes	Peak Z-score	Peak MNI coordinate		
				X	Y	Z
Left SMA	6	97	−3.75	−24	12	60
Left frontal eye field	8	76	−3.45	−20	10	80
Right insular	13	160	3.79	39	9	9
Right M1		149	3.54	54	10	11
Right frontal eye field	8	54	−3.34	23	−10	70
Right dlPFC	38	42	−3.57	−54	12	−8
Left HP	24	234	−4.46	−12	−24	−6
Left PHP	23	121	−3.89	−10	−22	0
Left mammillary body	23	111	−3.65	−16	−19	22
Left thalamus		56	−2.79	12	34	0
Right PCC	21	76	−4.01	−3	−6	30
Left ACC		45	−2.58	4	5	−45
Right supramarginal gyrus	40	387	−4.34	57	−54	24
Right angular gyrus	22	134	−3.78	60	−34	20
Right precuneus	42	145	−3.89	45	−20	−30
Right thalamus	13	84	−4.09	24	−27	4
Right insular		76	−3.75	23	30	10
Right PHP	35	81	−4.06	24	−24	−18
Right HP	28	45	−3.06	30	−10	−29
Left dmPFC	24	99	−3.78	0	33	18
Left PAG	31	117	−4.31	−9	−48	37
Right cerebellar anterior lobe		234	−4.76	9	−60	40
Left cerebellar anterior lobe		320	−4.32	−18	56	33
Lateral occipital gyrus	10	87	−3.54	2	33	−18

FDR: false discovery rate; MNI: Montreal Neurological Institute; SMA: supplementary motor area; dlPFC: dorsolateral prefrontal cortex; HP: hippocampus; PHP: parahippocampus; pACC: pregenual anterior cingulate cortex; ACC: anterior cingulate cortex; dmPFC: dorsomedial prefrontal cortex.

SHAM. These differences can be explained by the fact that the *de qi* sensations were rated at end of each functional scan, rather than during each block. Moreover, previous researchers found that too little autonomic arousal may fail to activate the dlPFC, while too much attention focused upon a task may limit dlPFC function and selection of optimal responses in people with elevated anxiety [50]. Taken together, we considered that moderate activity in the attention network may be important for acupuncture analgesia.

In animal studies, researchers found that the analgesic effects of manual acupuncture may have mainly resulted from a C-type afferent, by means of selective blockade of conduction in C- and Aδ-type afferents [4]. This effect seemed to act as a diffuse noxious inhibitory control, which also mediated C- and Aδ-type afferents and strongly alleviated the initial painful sensation [53]. On the other hand, a fMRI study showed that moderate-high thermal pain on the right forearm activated the left sensorimotor regions (S1 and M1), bilateral insula, and S2, while deactivating the right sensorimotor regions (S1 and M1) [54]. However, a clinical study in carpal tunnel syndrome subjects showed that acupuncture in the right hand yielded significant deactivation in the left S1, supplementary motor area, and anterior insula compared with noninsertive cutaneous stimulation, although this was not found to be the case in healthy control subjects [55]. Our results in ALBP subjects showed that ACUP in the right leg deactivated the left sensorimotor regions (S2, M1, and insula) while activating the right sensorimotor regions (S1, M1, and insula). One reasonable explanation for our result is that the acupuncture stimuli may have inhibited the ipsilateral ascending nociceptive inputs and facilitated the contralateral inputs to some extent.

5. Limitation

This fMRI-based study of the analgesic mechanism of acupuncture provides a good foundation for future research. However, the study also has some limitations. First, the types of data differed, particularly, the baseline and the pain state data, were rs-fMRI type data, whereas the ACUP and SHAM data were task fMRI type data. Therefore, we could only compare the pain state data with the baseline in the ReHo model and compare the ACUP or SHAM status data with the OFF status data in the GLM model. We could not compare

the rs-fMRI data with the task fMRI data. The data would be much better and more persuasive if they were of the same type and could be analyzed using the same method. Second, because each person's physique was different, the research subjects experienced different sensations of pain; it would be much better if we could divide the subjects into different groups according to their scores and for detailed analysis, similar to the *de qi* point. Third, although SHAM induced fewer deactivations than ACUP, it also yielded a somatic stimulatory effect on the ALBP model; to prove the effect of acupuncture, we could test other acupoints in the subjects and compare the results with the SHAM data.

6. Conclusion

In the present study, we found that ACUP induced more deactivations and fewer activations compared with SHAM. Furthermore, ACUP mainly induced deactivations in the limbic system and DMN, while mainly evoking activations in the attentional and somatosensory systems. Our results revealed that acupuncture analgesia may affect sensory, affective, cognitive, and autonomic functions, suggesting that acupuncture treatment exceeds somatosensory-guided mind-body therapy for ALBP. In addition, our experimental ALBP model may help to bridge the gap between clinical and experimental pain studies involving acupuncture treatment.

Experimental Site

All MRI scanning was conducted at the MRI Clinical Imaging Department at Zhujiang Hospital (Guangzhou, China).

Conflict of Interests

All authors report no conflict of interests.

Authors' Contribution

Yu Shi and Ziping Liu contributed to the work equally and should be regarded as co-first authors.

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Research Article

Effect of Repeated Electroacupuncture Intervention on Hippocampal ERK and p38MAPK Signaling in Neuropathic Pain Rats

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Results of our past studies showed that hippocampal muscarinic acetylcholine receptor (mAChR)-1 mRNA and differentially expressed proteins participating in MAPK signaling were involved in electroacupuncture (EA) induced cumulative analgesia in neuropathic pain rats, but the underlying intracellular mechanism remains unknown. The present study was designed to observe the effect of EA stimulation (EAS) on hippocampal extracellular signal-regulated kinases (ERK) and p38 MAPK signaling in rats with chronic constrictive injury (CCI) of the sciatic nerve, so as to reveal its related intracellular targets in pain relief. After CCI, the thermal pain thresholds of the affected hind were significantly decreased compared with the control group ($P < 0.05$). Following one and two weeks' EAS of ST 36-GB34, the pain thresholds were significantly upregulated ($P < 0.05$), and the effect of EA2W was remarkably superior to that of EA2D and EA1W ($P < 0.05$). Correspondingly, CCI-induced decreased expression levels of Ras, c-Raf, ERK1 and p-ERK1/2 proteins, and p38 MAPK mRNA and p-p38MAPK protein in the hippocampus tissues were reversed by EA2W ($P < 0.05$). The above mentioned results indicated that EA2W induced cumulative analgesic effect may be closely associated with its function in removing neuropathic pain induced suppression of intracellular ERK and p38MAPK signaling in the hippocampus.

1. Introduction

It has been well-documented that patients with chronic pain often experience sustained chronic psychological and physical stress and exhibit increased anxiety, depression, and deficits in working memory [1–3]. Results of a pilot study showed that in elderly patients with chronic pain, a reduced hippocampal volume and lower levels of hippocampal N-acetylaspartate to creatine ratios (NAA/Cr) were found [4]. The hippocampus, an important region of the limbic system, has been shown to be complicated in pain processing, particularly under chronic pain conditions [1–3].

Animal studies showed hippocampal abnormalities in animal models of chronic pain including short-term working memory dysfunction [5], recognition memory deficits [6], abnormal cytokine (IL-1β mRNA) expression [7, 8], deficits in long-term potentiation (LTP) [6], impaired enriched-environment neurogenesis [9], and altered synaptic plasticity [10]. Increasing evidence has demonstrated the involvement of hippocampus in acupuncture analgesia [11–13] and acupuncture signal processing [14, 15].

Our experimental studies demonstrated that in chronic constrictive injury- (CCI-) induced neuropathic pain rats, the resultant cumulative analgesic effect of repeated electroacupuncture stimulation (EAS) of Zusanli (ST36)-Yanglingquan (GB34) is closely associated with its effects in upregulating the decreased hippocampal synaptophysin immunoactivity [16], muscarinic acetylcholine receptor (mAChR)-1 mRNA and choline acetyl transferase (ChAT) mRNA expression [17], and improving synaptic plasticity of nerve cells in the hippocampal CA3 region shown by electron transmission microscope [18]. Differential proteomics analysis and

Western blotting validation indicated that the 19 hippocampal differentially-depressed proteins involving repeated EAS-induced pain relief are those participating in metabolic, physiological, and cellular processes, and so forth, and one of the top three canonical pathways identified is "mitogen-activated protein kinase (MAPK) signaling" [19].

MAPK is an important protein molecule for intracellular signal transduction and is involved in many physiological and pathological processes of biological activity. The MAPK family mainly includes extracellular signal-regulated kinases (ERK), p38 MAPK, and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), which represent three separate signaling pathways [20, 21]. The MAPKs signaling cascades from extracellular stimuli into a variety of intracellular responses are involved in various cellular functions by sequential activation of MAPKKK, MAPKK, MAPK, and transcription factors [22]. ERK signaling pathway is a cascade involving sequential activation of Ras, Raf, mitogen-activated protein kinase (MEK), ERK, p38MAPK, MKK3, 6, and p38 ($\alpha, \beta, \gamma, \delta$) [20]. By using MAPK inhibitors targeting ERK, p38 MAPK, and JNK in combination with LTP recording in the dorsal hippocampus formation (HF), Liu et al. [23] demonstrated that the specific members of the MAPK family might mediate pain-associated spatial and temporal plasticity in the HF. In addition, it has been shown that after peripheral nerve injury, ERK and p38 MAPK were activated and increased in their expression levels in the spinal dorsal horns [24–26]. However, there has been no any research on the effect of repeated EAS on changes of ERK signaling pathway and p38 MAPK in the hippocampus in neuropathic pain animals. For this reason, the present study was designed to investigate the relationship between EAS-induced cumulative analgesia and activities of ERK and p38MAPK signaling in the hippocampus in CCI rats for revealing the underlying intracellular mechanism of EAS analgesic target.

2. Materials and Methods

2.1. Ethic Statement. The protocols of the present study were approved by the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences. The study was carried out in accordance with the recommendation in the Guidelines for Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication number 80-23, revised 1996). All surgical operations were performed under anesthesia, and all efforts were made to minimize animals' sufferings.

2.2. Animals and Grouping. Adult male Wistar rats (200–250 g), purchased from Beijing Union Medical College, were acclimatized to standard laboratory conditions (about 12 h alternate light-dark cycle) of our institute's environment first for a week and were given free access to standard chow pellet diet and water. The rats were randomly assigned to 5 groups: control, model (chronic constrictive injury, CCI), CCI + EA2D (days), CCI + EA1W (week), and CCI + EA2W, with 14 rats in each group.

2.3. Chronic Neuropathic Pain. The chronic pain model was established by ligature of the left sciatic nerve with reference to modified Bennett's and Xie's methods [27]. Under anesthesia (with mixture solution of urethane 28 mg/100 g plus chloralose (Sigma, 3.3 mg/100 g)) and routine sterilization, the left sciatic nerve was exposed at the midthigh level by blunt dissection through the biceps femoris muscle. Four constrictive ligatures (4–0 surgical suture) were tied around the nerve at the distal end close to the bifid site at spaces of about 1.0 mm apart. The ligature was alright till the local moderate muscular contraction of the leg could be seen clearly. After local application of antibiotic (sodium penicillin, 9,000–10,000 U/rat), the muscle and skin were sutured in layers. For rats of the control group, the left sciatic nerve was just exposed without ligature. For reducing experimental variability, all the operations were finished by the same one operator.

2.4. Electroacupuncture Treatment. Bilateral "Zusanli" (ST36) and "Yanglingquan" (GB34) were punctured with stainless-steel acupuncture needles (Gauge 28, 0.20 mm in diameter) to a depth of about 4 mm, respectively, and stimulated electrically by using a HANS EA Stimulator (LH202, made in China). EA (2/15 Hz, 1 mA) was given to rats for 30 min, once a day, continuously for one week (from day 12 on after CCI), 2 weeks (from day 4 on after CCI), and 2 days (from day16 on after CCI), respectively.

2.5. Thermal Pain Threshold Detection. When thermal hyperalgesia test was conducted, the animal was put into a black cloth bag with the hindlimbs and tail exposed to move freely. A mobile radiant heat source (a high-intensity light beam of radiant heat dolorimeter) was focused onto the plantar surface of the hindpaw. The paw withdrawal latency (PWL) (i.e., pain threshold, PT) of the rat's bilateral footplates was detected 3 times, with an interval of about 5 min between two detections. In order to avoid potential tissue damage, the cutoff time of the radiant heat radiation was set at 20 sec. The mean PT before CCI operation was used as the control value, and 4 days after CCI operation, PT was detected again. For rats of CCI + EA groups, PT was detected on the following day for observing the posteffect of EA. In order to minimize the animal individual difference, the difference value of PWL between the healthy and the affected footplates was used as the pain score.

2.6. Western Blot. The right hippocampus was taken to be frozen in liquid nitrogen and stored at −80°C until use. Total protein was extracted first from the tissue in RIPA Lysis Buffer containing protease and phosphatase inhibitors (Roche) by using a tissue homogenizer. The tissue lysate was then centrifuged at 13000 rpm at 4°C for 20 min, and protein concentration of supernatants was determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific). Equivalent amount of protein (50 μg/tissue lysate) in each sample was loaded per lane and separated by 5% or 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for about 60 min at 90/160 V and then electrotransferred onto

polyvinylidene difluoride (PVDF) membrane for 150 min at 90 m A. The membranes were blocked with 5% bovine serum albumin (BSA, Amresco, USA) solution for 30 min at room temperature. The membranes were incubated with primary antibody Ras protein (1 : 5000, Cell Signaling Technology), c-Raf protein (1 : 2000, Cell Signaling Technology), MEK1 protein (1 : 10000, abcam), P44/42 (1 : 10000, Cell Signaling Technology), P-P44/42 (1 : 5000, Cell Signaling Technology), P38 (1 : 1000, abcam), and P-P38 (1 : 2000, Epitomics) at 4°C overnight. After washing, the membranes were incubated with secondary antibody (1 : 20000 diluted with goat anti-rabbit Immunoglobulin (Ig) G or 1 : 10000 diluted with goat anti-mouse IgG) conjugated to horseradish peroxidase (Jackson Immuno Research Laboratories) for 1 h at room temperature on the following day. The membranes were developed using an enhanced chemiluminescence (ECL) detection system to transfer to film. For densitometric analyses, the blots were scanned and quantified using Total Lab Quant analysis software (TotalLab Limited, England), and the result was expressed as the ratio of target gene immunoreactivity to β-action immunoreactivity.

2.7. RNA Isolation and Quantitative Real-Time PCR. The right hippocampus samples were excised and ground into powder in liquid nitrogen. Total RNA was isolated from hippocampus with Trizol (CW0581, CWbio. Co. Ltd., Beijing, China) and then reversely transcribed using a cDNA Synthesis Kit (CW0744, CWbio. Co. Ltd., Beijing, China). The reverse-transcribed products were amplified. The primer sequences used were as follows: ERK1: forward: 5′-CGTTCAGATGTC-GGTGTC-3′, reverse: 5′-AAAGGAGTCAAGAGTGGG-3′; ERK2: forward: 5′-CCAGAGTGGCTATCAAGAAG-3′, reverse: 5′-GGATGTCTCGGATGCCTA-3′; p38 MAPK: forward: 5′-GTACCTGGTGACCCATCTC-3′, reverse: 5′-GATTATGTCAGCCGAGTGTAT-3′; β-actin: forward: 5′-GGAGATTACTGCCCTGGCTCCTA-3′, reverse: 5′-GAC-TCATCGTACTCCTGCTTGCTG-3′. Quantitative real-time- (QRT-) PCR was performed in 96-well plates using the QRT-PCR detection systems (AB7500, Applied Biosystems, USA). Three different biological replicates for each sample were performed. All the cDNA samples were amplified in triplicate from the same RNA preparation and the mean value was calculated. Each reaction included 2 μL of cDNA, 10 μL of REALSYBR Mixture (2x), 0.8 μL (10 μmol/μL) of both forward and reverse primers, and 7.2 μL of PCR-grade water, equating to a final volume of 20 μL. PCR was performed under following conditions: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 60 s at 60°C. Then, the fluorescence acquisition after each cycle was performed. Finally, a dissociation curve was generated by increasing temperature from 65°C to 95°C in order to verify primer specificity. All samples for each reference gene were run on the same plate to avoid between-ran variations. The relative expression was calculated in accordance with the ΔΔCT method. Relative mRNA levels were expressed as $2^{-\Delta\Delta CT}$ values.

2.8. Statistical Analysis. The data collected in the present study were presented as mean ± standard deviation (mean ± SD) and analyzed by two-way repeated measures ANOVA, followed by *post hoc* test for least significant difference (LSD) to determine differences between every two groups. Statistical significance was accepted with $P < 0.05$.

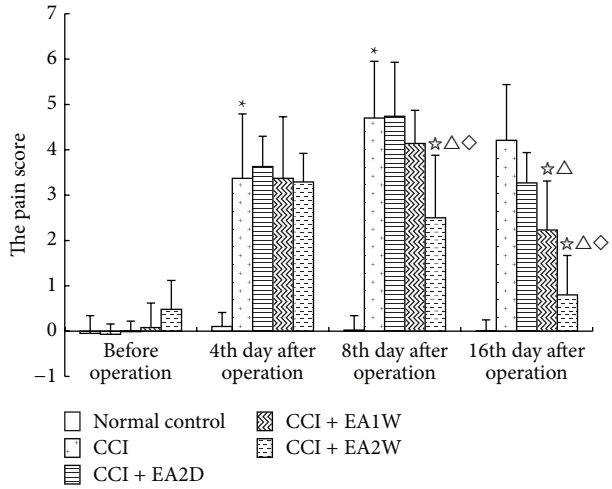

Figure 1: Effect of EA of ST36-GB34 on pain scores in CCI rats of different groups. Thermal pain thresholds after injury and EA are presented as mean ± SD ($n = 11$ in each group; $^{*}P < 0.05$ compared with the sham control group; $^{☆}P < 0.05$, compared with the CCI group; $^{△}P < 0.05$, compared with the CCI + EA2D group; $^{◇}P < 0.05$, compared with the CCI + EA1W group). Pain score = the paw withdrawal latency (PWL) of the healthy side (right) − PWL of the affected side (left).

3. Results

3.1. Effect of EA on Pain Response after CCI. The pain score is referred to the paw withdrawal latency of the difference between the healthy and the surgical footplates in the present paper. Results (Figure 1) indicated that before CCI, the pain scores of the control (sham operation), CCI model, CCI + EA2D, CCI + EA1W, and CCI + EA2W groups had no significant difference ($P > 0.05$). After CCI, the pain scores of the CCI group were evidently higher than those of the control group ($P < 0.05$), suggesting a hyperalgesia after CCI. On day 4 after CCI, the pain scores of the model group and those of the CCI + EA2D and CCI + EA1W and CCI + EA2W groups were comparable ($P > 0.05$), while on day 8, the pain scores of the CCI + EA2W group and, on day 20, those of the CCI+EA1W and CCI + EA2W were obviously lower than those of the model group ($P < 0.05$), and the effect of the CCI + EA2W group was significantly better than that of the CCI + EA2D and CCI + EA1W groups ($P < 0.05$), suggesting a cumulative analgesic effect of repeated EAS of ST36-GB34.

3.2. Effect of EA on Expression of Hippocampal Ras and C-Raf Protein in Different Groups. Ras is a membrane-associated guanine nucleotide-binding protein that is normally activated in response to the binding of extracellular signals [28], and the Raf kinase mediates the transduction of proliferative and differentiative signals from a variety of

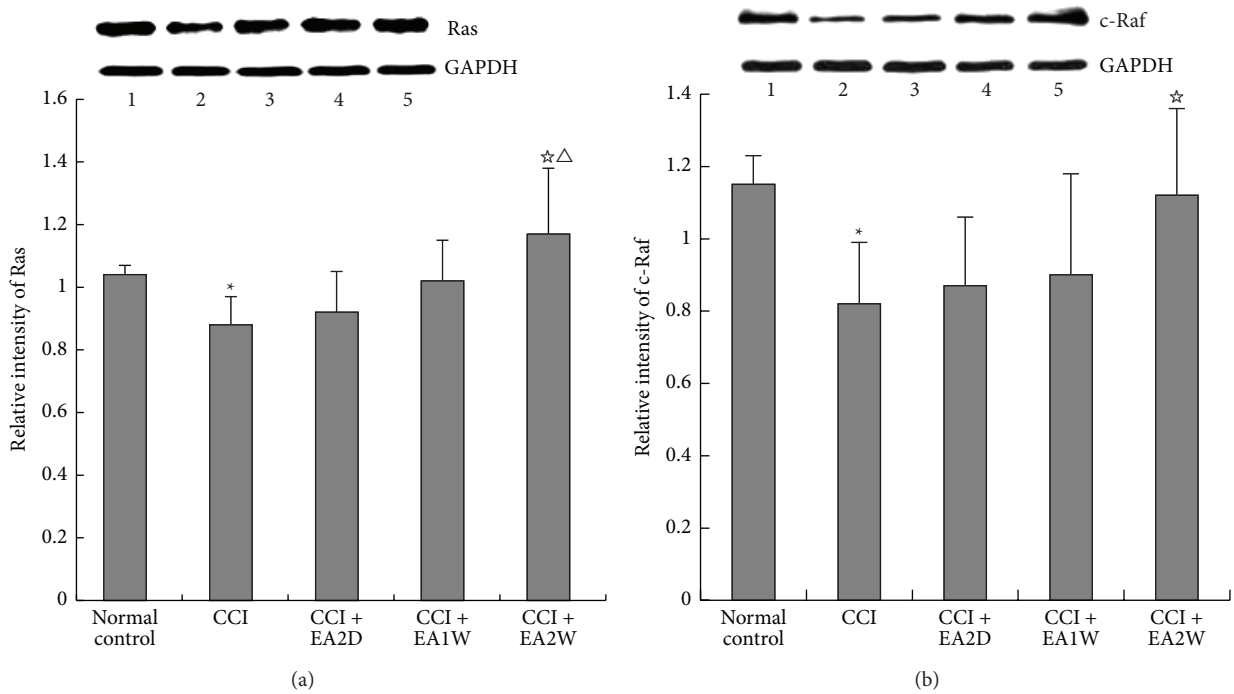

Figure 2: Effect of EAS on expression levels of hippocampal Ras and c-Raf proteins in different groups. After EA treatment, hippocampus tissues were prepared for assaying expression levels of Ras, c-Raf, and other related kinases (MEK, ERK, p38 MAPK) by Western blot. Data are presented as mean ± SD ($^{*}P < 0.05$, compared with the sham control group; $^{☆}P < 0.05$, compared with the CCI group; $^{\triangle}P < 0.05$, compared with the CCI + EA2D group; $n = 5$ in each group). (a) Top panel shows immunoblots of Ras and c-Raf proteins and GAPDH in different groups: (1) sham control group, (2) CCI group, (3) CCI + EA2D group, (4) CCI + EA1W group, and (5) CCI + EA2W group. GAPDH: glyceraldehyde-3-phosphate dehydrogenase (housekeeping gene); (b) histograms show the relative expression of Ras and c-Raf proteins in the 5 groups.

cell surface receptors to the nucleus and is the entry point to the MAPK/ERK-1/2 signaling pathway, which controls fundamental cellular functions [29].

Following CCI, hippocampal Ras and c-Raf protein expression levels were significantly downregulated in comparison with those of the control group ($P < 0.05$, Figures 2(a) and 2(b)). After EAS of ST36 and GB34, both Ras and c-Raf expression levels were considerably upregulated only in the CCI + EA2W group ($P < 0.05$), rather than in the CCI + EA2D and CCI + EA1W groups ($P > 0.05$) in spite of mild upregulation. The effect of the CCI + EA2W group in upregulating Ras protein was significantly better than that of the CCI + EA2D and CCI + EA1W groups ($P < 0.05$). No significant difference was found between the CCI + EA2D and CCI + EA1W groups ($P > 0.05$).

3.3. Effect of EA on Hippocampal MEK and p-MEK1/2 Protein Expression in Different Groups. MEK1/2 (MKK1/2) are the upstream kinases of ERK signaling. Compared with the control group, the expression levels of hippocampal MEK and p-MEK1 proteins had no significant changes in the CCI, CCI + EA2D, CCI + EA1W, and CCI + EA2W groups ($P > 0.05$, Figure 3(a)), while that of p-MEK2 protein was significantly downregulated after CCI ($P < 0.05$, Figure 3(b)). Following EAS of ST36-GB34, p-MEK2 expression had a slight upregulation in the three EAS groups ($P > 0.05$) without significant differences among the three groups ($P > 0.05$).

3.4. Effect of EA on Hippocampal ERK and p-ERK mRNA and Protein Expression. Like MEK, ERK exists in two isoforms (1 and 2). In order to identify changes of hippocampal ERK1/2 in both mRNA and protein expression levels, we conducted real-time PCR and Western blot measurements. Compared with the control group, the expression levels of hippocampal ERK1/2mRNA and ERK1/2 protein in the CCI group had no significant changes ($P > 0.05$), except for a marked upregulation of ERK1 protein expression in the CCI + EA2W group in comparison with the CCI group ($P < 0.05$, Figures 4(a) and 4(b)). Further tests revealed that the relative expression of p-ERK1/2 protein was considerably downregulated in the CCI group compared with the control group ($P < 0.05$, Figure 4(c)) and obviously upregulated in the CCI + EA2D, CCI + EA1W, and CCI + EA2W groups after EAS ($P < 0.05$). There was no significant difference among the three EAS groups in hippocampal p-ERK1/2 protein expression levels ($P > 0.05$, Figure 4(c)).

3.5. Effect of EA on Hippocampal p38 MAPK mRNA and Protein Expression. Activation of MAPK is the final step of intracellular phosphorylation cascade reactions in response to extracellular signal. Compared with the control group, hippocampal p38MAPKmRNA and p-P38MAPK protein expressions were significantly and moderately downregulated, respectively, in the CCI group ($P < 0.05$, Figures 5(a) and 5(c)). Following EA of ST36-GB34, both

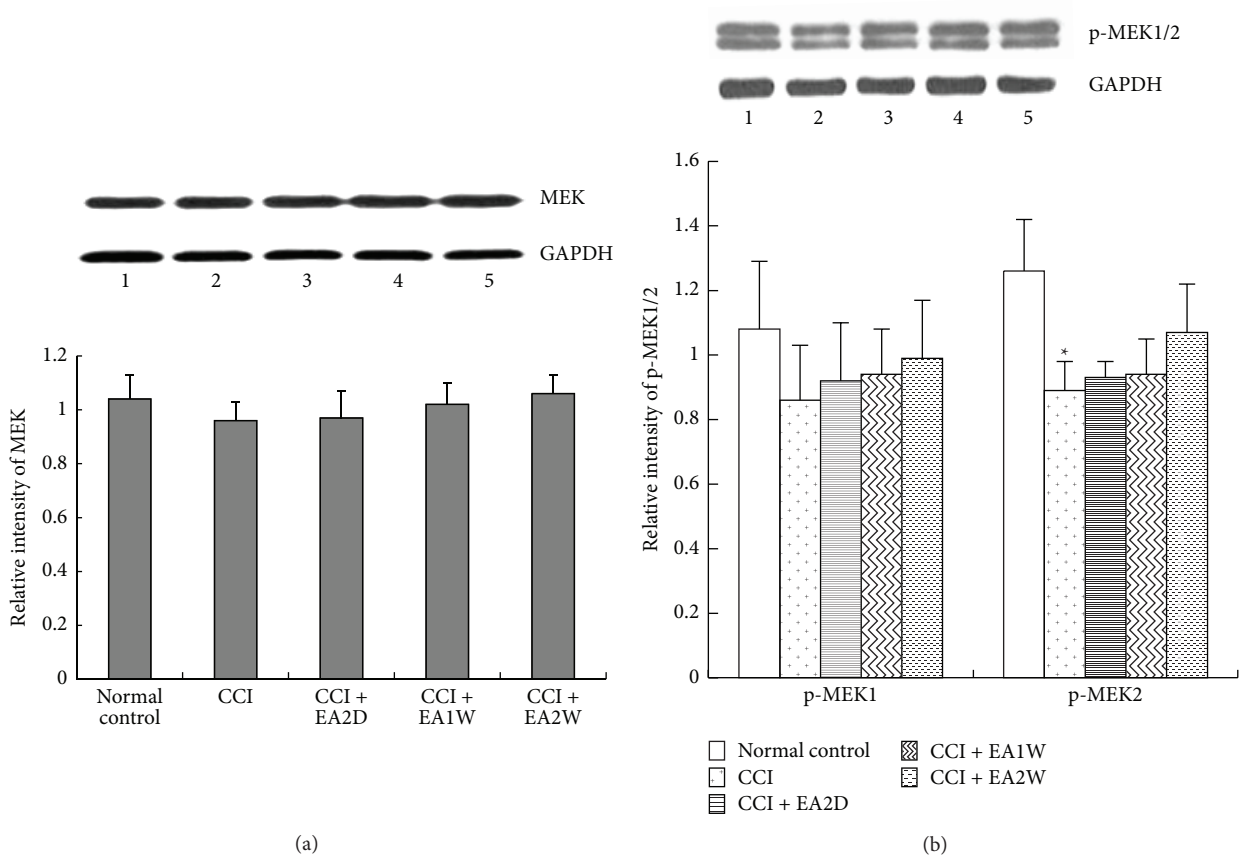

Figure 3: Effect of EA on expression levels of hippocampal MEK, p-MEK proteins in different groups. Data are presented as mean ± SD ($^{*}P < 0.05$, compared with the sham control group; $n = 5$ in each group). (a) Upper panel shows representative immunoblots of MEK protein in the 5 groups: (1) sham control group, (2) CCI group, (3) CCI + EA2D group, (4) CCI + EA1W group, and (5) CCI + EA2W group; lower histograms show the relative expression levels of MEK protein in the 5 groups. (b) The lower histograms show the relative expression levels of p-MEK1 and p-MEK2 proteins in the five groups; upper panel shows the representative immunoblots of MEK1/2 proteins and GAPDH in different groups.

p38MAPKmRNA and p-P38MAPK protein were obviously upregulated only in the CCI + EA2W group ($P < 0.05$). There were no significant changes of hippocampal p38MAPK protein expression in the five groups and p38MAPK mRNA and p-p38MAPK protein expression in the CCI + EA2D and CCI + EA1W groups ($P > 0.05$, Figures 5(a), 5(b), and 5(c)).

4. Discussion

Results of the present study showed that following CCI, the pain threshold of the affected paw was significantly lowered and the difference values of PWL of the bilateral paws (pain scores) were apparently increased, peaking on day 8 after CCI, which is similar to Bennett's and Xie's outcomes [27]. Following EAS of ST36-GB34, the pain threshold was markedly increased in both EA1W and EA2W groups, but not in the EA2D group, presenting a cumulated analgesic effect after repeated EAS, which are identical to our results of past studies [17, 30, 31] and related reports [32, 33].

Correspondingly, after CCI, the expression levels of intracellular Ras, c-Raf, p-MEK proteins, ERK2 mRNA, p-ERK1/2 protein, and p38MAPK mRNA were obviously downregulated and that of p-p38MAPK protein was moderately downregulated in spite of the fact that there was no statistical significance. It suggests an inhibition of hippocampal ERK/MAPK signaling after CCI in neuropathic pain rats. These results of hippocampal molecules are also basically identical to those of Mutso et al. report [10] which showed reduced ERK expression and phosphorylation in the hippocampus in spared nerve injury (SNI) (tight ligature and severing of the tibial and common peroneal nerves) mice and to Liu and colleagues' study [23] about an involvement of ERK and p38MAPK in pain processing in the dorsal hippocampus formation, in which ERK and p38 MAPK seemed to play opposing roles, with the former positively involved and the latter negatively involved. CCI may be considered to be chronic stress stimulation and chronic pain often resulting in depression. Thus, some molecular changes of the hippocampus under chronic stress and depression conditions may also be used as references. It was reported that chronic stress exposure caused a reduction in p-ERK and p-CREB expression in the hippocampus of rats [34, 35]. In terms of depression caused by chronic pain [1, 10, 36–38],

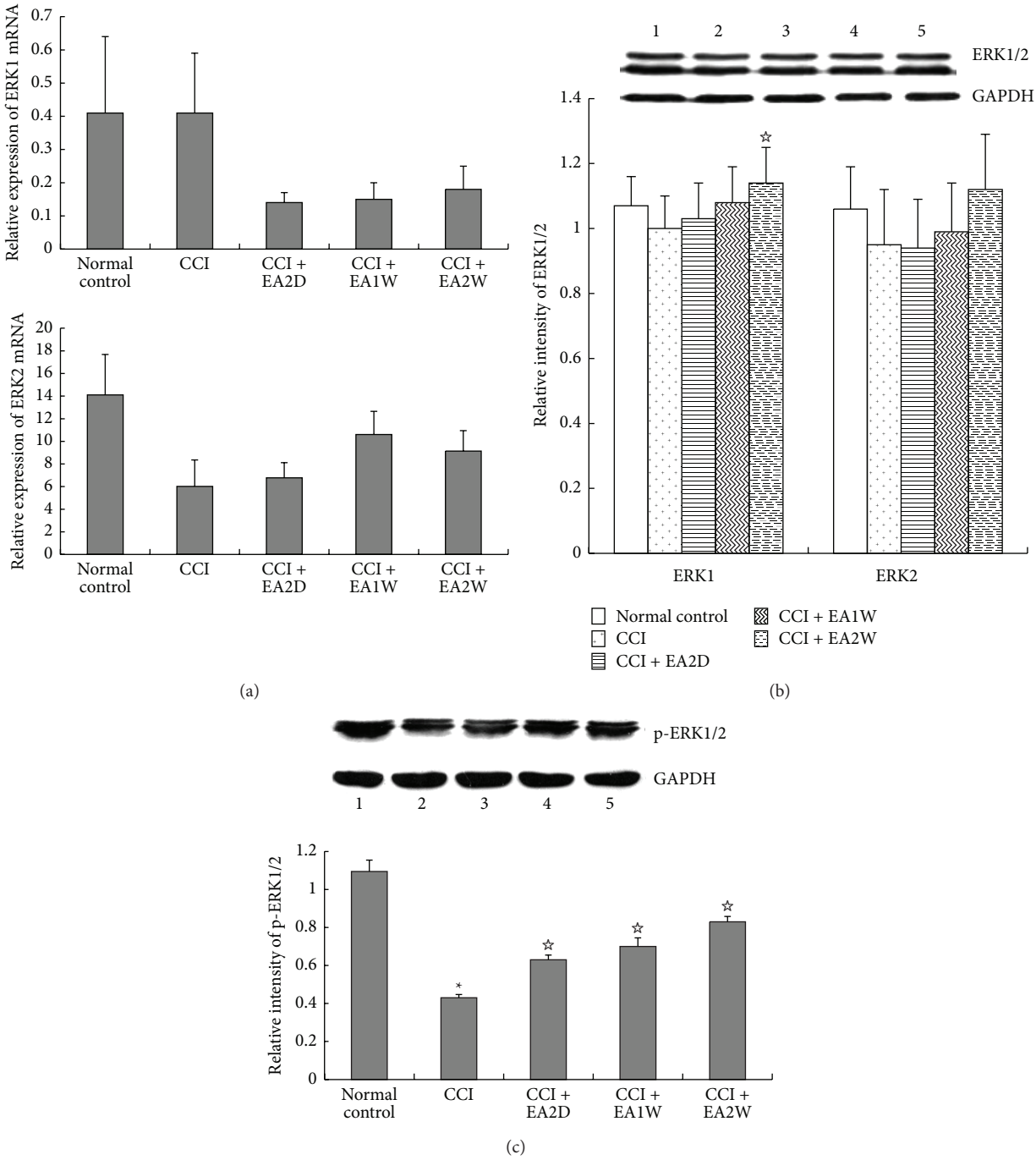

Figure 4: Effect of EA on expression levels of hippocampal ERK1/2mRNA and ERK1/2 and p- ERK1/2 protein in different groups. Hippocampal ERK1/2mRNA expression levels were assessed by real-time PCR and ERK1/2 protein expressions were detected by Western blot. Data are presented as mean ± SD ($^{*}P < 0.05$, compared with the sham control group; $^{☆}P < 0.05$, compared with the CCI group; $n = 6$ in each group for real-time PCR; $n = 5$ for each group for western blot); (a) histograms show the expression levels of ERK1/2 mRNA. (b) The top panel shows the representative immunoblots of ERK1/2 proteins in the 5 groups: (1) sham control group, (2) CCI group, (3) CCI + EA2D group, (4) CCI + EA1W group, and (5) CCI + EA2W group. The histograms show relative expression levels of ERK1/2 proteins in the 5 groups. (c) The upper panel shows the representative immunoblots of p-ERK1/2 proteins in the 5 groups. The lower bar graph shows the relative expression of p-ERK1/2 proteins in the 5 groups.

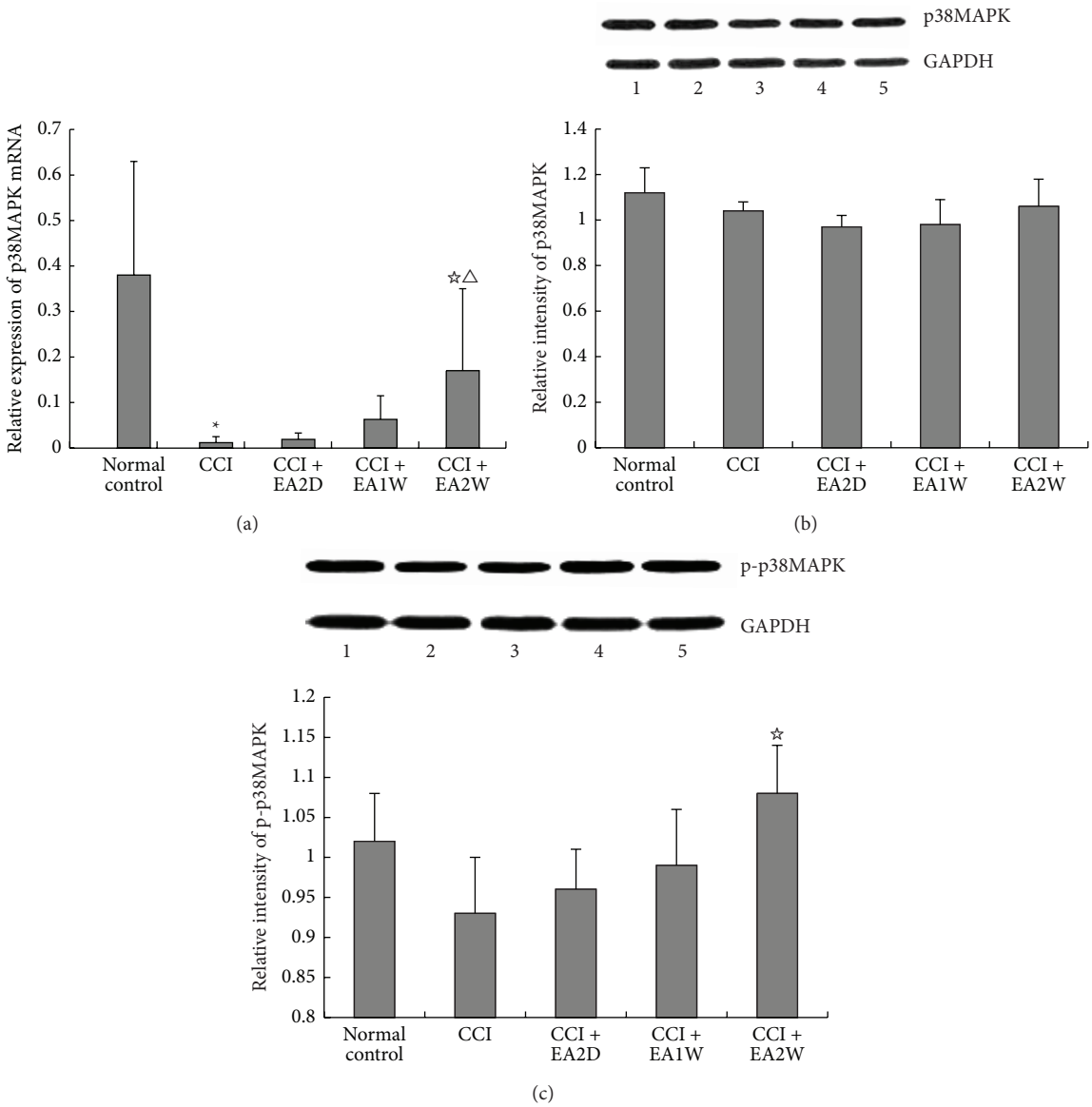

Figure 5: Effect of EA on expression levels of hippocampal p38MAPKmRNA and p38MAPK and p-p38MAPK proteins in different groups. Hippocampal p38MAPK mRNA and p-38MAPK and p-p38MAPK protein expression levels were assayed by real-time PCR and Western blot, respectively. Data are presented as mean ± SD ($^{*}P < 0.05$, compared with the sham control group; $^{☆}P < 0.05$, compared with the CCI group; $^{△}P < 0.05$, compared with the CCI + EA2D group; $n = 6$ in each group for real-time PCR; $n = 5$ for each group for Western blot). (a) Histograms of real-time PCR show the expression levels of p38MAPKmRNA in the 5 groups; (b) the top panel shows the representative immunoblots of p38MAPK protein and GAPDH in (1) sham control group, (2) CCI group, (3) CCI + EA2D group, (4) CCI + EA1W group, and (5) CCI + EA2W group. The histograms show the relative expression levels of p38MAPK protein in the 5 groups. (c) Upper panel shows the representative immunoblots of p-p38MAPK in (1) normal control group, (2) CCI group, (3) CCI + EA2D group, (4) CCI + EA1W group, and (5) CCI + EA2W group. The histograms show the relative expression levels of p-p38MAPK protein in the 5 groups.

it was demonstrated that chronic unpredictable stress (CUS) suppressed p-ERK, p-ERK1/2, and p-CREB expression in the hippocampus.

On the other hand, controversial results do exist; for instance, it was reported that 14 days of stress induced an increase in p-ERK1/2 and p-CREB expression in the hippocampus in rats with infraorbital nerve injury [39]. Under acute conditions, Guo et al. [22] observed that in the hippocampus of naïve rats, intraplantar saline or bee venom injection mimicking transient or persistent pain equally initiated an intense and long-lasting activation of hippocampal ERKs and ERK1 which were more remarkably activated than ERK2 in the hippocampus. The possible explanations for the discrepancy may lie in the difference in stress category, duration, and other experimental procedures, and the acute pain is quite different from chronic pain in the underlying mechanisms. Moreover, in the dorsal horns of the spinal cord, the ERK signaling pathway plays an important role in the

genesis and maintenance of pain, which exhibited upregulation of the expression of ERK and phosphorylated ERK proteins under peripheral nerve and tissue injury conditions [27, 40, 41].

Just as those mentioned above, p38 MAPK, an important member of the MAPKs, plays an important role in the development of central sensitization in responding to chronic nociceptive stimulation shown at the spinal cord level. Following peripheral nerve injury, p38MAPK and ERK were activated in spinal microglia, and JNK was activated in astrocytes [24, 42]. However, in the hippocampus, there has been no direct evidence for its involvement in pain processing. In view of chronic neuropathic pain induced complications as persistent stress, depression, deficits of memory, and abnormal neural plasticity changes, some findings may be used as reference evidence supporting our results. For example, as a mediator of cellular stresses, p38MAPK was implicated in depression induced by forced swim tests and tail suspension tests, exhibiting an intensive phosphorylation of PKC-dependent ERK1, ERK2, JNK, and p38MAPK in the hippocampus [43]. However, in CUS rats with impaired spatial memory, significantly decreased p-CREB and pJNK levels, but without statistical changes in CREB, ERK1/2, p-ERK1/2, p38MAPK, p-P38MAPK, and JNK levels, were found in the hippocampus [44].

Regarding the effect of EAS of bilateral ST36-GB34 on hippocampal ERK and p38MAPK signaling in the present study, following two weeks' EAS, along with the appearance of cumulative analgesia, the CCI-induced decreased expression levels of Ras, c-Raf, ERK1, p-ERK1/2 proteins, and p38 MAPK mRNA and p-pMAPK protein were considerably and gradually upregulated in the hippocampus, denoting a normalizing trend of functional activities of nerve cells under EAS-induced pain relief conditions. Most of those proteins were upregulated but had no significant changes after 2 days and one week's EAS, suggesting that two weeks' EAS has a cumulative effect in upregulating the activities of ERK and p-38 MAPK signaling along with the appearance of cumulative analgesic effect. These results are also consistent with our past results about expression levels of cellular membrane receptors including mAChR1 mRNA and protein [17] and presynaptic synaptophysin [16] in which two weeks' EAS evidently suppressed CCI-induced decrease of their expression in CCI rats. These results indicate that the EAS targets multiple signal transmission sites from extracellular to intracellular events during cumulative analgesia induction, and intracellular ERK and p-38 MAPK signal pathways play an important role in this pain processing. As we know that mAChRs are attributed to G protein-coupled receptors (GPCRs) which are critical players in converting extracellular stimuli into intracellular signals in response to various signaling inputs, and these signal inputs have to be integrated for the processing of complex biological responses. Chan et al. proved that G protein signals can be integrated at the level of MAPK, resulting in differential effects on ERK, JNK, and p38 MAPK in human brain neuroepithelioma cells as a neuronal model [45]. Despite a great variety of components of the MAPK/ERK signaling cascade, the architecture of the signal pathway is usually known as the Ras-Raf-MEK-ERK pathway [46]. Combining our past partial research results, a complete network linking the presynaptic synaptophysin, mAChR, and Ras-Raf-MEK-ERK pathway and synaptic remodeling [18] may participate in the cumulative analgesic effect of EAS in neuropathic pain.

There have been no similar reports available about the effect of EAS on hippocampal ERK and MAPK signaling in neuropathic pain animal models up to now. Therefore, we have no way to compare our outcomes with others' outcomes. However, some results may be used as a reference. For example, in depression model rats, EA could reverse CUS induced considerable upregulation of p-ERK expression, ratio of p-ERK1/2 to ERK1/2 and the ratio of p-CREB to CREB in the hippocampus [47], or enhanced the activation of hippocampal ERK signaling pathway [48], suggesting an involvement of hippocampal ERK–CREB signaling in EAS-induced antidepressant-like effects. At the spinal level, EAS could suppress complete Freund's adjuvant- (CFA-) induced activation or phosphorylation of p38MAPK in rats with inflammatory pain [49, 50]. In contusion injury induced below-level neuropathic pain rats, acupuncture stimulation of Shuigou (GV26) and Yanglingquan (GB34) relieved mechanical allodynia and thermal hyperalgesia and simultaneously inhibited neuropathic pain induced activation of p38MAPK and ERK in microglia at the L4-5 spinal cord. Injection of p38MAPK or ERK inhibitors attenuated neuropathic pain [51]. These results denote that intracellular ERK and p-38 MAPK signaling pathways in the central nervous system are involved in nociceptive information processing in chronic pain model animals.

5. Conclusion

In conclusion, results of the present study once again demonstrated the cumulative analgesic effect of repeated EAS of ST36-GB34 in CCI-induced neuropathic pain rats and reduce CCI-induced downregulation of Ras, c-Raf, ERK1, p-ERK1/2 proteins, and p38 MAPK mRNA and p-pMAPK protein in the hippocampus, suggesting an involvement of both ERK and p38 MAPK signaling of hippocampal nerve cells in EAS-induced pain relief. It is sure that this conclusion should be further confirmed by other approaches in the future.

Conflict of Interests

None of the authors has any other conflict of interests related to this paper.

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Review Article

Eye Acupuncture Treatment for Stroke: A Systematic Review and Meta-Analysis

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There were applications of eye acupuncture for stroke patients. Unfortunately, similar to many other Traditional Chinese Medicine (TCM) treatments, it lacks comprehensive evaluation and system review for its effect and safety. *Objective*. This study is a systematic review to appraise the safety and effectiveness of eye acupuncture for stroke. *Methods*. "Eye acupuncture therapy" in eleven databases was searched by randomized controlled trials and quasi-randomized controlled trials. The search activity was ended in April 2014. The data were extracted and assessed by three independent authors. Rev Man 5.0 software was used for data analysis with effect estimate presented as relative risk (RR) and mean difference (MD) with a 95% confidence interval. *Results*. Sixteen trials (1120 patients) were involved with generally poor methodological quality. The study indicated that when eye acupuncture was combined with western medicine compared to western medicine, there was a significant difference in the areas of mental state, swallow function, and NDS. When eye acupuncture was combined with western medicine and rehabilitation compared to western medicine and rehabilitation, there was significant difference in the changes of SSS, FMA, and constipation symptoms evaluation. No adverse events or side effects have been reported. *Conclusions*. The current evidence is insufficient and the rigorously designed trials are warranted.

1. Introduction

Stroke is a neurological deficit that attributed to an acute focal central nervous system damage caused by vascular problems, such as cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage. It is a major cause of disability and death worldwide [1]. The burden of ischaemic and haemorrhagic stroke have increased between 1990 and 2010 in terms of the absolute number of people with incident ischaemic and haemorrhagic stroke (37% and 47% increase, resp.), number of deaths (21% and 20% increase), and Disability Adjusted of Life Years (DALYs) lost (18% and 14% increase) [2]. Eye acupuncture is a specialized and clinic approved acupuncture treatment. It was invented by Doctor Jing-Shan Peng, the professor of Liao Ning University of Traditional Chinese Medicine, in the early 1970s.

The idea of eye acupuncture was inspired by TCM theory. In his eye acupuncture theory, for the purpose of both diagnosis and treatment of disease, Dr. Peng divided the eye into four regions, eight areas, and thirteen points [3]. Eye acupuncture therapy is thought to be a kind of microacupuncture because it is believed that the stimulations of the eye around the orbital margin can open the meridians, invigorate blood, stop pain, calm the "Shen," and regulate "Zang Fu" function [4].

Standardized manipulation of eye acupuncture is various [5]. It could be the vertical insertion within the orbital

cavity, horizontal insertion outside the orbital cavity, pricking acupuncture, double insertion, and successive insertion within and outside the orbital cavity.

Since it was invented, the eye acupuncture has been practiced in Liaoning University of Traditional Medicine for more than 40 years. Thousands of stroke patients received this special treatment. Eye acupuncture has produced a tremendous clinical significance. Today, eye acupuncture is widely used in clinical treatment including: cerebrovascular disease, pain, neurological disorders, and mental disease [6, 7].

There are about 400 trials related to the eye acupuncture stored in the database of China National Knowledge Internet (CNKI). It seems that there is a large data of applications of eye acupuncture treatment for stroke. The problem is that, similar to other effective TCM treatments, it still lacks comprehensive evaluation and system review. Thus, systematic review and meta-analysis of eye acupuncture treatment are necessary and will have a great significance for study in stroke related treatment and rehabilitation.

2. Material and Methods

2.1. Protocol and Registration. A protocol of this systematic review was published in "eye acupuncture therapy for stroke: a systematic review of randomized controlled trials" (http://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42014009632#.VHqcSNJPgoE).

2.2. Inclusion Criteria. As interventions, randomized controlled trials (RCT) and quasi-randomized controlled trials (Quasi-RCT) of eye acupuncture were included in this study. There was no limitation on language of publication or publication type.

According to the clinical criteria of the World Health Organization (WHO 1970), patients without limitations on age or gender were included if they were diagnosed as stroke patients. Patients were confirmed by purely clinical features or by the result of computed tomography (CT) or magnetic resonance imaging (MRI). Patients with ischemic as well as hemorrhagic stroke but not subarachnoid hemorrhage or subdural hematoma were considered for inclusion in the review.

The interventions include eye acupuncture and combined treatments, such as eye acupuncture combining with western medicine treatment, herbal treatment, rehabilitation therapy, or other alternative treatments. The controls could be western medicine treatment, herbal treatment, rehabilitation therapy, or other alternative treatments. Trials would be excluded if it related to any acupuncture treatment other than eye acupuncture in order to eliminate the influence of different acupuncture methods.

2.3. Identification and Selection of Studies. The relevant articles in the following databases were searched: Cochrane stroke Group Trials Register, The Chinese Stroke Trials Register, The Chinese Acupuncture Trials Register, MEDLINE, EMBASE, Alternative Medicine Database, Cumulative Index to Nursing and Allied Health Literature (CINAHL), The Chinese Biological Medicine Database (Sino Med), China National Knowledge Infrastructure (CNKI), VIP Database, and Wan fang Database.

The search activity was ended in April 2014. The following search terms were included: Ischemic stroke, Cerebral infarction, Cerebral hemorrhage, Cerebrovascular accident (CVA), Eye acupuncture, Random; Chinese phrases "zhong feng," "nao cu zhong," "nao xue guan bing," "ban shen bu sui," "pian tan," "nao geng si," "nao geng se," "nao chu xue," "nao yi xue," "nao xue shuan," "nao shuan se," "qiang xi xing geng si," "yan zhen," and "sui ji."

2.4. Data Extraction and Quality Assessment. The literature searching (BZH, ZYY), study selection (ZZX, ZYY), and data extraction (BZH, ZYY) were conducted by three independent authors. The extracted data include the name of author, title of study, year of publication, study size, age and gender of the participants, outcomes, adverse effects, prick depths of eye acupuncture, and eye acupoints for each study. Disagreement was resolved by discussion, and consensus was reached through a third party (LCR).

2.5. Data Analysis. Rev Man 5.0 software was used for data analysis. The effect estimates were presented as relative risk (RR) and mean difference (MD) with a 95% confidence interval. If a sufficient number in randomized trials were identified, the subgroup analyses for the outcomes, such as ADL, MRS, OHS, NIHSS, CSS, MMT, HAMD, MMSE, and WST, would be carried out.

Meta-analysis could be performed if the trials had a good homogeneity on study design, participants, interventions, controls, and outcome measures. Heterogeneity [8] between studies could be investigated by I^2 statistic which quantifies inconsistency across studies. If an I^2 was larger than 50%, it could indicate the possibility of heterogeneity. Both fixed effect model and random effect model would be used if there was a possibility of statistical heterogeneity among trials. The fixed effect model would be used for meta-analysis, if I^2 is less than 50%. The missing data could be obtained from the original trial authors. If a sufficient number of randomized trials were identified, the sensitivity analyses would be performed to explore the influence of trial quality for effect estimates. The adequacy of generation of allocation sequence, concealment of allocation, doubles blinding, and use of intention-to-treat (yes or no) were included as the quality components of methodology.

3. Results

3.1. Description of Studies. 16 randomized trials [9–24] were included in this review. Five trials were reported as thesis [11, 13, 14, 19, 22], and the remaining 11 trials were published in Chinese journals. A flow chart depicting the search process and study selection is shown in Figure 1. 16 RCTs and a total of 1120 stroke patients were involved in this review (69 patients per trial).

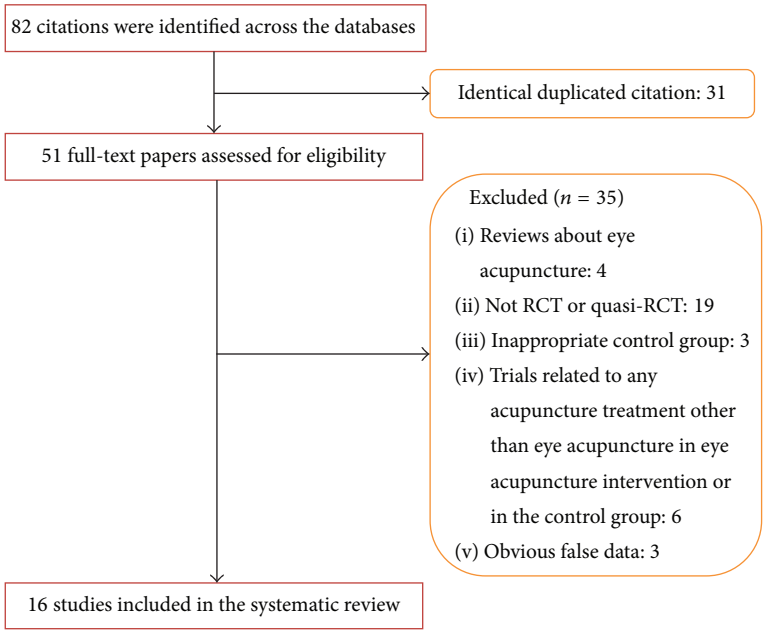

Figure 1: Flow chart of study selection.

The intervention time point for ischemic stroke and hemorrhagic stroke in this study was varying from 1–3 days to more than 6 months.

The content of intervention includes eye acupuncture, eye acupuncture combined with western medicine, TCM herbal treatment, and rehabilitation. The control included western medicine, TCM herbal treatment, and rehabilitation.

The outcomes were different. As the primary outcome, CSS (Chinese Stroke Scale) was reported in 7 trials [9, 11, 13–15, 20, 22]. Activities of Daily Living (ADL) were reported in three trials [12, 17, 22]. HAMD (Hamilton Depression Scale) and WST (water swallow test) were reported, respectively, in two trials [16, 23]. The first defecation time and constipation symptoms were evaluated in one trial [19]. SSS (Scandinavian Stroke Scale) and FMA (Fugl-Meyer Scale) were assessed in one trial [17]. MMSE (Mini-Mental State Examination) was reported in one trial [18]. The ranked data for effect judgment based on clinic neurological function deficit scale (NDS) was applied in one trial [10]. As secondary results, the level change of ET and that of CGRP were reported in three trials [10, 13, 14, 24] and the level change of FIB [9] was reported for pathological improvement. The change of CRP level was observed in one trial [15]. VEGF (vascular endothelial growth factor) at the end of treatment was detected in one trial [25]. The characteristic of all included studies has been presented in Table 1.

3.2. Methodological Quality. The study shows that the quality of all included trials is poor. Five trials [10, 13–15, 22] used random number table to allocate treatment. Three trials [11, 19, 21] were quasi-randomized. In these 3 trials, the patients were allocated alternately according to the visiting time point with the doctors in hospital. Nine trials did not describe the details of sequence generation. Neither adequate concealment nor blinding method was used in all trials. No follow-up document was provided. Protocols were not available. The missing data in three trials [12, 15, 25] were not available. Methodological quality has been summarized in Figure 2.

3.3. Effects of Interventions. Results of meta-analysis were listed in Table 2 (estimate effect of included trials in meta-analyses).

3.3.1. Changes of CSS at the End of Treatment. The outcome of CSS at the end of the treatment was measured in 8 trials [9, 11, 13–15, 20, 22] with 452 patients. When eye acupuncture is combined with western medicine compared to western medicine [9, 13–15], there was an obvious difference (MD −4.24, 95% CI −5.59 to −2.89 Fixed, I^2 = 31% Fixed). One trial [11] compared the eye acupuncture combined with TCM herbal treatment to TCM herbal treatment, and there was a clear difference (MD −2.89, 95% CI −4.15 to −1.63). There was a significant difference between eye acupuncture combined with rehabilitation and western medicine versus rehabilitation with western medicine [22] (MD −2.40, 95% CI −3.76 to −1.04). There was no significant difference between eye acupuncture combined with rehabilitation and rehabilitation [20] (RR −2.40, 95% CI −4.87 to 0.07).

3.3.2. Changes of ADL at the End of Treatment. The change of ADL score was measured in 3 trials [12, 17, 22] with 207 patients. Two of these trials [17, 22] were collected on continuous variable with 140 patients. The data in the other trial [12] were not available. There was a significant difference when eye acupuncture was combined with rehabilitation and western medicine versus rehabilitation and western medicine [17] (MD 17.60, 95% CI 14.19 to 21.01). One trial [22]

Table 1: Characteristic of all included trials.

Study ID	Study type	Sample size (T/C, male/female)	Age (yr, T/C)	Ischemic or hemorrhagic	Intervention		Duration	Area of eye acupuncture intervention	Prick depth	Outcomes
					Eye acupuncture intervention	Control intervention				
Wang et al. (2008) [9]	RCT	T: 60 (32/28) C: 60 (29/31)	T: 66 (42~70) C: 64 (41~70)	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Shuxuening injection (extract of Ginkgo) (2) Citicoline Injection (3) low-dose aspirin	14 days	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, spleen, and heart	NA	CSS; FIB
Zhou et al. (2011) [10]	RCT	T: 60 (46/16) C: 60 (42/18)	NA	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Sodium Ozagrel injection (2) Citicoline injection (3) Low-dose aspirin	15 days	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, spleen, and heart	2 mm along the cavity orbital	NDS; ET; CGRP
Liu (2010) [11]	Q-RCT	T: 28 C: 28	NA	Ischemic	(1) Eye acupuncture (2) Buyang Huanwu Decoction	Buyang Huanwu decoction	3 weeks	Major acupoints: upper jiao, lower jiao, spleen, and heart	NA	CSS
Pang (2006) [12]	RCT	T: 34 C: 34	T: (40~70) C: (40~70)	Ischemic and hemorrhagic	(1) Eye acupuncture (2) Rehabilitation training (3) Basic treatment: medicine was not available	(1) Rehabilitation training (2) Basic treatment: medicine was not available	>38 days	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, and heart	9~10.5 mm in orbit	ADL
Cui (2009) [13]	RCT	T: 8 (5/3) C: 10 (6/4)	T: 51.31 ± 13.25 (40~75) C: 51.59 ± 12.89 (40~75)	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Sodium Ozagrel injection (2) Citicoline injection (3) Low-dose Aspirin	2 weeks	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, heart, spleen, and large intestine	3 mm along the cavity orbital	CSS; ET; CGRP
Li (2010) [14]	RCT	T: 23 (12/11) C: 25 (13/12)	T: 52.29 ± 14.89 (40~75) C: 52.46 ± 13.35 (40~75)	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Sodium Ozagrel injection (2) Citicoline injection (3) Low-dose dose aspirin	2 weeks	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, heart, spleen, and large intestine	2 mm along the cavity orbital	CSS; ET; CGRP
Wang et al. (2007) [15]	RCT	T: 45 (24/21) C: 45 (26/19)	T: 63.24 (40~70) C: 64.98 (43~70)	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Shuxuening injection (2) Citicoline injection (3) Low-dose aspirin	14 days	Main eye acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, heart, spleen, and stomach	NA	CSS; CRP
Li and Wang (2009) [16]	RCT	T: 50 C: 50	T: (42~75) C: (42~75)	Ischemic	(1) Eye acupuncture (2) Basic treatment: medicine was not available	Basic treatment: medicine was not available	2 weeks	Major acupoints: upper jiao	7.5 mm in orbit	WST

Table 1: Continued.

Study ID	Study type	Sample size (T/C, male/female)	Age (yr, T/C)	Ischemic or hemorrhagic	Intervention		Duration	Area of eye acupuncture intervention	Prick depth	Outcomes
					Eye acupuncture intervention	Control intervention				
Chen et al. (2007) [17]	RCT	T: 40 (24/16) C: 40 (22/18)	T: 68.1 ± 8.2 (40~80) C: 67.3 ± 11.1 (40~80)	Ischemic	(1) Eye acupuncture (2) Rehabilitation training based on the Bobath (3) Basic treatment	(1) Rehabilitation training based on the Bobath (2) Basic treatment (a) t-PA (b) Aspirin (c) Mannitol injection	3 months	Major acupoints: upper jiao, lower jiao. Minor acupoints: liver, gallbladder, kidney, heart, spleen, and middle jiao	NA	SSS; ADL; FMA
Li (2009) [18]	RCT	T: 25 C: 25	T: (50~75) C: (50~75)	Ischemic	(1) Eye acupuncture (2) Basic treatment: medicine was not available	Basic treatment: medicine was not available	2 weeks	Major acupoints: upper jiao, kidney, and spleen	7.5 mm in orbit	MMSE
Xi (2011) [19]	Q-RCT	T: 30 (16/14) C: 30 (18/12)	T: (35~75) C: (35~75)	Ischemic and hemorrhagic	(1) Eye acupuncture (2) Basic treatment: medicine was not available (3) Rehabilitation training: methods were not available	(1) Basic treatment: medicine was not available (2) Rehabilitation training: methods were not available	7 days	Major acupoints: lower jiao, lung, and spleen	NA	First defecation time; constipation symptoms evaluation
Jiang (2009) [20]	RCT	T: 30 C: 30	T: (40~70) C: (40~70)	Ischemic and hemorrhagic	(1) Eye acupuncture (2) Rehabilitation training	Rehabilitation training	48 days	Major acupoints: upper jiao, lower jiao, kidney, and liver	2 mm along the cavity orbital	CSS
Ren and Lin (2005) [21]	Q-RCT	T: 30 (21/9) C: 28 (20/8)	T: 54 (32~78) C: 53 (37~83)	Ischemic and hemorrhagic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) 20% mannitol (2) Cerebrolysin (3) Huatuo Zaizao pill (4) Hemorrghagic: PAMBA Nimodipine Nifedipine (5) Ischemic: Low molecular dextran ATP Cytochrome C for injection Dicoumarin Nimodipine Aspirin	30 days	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, gallbladder, kidney, and heart	2 mm along the cavity orbital	Treatment efficiency
Gao (2012) [22]	RCT	T: 30 (18/12) C: 30 (20/10)	NA	Ischemic	(1) Eye acupuncture (2) Basic treatment	Basic treatment (1) Aspirin or Clopidogrel Other medicines were not available	14 days	Major acupoints: liver, gallbladder, kidney, and heart.Minor acupoints: upper jiao, lower jiao, heart, spleen, stomach, large intestine, and bladder	2 mm along the cavity orbital	ADL; CSS
Huang (2013) [23]	RCT	T: 80 (43/37) C: 76 (40/36)	T: 61.10 ± 10.12 (40~76) C: 55.72 ± 9.02 (40~76)	Ischemic and hemorrhagic	(1) Eye acupuncture (2) Neurostan	Neurostan	8 weeks	Major acupoints: liver, middle jiao, heart Minor acupoints: kidney, spleen, and gallbladder	2 mm along the cavity orbital	HAMD

Table 1: Continued.

Study ID	Study type	Sample size (T/C, male/female)	Age (yr, T/C)	Ischemic or hemorrhagic	Intervention		Duration	Area of eye acupuncture intervention	Prick depth	Outcomes
					Eye acupuncture intervention	Control intervention				
Xu et al. (2006) [24]	RCT	T: 34 (18/16) C: 26 (16/10)	T: 62.5 ± 17.5 C: 64.2 ± 7.7	Ischemic	Eye acupuncture	Basic treatment: the medicine might be Xingding injection, compound danshen injection, and Deproteinized calf blood injection	22 days	Major acupoints: upper jiao, lower jiao Minor acupoints: liver, kidney, and heart	NA	ET
Dong (2009) [25]	RCT	T: 38 (20/18) C: 34 (18/16)	T: 63.2 ± 12.5 C: 65.1 ± 8.6	Ischemic	Eye acupuncture	Basic treatment: the medicine might be Deproteinized calf blood injection, Shuxuening injection (extract of Ginkgo), Sanqi Panax Notoginseng injection	7 days	Major acupoints: upper Jiao, lower Jiao. Minor acupoints: liver, kidney, and heart	NA	VEGF

Notes: (1) ADL: Activities of Daily Living. (2) CGRP: calcitonin gene related peptide. (3) CRP: C-reactive protein. (4) CSS: Chinese Stroke Scale. (5) ET: endothelin. (6) FIB: fibrinogen. (7) FMA: Fugl-Meyer Scale. (8) HAMD: Hamilton Depression Scale. (9) MMSE: Mini-Mental State Examination. (10) SSS: Scandinavian Stroke Scale. (11) NDS: clinic neurological function deficit scale. (12) VEGF: vascular endothelial growth factor.

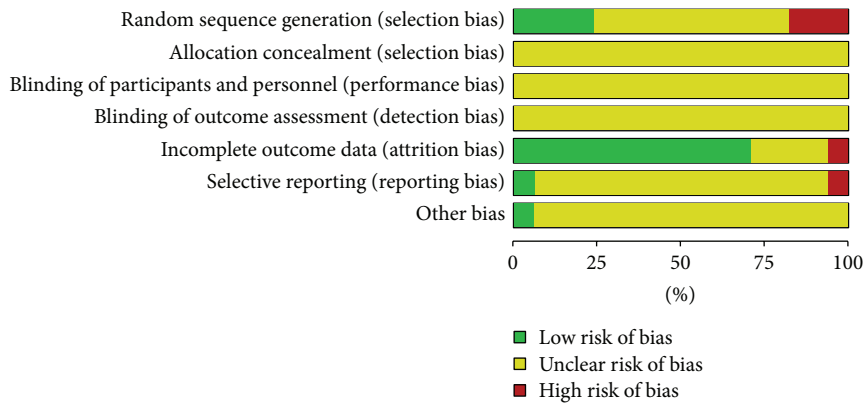

Figure 2: Methodological quality.

indicated that there was a significant difference between eye acupuncture combined with western medicine treatment and western medicine treatment (MD 4.67, 95% CI 1.45 to 7.89).

3.3.3. Changes of SSS at the End of Treatment. The SSS score at the end of treatment was applied in 1 trial [17] with 80 patients. There was a significant difference between acupuncture combined rehabilitation and western medicine versus rehabilitation and western medicine (MD 12.41, 95% CI, 8.92 to 15.90).

3.3.4. Changes of FMA Assessment at the End of Treatment. The FMA assessment at the end of treatment was applied in 1 trial [17] with 80 patients. When eye acupuncture was combined with rehabilitation and western medicine versus rehabilitation and western medicine, there was a significant difference (MD 8.31, 95% CI, 3.15 to 13.47).

3.3.5. Changes of HAMD Score at the End of Treatment. The changes of HAMD at the end of treatment were observed in 1 trial [23] with 156 patients. There was no significant difference between eye acupuncture combined with western medicine and western medicine (MD −0.82, 95% CI −1.87 to 0.23).

3.3.6. Changes of MMSE at the End of Treatment. The changes of MMSE at the end of treatment were observed in 1 trial [18] with 50 patients. There was a significant difference between eye acupuncture combined with western medicine treatment and western medicine treatment (MD 1.60, 95% CI 0.28 to 2.92).

3.3.7. Changes of SWT at the End of Treatment. The changes of SWT at the end of treatment were observed in 1 trial [16] with 100 patients. There was a significant difference between the eye acupuncture combined with western medicine and western medicine (RR 1.24, 95% CI 1.03 to 1.49).

3.3.8. Changes of Constipation Symptoms and First Defecation Time Evaluation at the End of Treatment. The first defecation time and constipation symptoms at the end of treatment were evaluated in 1 trial [19] with 60 patients. In the comparison of eye acupuncture combined with rehabilitation and western medicine versus rehabilitation and western medicine, there was a significant difference in the constipation symptoms evaluation (MD −4.78, 95% CI −5.14 to −4.42) as well as the first defecation time (MD −1.03, 95% CI −1.46 to −0.60).

3.3.9. Changes of NDS. The changes of NDS score at the end of treatment were checked in 1 trial [10] with 120 patients. There was a significant difference between eye acupuncture combined with western medicine and western medicine (RR 1.08, 95% CI 0.93 to 126).

3.3.10. Changes of ET Level at the End of Treatment. The changes of ET level at the end of treatment were checked in 4 trials [10, 13, 14, 24] with 246 patients.

There was no significant difference between eye acupuncture combined with western medicine treatment and western medicine treatment in 2 trials [10, 14] (MD, 30.40, $I^2 = 100\%$, 95% CI −43.65 to 104.46 Random). There was no significant difference between eye acupuncture combined with rehabilitation and rehabilitation in 1 trial [13] (MD −10.71, 95% CI −28.9 to 6.67). There was a significant difference between eye acupuncture and western medicine treatment in 1 trial [24] (MD, −0.64, 95% CI −1.17 to −0.12).

3.3.11. Changes of CGRP Level at the End of Treatment. There was no significant difference between eye acupuncture combined with western medicine treatment and western medicine treatment (MD 1.48, 95% CI −5.31 to 8.27, $I^2 = 28\%$, Fixed).

3.3.12. Changes of FIB Level at the End of Treatment. The changes of FIB level at the end of treatment were observed in 1 trial [9] with 120 patients. There was a significant difference between eye acupuncture combined with western medicine treatment and western medicine treatment (MD −0.72, 95% CI, −1.09 to −0.35).

3.3.13. Changes of CRP Level at the End of Treatment. The changes of CRP level at the end of treatment were observed in 1 trial [15] with 90 patients. There was a significant difference between eye acupuncture combined with western medicine

Table 2: Estimated effect sizes of included trials in meta-analyses.

Trials	Participants	Estimate effects
(1) **Changes of CSS scores for neurological assessment**		
(1.1) Eye acupuncture combined with western medicine versus western medicine		
Wang et al. (2008) [9]	120	MD −5.56 [−7.15, −3.97]
Cui (2009) [13]	18	MD −3.23 [−9.14, 2.68]
Li (2010) [14]	48	MD −2.44 [−5.44, 0.56]
Wang et al. (2007) [15]	90	MD −3.84 [−5.35, −2.33]
Subtotal MD −4.24, 95% CI −5.59 to −2.89 I^2 = 31% fixed		
(1.2) Eye acupuncture combined with TCM herbal treatment versus TCM herbal treatment		
Liu (2010) [11]	56	MD −2.89 [−4.15, −1.63]
(1.3) Eye acupuncture combined with rehabilitation versus rehabilitation		
Jiang (2009) [20]	60	RR −2.40 [−4.87, 0.07]
(1.4) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Gao (2012) [22]	60	MD −2.40 [−3.76, −1.04]
(2) **Changes of ADL at the end of treatment**		
(2.1) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Chen et al. (2007) [17]	80	MD 17.60 [14.19, 21.01]
(2.2) Eye acupuncture combined with western medicine versus western medicine		
Gao (2012) [22]	60	MD 4.67 [1.45, 7.89]
(3) **Changes of SSS score at the end of treatment**		
(3.1) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Chen et al. (2007) [17]	80	MD 12.41 [8.92, 15.90]
(4) **Changes of FMA assessment at the end of treatment**		
(4.1) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Chen et al. (2007) [17]	80	MD 8.31 [3.15, 13.47]
(5) **Changes of HAMD score at the end of treatment**		
(5.1) Eye acupuncture combined with western medicine versus western medicine		
Huang (2013) [23]	156	MD −0.82 [−1.87, 0.23]
(6) **Changes of MMSE assessment at the end of treatment**		
(6.1) Eye acupuncture combined with western medicine versus western medicine		
Li (2009) [18]	50	MD 1.60 [0.28, 2.92]
(7) **Changes of SWT assessment at the end of treatment**		
(7.1) Eye acupuncture combined with western medicine versus western medicine		
Li and Wang (2009) [16]	100	RR 1.24 [1.03, 1.49]
(8) **Changes of NDS**		
(8.1) Eye acupuncture combined with western medicine versus western medicine		
Zhou et al. (2011) [10]	120	RR 1.08 [0.93, 1.26]
(9) **Constipation symptoms evaluation at the end of treatment**		
(9.1) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Xi (2011) [19]	60	MD −4.78 [−5.14, −4.42]
(10) **First defecation time at the end of treatment**		
(10.1) Eye acupuncture combined with rehabilitation and western medicine versus rehabilitation combined with western medicine		
Xi (2011) [19]	60	MD −1.03 [−1.46, −0.60]

Table 2: Continued.

Trials	Participants	Estimate effects
(11) **Changes of ET level at the end of treatment**		
(11.1) Eye acupuncture combined with western medicine versus western medicine		
Zhou et al. (2011) [10]	120	MD −7.03 [−9.48, −5.12]
Li (2010) [14]	48	MD 68.27 [61.03, 75.51]
Subtotal 168 MD 30.40 95% CI −43.65 to 104.46 I^2 = 100% random		
(11.2) Eye acupuncture combined with rehabilitation versus rehabilitation		
Cui (2009) [13]	18	MD −10.71 [−28.09, 6.67]
(11.3) Eye acupuncture versus rehabilitation		
Xu et al. (2006) [24]	60	MD −0.64 [−1.17, −0.12]
(12) **Changes of CGRP level at the end of treatment**		
(12.1) Eye acupuncture combined with western medicine versus western medicine		
Zhou et al. (2011) [10]	120	MD 5.67 [4.03, 7.31]
Li (2010) [14]	48	MD 1.48 [−5.31, 8.27]
Subtotal 168 MD 1.48 95% CI −5.31 to 8.27 I^2 = 28% fixed		
(13) **Changes of FIB level at the end of treatment**		
(13.1) Eye acupuncture combined with western medicine versus western medicine		
Wang et al. (2008) [9]	120	MD −0.72 [−1.09, −0.35]
(14) **Changes of CRP level at the end of treatment**		
(14.1) Eye acupuncture combined with western medicine versus western medicine		
Wang et al. (2007) [15]	90	MD −5.86 [−7.54, −4.18]
(15) **Changes of VEGF level at the end of treatment**		
(15.1) Eye acupuncture versus western medicine		
Dong (2009) [25]	60	MD 0.02 [−0.49, 0.53]

treatment and western medicine treatment (MD −5.86, 95% CI, −7.54 to −4.18).

3.3.14. Changes of VEGF Level at the End of Treatment. The changes of VEGF level at the end of treatment were observed in 1 trial [25] with 60 patients. There was no significant difference between eye acupuncture and western medicine (MD 0.02, 95% CI, −0.49 to 0.53).

Ignoring the methodological quality of included trials, the results showed some effect in independency and symptom alleviation. As eye acupuncture is combined with western medicine versus western medicine, effects appeared in the outcomes of CSS, ADL, FIB, SWT, CRP, and FIB. Eye acupuncture combined with TCM herbal treatment showed more effectiveness than TCM herbal treatment in the outcome of CSS. The outcomes of ADL have showed superiority of eye acupuncture combined with rehabilitation compared to rehabilitation. The outcomes of ADL, SSS, FMA, constipation symptoms, and first defecation time were more effective in eye acupuncture combined with rehabilitation and western medicine as compared to rehabilitation and western medicine.

3.4. Adverse Event. No adverse events or side effects have been reported during or after the eye acupuncture treatment according to the trials.

4. Discussion

The focal points in this study are the safety and effectiveness of eye acupuncture for stroke. The study demonstrated that eye acupuncture is a safe and effective treatment for stroke patients on symptoms alleviation and the dependency in the results of CSS, SSS, ADL, FMA, MMSE, HAMD, WST, and first defecation time as well as the biochemistries tests (CRP, ET, VEGF, and CGRP).

However, there were several limitations of this review. The quality of the included studies was poor because there were a mass of trials either having high or unclear risk of bias. 13% trials of random sequence generation results were of high risk and 53% trials were unclear. One trial was high risk and the rest were unclear in blinding and the same result appeared again in allocation concealment. No trials about adverse events or death were mentioned so it was impossible to get any information about safety and no trials have had the follow-up observation either. There were some therapeutic effects, but the outcomes did not focus on commonly used evaluation standards.

There were eight areas and thirteen points for eye acupuncture, but it was noticeable that the location of eye acupoints is different in trials according to the mentioned intervention methods. We wish that the locations of eye acupoints could be unified according to Standardized Manipulations of Eye Acupuncture [5]. Furthermore, trials of

eye acupuncture therapy should follow the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) [26] to confirm the effect in stroke and facilitate a meta-analysis. It has been recommended that primary outcome measures for stroke should be at the level of Activities of Daily Living and the outcome should be assessed at 6 months [27].

There was no data indicating adverse events or death. But considering the position of needling, prick depths, sense of fear that patients might confront, and other potential risks for stroke, the author strongly suggests safety evaluation and psychology evaluation should be carried out for eye acupuncture.

Conflict of Interests

The authors declare that they have no conflict of interests in the research.

Authors' Contribution

Zeng-Hua Bai and Zhi-Xing Zhang have equally contributed to this work.

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Research Article

Effect of Acupuncture on Functional Connectivity of Anterior Cingulate Cortex for Bell's Palsy Patients with Different Clinical Duration

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Acupuncture is widely used in the treatment of Bell's palsy (BP) in many countries, but its underlying physiological mechanism remained controversial. In order to explore the potential mechanism, changes of functional connectivity (FC) of anterior cingulate gyrus (ACC) were investigated. We collected 20 healthy (control group) participants and 28 BP patients with different clinical duration accepted resting state functional MRI (rfMRI) scans before and after acupuncture, respectively. The FC of ACC before and after acupuncture was compared with paired t-test and the detailed results are presented in the paper. Our results showed that effects of the acupuncture on FC were closely related to clinical duration in patients with BP, which suggested that brain response to acupuncture was closely connected with the status of brain functional connectivity and implied that acupuncture plays a homeostatic role in the BP treatment.

1. Introduction

Bell's palsy is a unilateral idiopathic and mostly transient facial paralysis resulting from dysfunction of the cranial nerve VII (the facial nerve), with a pure peripheral deefferentation [1]. Previous researches have demonstrated that the cortical reorganization may complement the recovery from facial nerve palsy with the change of functional connectivity (FC), mainly demonstrated as disruption at the early stage and enforcement at the later stage [2] in the areas related to error detection, sensorimotor integration, motor integration, and control. The FC [2], defined as the temporal correlation between spatially remote neurophysiological events, has become a significant method for studying neuroplasticity to detect changes during cortical reorganization.

Acupuncture, as an alternative and complementary therapeutic intervention, is playing an increasingly important role in treating BP [3, 4]. However, effectiveness and underlying mechanism of acupuncture for treating BP remain controversial and need further investigation. By now, only a few studies have investigated the effect of acupuncture on resting state functional magnetic resonance imaging (rfMRI) FC of BP patients. Our preliminary research concerning the instant effect of acupuncture in BP treatment [5] reported negative activation in the early stage and positive activation in the later stage of BP. Besides, another investigation [6] of our group showed that changes in FC of the primary somatosensory cortex (SI) induced by acupuncture varied with clinical durations of BP, mainly displayed as decreased connectivity in the early stage but increased connectivity in the later stage. Furthermore, our rfMRI FC studies [7] reported that FC of ACC showed positive correlation with the duration of BP, which suggested that ACC may play a crucial role in the process of cortical reorganization during the recovery from BP. Based on these critical conclusions, in order to probe the effect of acupuncture on the FC of BP

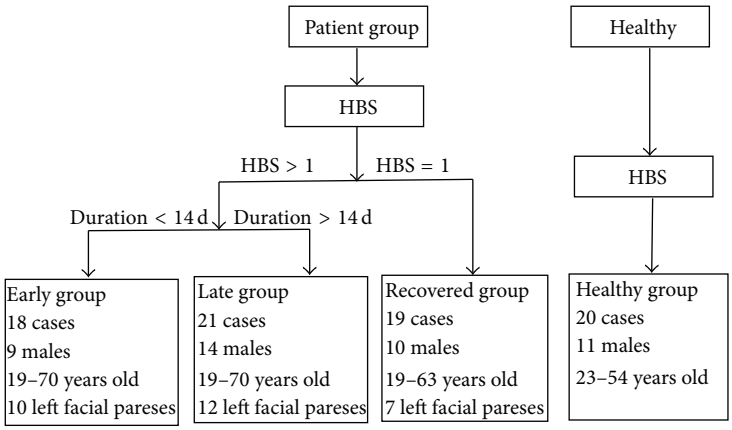

Figure 1: Healthy group and four subgroups of patient group classified based on House-Brackmann score (HBS) and disease duration.

patients and further reveal the underlying role of acupuncture in the cortical reorganization in the recovery process of BP, we investigated longitudinal changes of FC (before versus after acupuncture) of bilateral ACC for BP patients with different clinical duration.

The hypothesis of the present research was that acupuncture may have different effect on the FC of ACC for patients with different clinical duration during the recovery of BP.

2. Materials and Methods

2.1. Subjects. All subjects recruited in this study were right-handed with no histories of drug abuse and no mental, central nervous system or other serious disease. The subjects were divided into two groups: the patient group (28 cases, totally 58 times MRI scanning) and the healthy control group (20 cases, totally 20 times MRI scanning).

The patient groups, including patients with left and right unilateral facial paresis, were further divided into three subgroups based on the disease duration and HBS scores (House-Brackmann facial nerve grading system, 1 = normal facial movement, and 6 = no movement, scored by an experienced acupuncturist with no prior knowledge about the data results). The 3 subgroups (stages) were the early group (18 cases, 9 males, 19–70 years old; duration < 14 d, HBS > 1; 10 left facial pareses), the late group (21 cases, 14 males, 19–70 years old; duration > 14 d, HBS > 1; 12 left facial pareses), and the recovered group (19 cases, 10 males, 19–63 years old; HBS = 1; 7 left facial pareses). Among the 28 subjects of the patient group with different clinical duration, 4 undertook MRI scanning only once, 18 undertook it twice, and 6 undertook it thrice. Manual acupuncture was applied to all patients thrice a week semi-individually at acupoints chosen by acupuncturists based on the individual symptoms in the course of acupuncture treatment. The acupoint Hegu (LI4) was selected in present experiment for all subjects since LI4 is usually selected as the main acupoints in the clinical treatment of BP. All healthy subjects (20 cases, 11 males, 23–54 years old) were either college students or the workers in the hospital. All subjects signed informed consent forms before participating in the experiment in accordance with the Human Research Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (see Figure 1).

2.2. fMRI Data Acquisition. The experiment was performed in the MRI room of the Medical Imaging Center, the First Affiliated Hospital of Anhui University of TCM. The Siemens Symphony 1.5 T MRI whole body scanner and standard head coil were used. All subjects were instructed to lie down with eyes closed and to stay awake. All lights in the scanning room were turned off to avoid unwanted visual stimulation.

Eight sequences were scanned: (1) pilot images; (2) T2-weighted images to rule out any disease of the brain; (3) T1-weighted 2D anatomical images with the axial position parallel to the AC-PC line; the images include 36 slices that covered the whole brain. T1-weighted spin-echo sequence was used, with TR/TE = 500/12 ms, FOV = 230 mm × 230 mm, slice thickness/interval = 3.0 mm/0.75 mm, and resolution = 192 × 144; (4) resting-state fMRI before acupuncture, namely, Run 1; the EPI BOLD sequence with TR/TE/FA = 3000 ms/30 ms/90° was used and FOV = 192 mm × 192 mm and resolution = 64 × 64; (5) resting-state fMRI with the same parameters during acupuncture, namely, Run 2; (6) resting-state fMRI with the same parameters after acupuncture, namely, Run 3; (7) task-state acupuncture fMRI: the scanning direction and the number of slices were the same as those of the resting-state fMRI, with TR/TE/FA = 4000 ms/50 ms/90°, namely, Run 4; (8) T1-weighted 3D anatomical images: the sagittal position was taken, and total of 176 slices were scanned which covered the whole brain. The spoiled gradient echo sequence was used, with TR/TE/FA = 2100 mm/3.93 mm/13°, FOV of 250 × 250 mm, slice thickness/spacing = 1.0 mm/0.5 mm and resolution of 256 × 256. It took about 60 minutes to complete all of the data acquisition. fMRI paradigms are shown in Figure 2.

2.3. Extraction of the Region of Interest. Bilateral ACC was extracted as region of interest (ROI) for FC analysis. The datasets were from 37 healthy volunteers with the task of mouth movement. The paradigm for motor task lasted 400

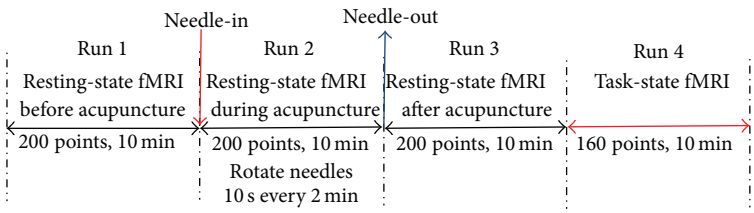

Figure 2: fMRI paradigms of the experiment.

seconds, which consists of 40-time task of opening and shutting mouth (mean time 7.8 ± 1.6 s) separated by 2-second duration. The volunteers were trained to open or shut mouth when seeing the word (outward/protrude) shown randomly on the display before scanning and were instructed to lie down on the scanning bed and keep their body static.

The experiment was completed in the Department of Biomedical Engineering of Kyung Hee University of South Korea. The Philips Achieva 3.0 T MRI whole body scanner and 8-channel head coil were used. A total of 3 sequences were scanned, which were (1) 2D structural image: TR/TE of 2000 ms/35 ms, voxel size of 2.785 mm × 2.875 mm × 4 mm, slice/volume of 34/180, and matrix 80 × 80; (2) EPI-BOLD: TR/TE of 2000 ms/35 ms, voxel size of 2.785 mm × 2.875 mm × 4 mm, slice/volume of 34/200, and matrix of 80 × 80; (3) T1-Weighted 3D structural image: TR/TE of 2000 ms/35 ms, slice/gap of 1.0 mm/1.0 mm, and matrix of 256 × 256.

To investigate the changed FC of bilateral ACC with related brain areas, the ROIs were extracted from the statistic activation maps obtained from motor task experiment. The maximum point of activation strength of bilateral ACC, along with its 33 neighbors, was selected as ROIs (a sphere with radius as 4 mm and voxel size 2 × 2 × 2 mm), as shown in Figure 1 of [7].

2.4. Paradigms of the Experiment. The acupuncture in the experiment was executed by a professional acupuncturist. Resting-state fMRI data before acupuncture (Run 1) lasts for 10 min (200 points). Then, the needle was inserted into the acupoints of LI4 on the contralateral hand of the paralyzed face and rotated to achieve De-Qi sensation. The second fMRI data (Run 2) was collected including 200 points for 10 min, during which the needle was rotated bidirectionally for 10 s every 2 min. Then the needle was pulled out and the third fMRI data after acupuncture (Run 3) including 200 points was obtained.

2.5. Data Preprocessing. Data analysis was performed using the FSL (Oxford Centre for Functional MRI of the Brain's (FMRIB's) Software Library), Freesurfer, and AFNI. The preprocessing was applied as follows. Anatomical images were reconstructed using Freesurfer recon-all and then tilt correction was done for functional and anatomical images using 3drefit and fslorient. Nonbrain removal was done using mri_watershed for anatomical images and BET for functional images. Motion correction was done using MCFLIRT (Motion Correction using FMRIB's Linear Image Regression Tool) and melodic to compensate for any head movements during the scan. The functional images were then coregistered to the high-resolution anatomical images reconstructed by Freesurfer. Afterwards, the functional images were registrated to standard MNI152 space (Montreal Neurological Institute) using FNIRT and FLIRT (affine transformation with FMRIB's Linear Image Registration Tool). Functional data were smoothed using a Gaussian kernel of FWHM 6 mm; and band-pass filter (0.007 HZ $< f <$ 0.1 HZ) was also performed to reduce the effect of low-frequency drift and high-frequency noise. Then, individual data of right-sided facial palsy patients were flipped along the y-axis so that all data could be processed unilaterally. The individual 4D data was then used for further group statistics and connectivity analysis.

2.6. Functional Connectivity Analysis. In our research, to compute the FC of ACC, the temporal signal series of cerebrospinal fluid, white matter was extracted firstly. Then, based on linear regression analysis, variances including 6 parameters obtained by rigid body correction of head motion and the signal of cerebral spinal fluid and white matter were removed. Finally, individual statistical maps were obtained based on the general linear model for further group analysis.

2.7. Group Analysis. Standard space parameters of individual subjects were imported to a high level analysis with FLAME (FMRIB's Local Analysis of Mixed Effects). With the aim of investigating the effects of acupuncture on FC of BP patients with different clinical durations, we conducted intergroup analysis through paired t-test before and after acupuncture for each group. The individual data with head movement more than 2 mm or 2° were excluded before group analysis. The significance threshold for FEAT was set at $z = 2.3$ and $P = 0.01$. All results were then corrected using cluster (based on theory of Gaussian Random Field, GRF) to obtain the activation maps. The results of intergroup analysis were corrected using Monte-Carlo simulations with $P = 0.01$, $\alpha = 0.05$, and cluster size = 68.

3. Results

There were no significant difference among subjects' age distributing and sample size of four groups. To address the significant differences in functional connectivity changes among different groups, the results of group analysis for each group were showed as follows.

Table 1: Group analysis of areas changed FC with right ACC after acupuncture for patient with Bell's palsy in the early group.

Region (BA)	Side	BA	Coordinate (MNI)			Z value	Cluster size
			Peak x (mm)	Peak y (mm)	Peak z (mm)		
Superior frontal gyrus	R	8	32	28	56	−4.151	143
Middle frontal gyrus	R	6	26	18	62	−3.643	126
Middle frontal gyrus	R	10	38	30	46	−3.515	77

BA: Brodmann area; L: left; R: right; the threshold was set at $P \leqq 0.01$; $\alpha \leqq 0.05$ (corrected with Monte-Carlo method).

Table 2: Group analysis of areas changed FC with right ACC after acupuncture for patient with Bell's palsy in the latter group.

Region (BA)	Side	BA	Coordinate (MNI)			Z value	Cluster size
			Peak x (mm)	Peak y (mm)	Peak z (mm)		
Superior temporal gyrus	R	22	58	2	−4	3.945	130
Insula	R	22	46	6	−4	3.633	102
Superior temporal gyrus	R	41	46	−34	12	3.806	88
Putamen	R		28	8	2	3.626	68

BA: Brodmann area; L: left; R: right; the threshold was set at $P \leqq 0.01$; $\alpha \leqq 0.05$ (corrected with Monte-Carlo method).

3.1. The Healthy Control Group. Paired t-test was done before and after acupuncture to find out significant changes induced by acupuncture effect on FC of bilateral ACC of the healthy group. In the healthy group, after being corrected with Monte-Carlo method ($P = 0.01$, $\alpha = 0.05$ and cluster size = 68), nostatistical significant differences were observed for bilateral ACC before and after acupuncture. The results imply that acupuncture has no significant effect on FC of bilateral ACC in healthy subjects.

3.2. The Early Group. Different results were observed for the early group. In the early group, after being corrected with Monte-Carlo method ($P = 0.01$, $\alpha = 0.05$, and cluster size = 68), significant changed FC were found after acupuncture. For the left ACC (ipsilateral to Bell's palsy), no remarkable changes of FC were found. Significant decreased connectivity of the right ACC (contralateral to BP) after acupuncture were observed in right superior frontal gyrus (SFG, BA 8), right middle frontal gyrus (MFG, BA 6), and right middle frontal gyrus (MFG, BA 10) (as shown in Figure 3 and Table 1).

3.3. The Latter Group. For the latter group, after being corrected with Monte-Carlo method ($P = 0.01$, $\alpha = 0.05$, and cluster size = 68), FC of ACC was also changed after acupuncture. For the left ACC (ipsilateral to Bell's palsy), no significant changes of FC were found. Significant increased FC of the right ACC were observed in right superior temporal gyrus (STG, BA 22), right insula (BA 22), right superior temporal gyrus (STG, BA 41), and right putamen (see Figure 4 and Table 2).

3.4. The Recovered Group. Similar with the healthy group, in the recovered group, the intergroup analysis results before and after acupuncture with Monte-Carlo correction ($P = 0.01$, $\alpha = 0.05$, and cluster size = 68) showed no significant functional connectivity changes of bilateral ACC.

4. Discussions

In this study, in order to assess the role of acupuncture during the recovery of BP, we investigated the FC changes of ACC induced by acupuncture for patients with different clinical duration. The results suggest that the acupuncture effects on the FC varied with clinical duration.

4.1. Overall Acupuncture Effect on ACC Connectivity in Bell's Palsy Patients with Different Clinical Durations. As well known, one of the most important characteristics of BP is the damaged efferent nerve (without afferent nerve) and the consequent impaired facial motor function of the affected side of the face. Therefore, the sensory feedback of the acute reduction of facial motor performance due to BP will be detected by brain, thus causing increased processing in brain areas responsible for the monitoring and integration of somatosensory and motor information. Previous functional neuroimaging studies have shown that the ACC is important in error detection and performance-monitoring functions, including executive function, response selection, and conflict monitoring [8].

The present results indicated that no changes were found for the FC status of bilateral ACC for both the healthy and the recovered groups after acupuncture. Presently, researches show that the therapeutic principles of acupuncture are not through relieving local condition of the diseased area but in the way of reestablishing the balance of the internal milieu (involving Ying/Yang, the Five Elements, and the Zhong-Fus) [9]. Therefore, for healthy subjects and the recovered group, their homeostatic can be considered to stay in/in return to a balance state; thus no significant effect on FC of ACC was observed after acupuncture.

Acupuncture-induced FC changes of the contralateral ACC were observed for both the early and the later groups. As we know, one of the most remarkable features of the human brain is its ability to adapt to new situations and to new information. Changes in the functional network connectivity

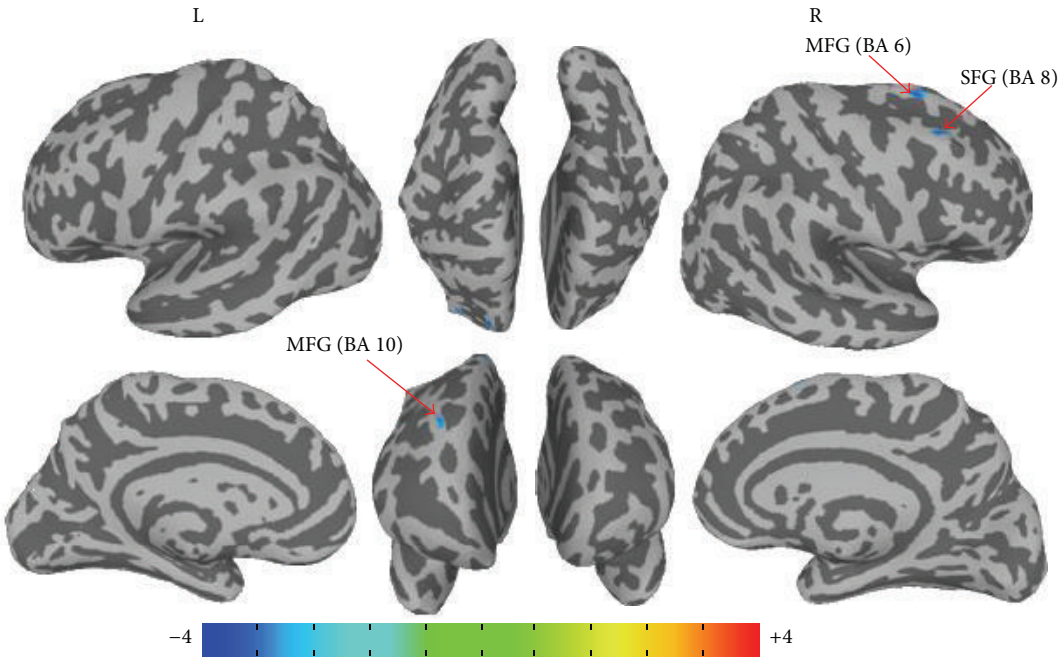

Figure 3: Changed functional connectivity of right ACC after acupuncture for patients with Bell's palsy at early stage. $P \leq 0.01$, $\alpha \leq 0.05$, corrected with Monte-Carlo method. BA: Brodmann area; SFG: superior frontal gyrus; MFG: right middle frontal gyrus; MFG: middle frontal gyrus.

status, which reflect the process of cortical reorganization of different brain areas, may give expression to this feature. It is suggested that [1] the functional reorganization caused by transient peripheral deefferentation, which can be interpreted as the compensatory effect of brain to the impaired motor performance of BP patients, happened after Bell's palsy. As a result of this process, the connectivity status of the brain changed and thus led to a different acupuncture response of brain. Generally speaking, compared with the healthy group, FC changes in the early and the late group imply the different acupuncture responses resulting from the changed FC status of BP patients. The results here are consistent with our previous research concerning the instant effect of acupuncture in BP treatment, which reported the conclusion that the brain responses to acupuncture differ at different pathological statuses and probably depend on the brain functional status [1].

Another remarkable characteristic of our results is that the acupuncture-induced changes of the intrahemispheric FC were found only for the contralateral ACC but not for the ipsilateral ACC for all groups. Previous investigations have consistently reported that the changed brain responses to acupuncture were mainly contralateral to the palsy area [2]. An fMRI study on BP [2] also revealed a significant acutely disrupted but unaltered interhemispheric connectivity of MI and other parts of the facial motor network at early stage of the palsy, followed by a return toward normal during the course of recovery. Their results are in accordance with ours. This can be explained since motor commands of paretic side are blocked in the acute stage of BP; then the error in the process of command execution was feed backed to the contralateral ACC and thus the FC status changed due to various adjustment and compensatory mechanism. No changes of the FC status of the ipsilateral ACC may indicate that the brain areas related to the healthy side remain in a good condition.

4.2. Changed Functional Connectivity of Sensorimotor Related Brain Areas. Compared with the Pre-Acu, decreased FC was found in sensorimotor related brain areas including SFG (BA 8) and MFG (BA 6, BA 10) after acupuncture. BA 8 and BA 6 are well known as the advanced motor center in planning, integration, and execution of motor function. The difficulty in movement of the paralyzed facial muscle in patients with BP may elicit FC changes of these motor related areas. Studies concerning the BP suggested that the impaired motor function (without a lesion in the brain) might initially lead to a disrupted connectivity within the cortical facial motor network, with a subsequent reorganization supporting functional recovery [1, 2]. Our research concerning acupuncture-induced FC changes of SI also reported decreased connectivity in the early stage and increased connectivity in the later stage [1]. Therefore, hypoactivation of motor related areas after acupuncture may result from disruption FC in the early stage of Bell's palsy.

Another sensorimotor correlated brain area with changed FC was observed in putamen at the latter group. While it has been concluded that the putamen has no specific specialization, it works with many other motor related structures to control many types of motor skills, including motor learning, motor performance and tasks [10], and motor preparation [11]. In addition, the putamen contains high

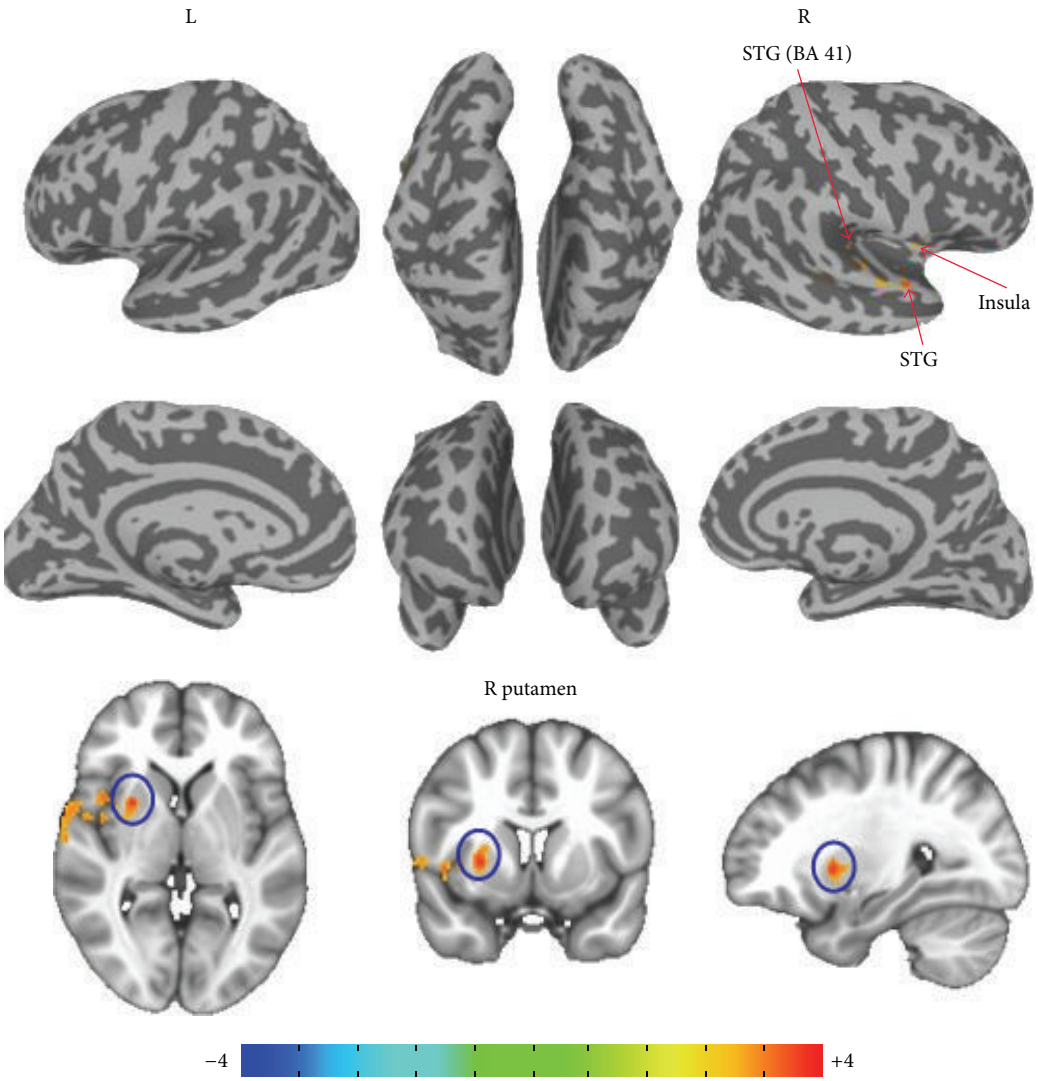

Figure 4: Changed functional connectivity of right ACC after acupuncture for patients with Bell's palsy at later stage. $P \leq 0.01$, $\alpha \leq 0.05$, corrected with Monte-Carlo method. BA: Brodmann area; STG: superior temporal gyrus; STG: superior temporal gyrus.

levels of opioid receptors and is considered to be involved in sensory and emotional components of pain. Meanwhile, fMRI research of patients with PD received acupuncture treatment demonstrated that the activated putamen was correlated with enhanced motor function of patients. Based on comprehensive analysis, increased FC in putamen may result from the cortical reorganization in motor correlated brain areas which were caused by the loss of facial motor control in BP patients.

In addition, increased FC was observed in superior temporal gurus (STG, BA 22, and BA 41), which is well known as the primary auditory cortex and is involved in auditory and language processing. Changed FC in temporal lobe reflects brain's potential compensatory mechanism because of facial motor difficulty in BP patients. Besides, changed FC in STG (BA 22) may also occur as cortical reorganization result from the difficulty in flexible pronunciation of those patients with serious facial paralysis.

4.3. Changed Functional Connectivity of Homeostatic Related Network. Changed connectivity between the contralateral ACC and insular cortex induced by acupuncture is also an interesting finding in our research. As well known, the insular cortex is involved in a wide range of functions including motor control and homeostatic regulation [12–15]. As reported in previous neuroimaging researches, the ACC and insular cortex play an important role in the network termed as the "homeostatic afferent processing network" [16]. The network represents all aspects of the physiological condition of the body and meanwhile provides crucial sensory input that is essential for maintaining homeostasis. The fMRI research in functional diarrhea reported that acupuncture brings functional connectivity changes to the homeostatic afferent processing network only in patients but not in the healthy group because of functional abnormality of the network in functional diarrhea patients. The results were in accordance with ours since the changed FC induced by acupuncture

within the homeostatic afferent processing network in BP patients may suggest the unbalanced functional state result from the Bell's palsy. All results imply the homeostatic regulation role of acupuncture that is emphasized in the traditional Chinese medical theory. But considering the high self-recovery rate of Bell's palsy and there was no control group in our experiment, the results might just reflect the self-recovery of the disorder instead of the acupuncture effect.

5. Conclusion and Limitation

The paper introduces the influence of acupuncture on FC of ACC for BP patients with different clinical duration and our results indicate that different changes were observed. Changed FC induced by acupuncture occurred in more than one functional neural system. The deactivation in the early group and positive activation in the latter group kept peace with the disrupted connectivity in the acute stage and subsequent reorganization during the recovery of the BP. This synchronization suggested the dependence of acupuncture effect on the functional status of brain and may imply the homeostatic role of acupuncture in BP treatment. Moreover, changed FC of "homeostatic afferent processing network" induced by acupuncture also reflected homeostatic regulation role of acupuncture.

Present results indicate that acupuncture might at least partly contribute to the functional recovery of BP arising from the cortical reorganization by changing the FC within the somatosensory motor network and other related areas. The evidence provided in this research for the effectiveness of acupuncture in treating BP is limited because there was no blank control group due to ethics restriction. Therefore, we cannot exclude the possibility that the results might just reflect the self-recovery of Bell's palsy. The paper may bring to light the underlying mechanism of acupuncture during the recovery of BP, although further researches are still needed.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

The Study of Dynamic Characteristic of Acupoints Based on the Primary Dysmenorrhea Patients with the Tenderness Reflection on *Diji* (SP 8)

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In TCM theory, acupoint is not a fixed point but a portal with dynamic characteristics where the channel *qi* enters and flows out. The dynamic characteristics have been verified primarily by detecting the tenderness reaction on *Diji* (SP 8) in primary dysmenorrhea patients. In this study, finger pressing and algometer were applied in *Diji* (SP 8) area of participants in menstrual period and nonmenstrual period, respectively, to detect the tenderness occurrence rate, the VAS score of the tenderest point, the tenderness threshold of the tenderest point, and the location of the tenderest point. The result suggests that the acupoint may not be a fixed location but a point in a dynamic state within a certain range in time and space varying with different physiological and pathological status.

1. Background

Acupoints are specific locations where *qi* and blood of meridians and *Zang-fu* organs infuse and also where diseases are reflected and the acupuncture needles are applied [1]. Therefore, acupoint is the essential factor in acupuncture diagnosis and treatment. Each acupoint has its own name, location, specificity, function, and so forth, while location is considered the most essential and fundamental. Without accurate and precise location, it is likely neither to examine the reaction of the acupoint in pathological condition, nor to find the point with therapeutic effects. Because of this, the standardization of the acupoint location is considered as the key in the history of Chinese acupuncture and moxibustion standardization. Since the official promulgation of the *location of acupoints* (national standard) in 1990, updated standards of the acupoint locations continued to be introduced one after the other in the years 2006 and 2010. The standardization of the acupoint location has to some extent normalized clinical operation and promoted the dissemination and development of the acupuncture and moxibustion worldwide. However what catches our attention is that while promoting the standard of the acupoint location, parts of the clinical practitioners stick too much to the fixed location of the acupoint and ignore the importance and necessity of searching and seeking acupoint along the meridian, leading to dissatisfactory clinical effect.

According to the description in the *Yellow Emperor's Inner Canon, the classic of Traditional Chinese Medicine*, acupoint is a portal where the channel *qi* enters and flows out and not a fixed point attaching to the skin, vessel, muscle, tendon, and bone. Therefore, acupoint is not isolated or static structure in the body. It is related to the movement of channel *qi*. It is not only able to reflect the changes of *qi* in channel, but also able to be used for adjusting the channel *qi*, showing dynamic characteristics. Whereas most of the researches on

Table 1: Comparison ages between observation group and control group (unit: age).

Group	Number of cases	Age		
		Smallest value	Largest value	M (QR)
Observation group	30	21	34	26 (5)
Control group	30	23	32	25 (2)
Z				−0.88
P				0.378

dynamic performance of acupoints have been theoretical, clinical research is lacking.

Diji (SP 8) is the most important and commonly used point for the treatment of dysmenorrhea. According to the theory of traditional Chinese medicine (TCM), *Diji* (SP 8) is the *Xi*-cleft point of the Spleen meridian of Foot *Taiyin*, where the meridian *qi* accumulates deeply and is suitable for treating acute pain and blood disease. Primary dysmenorrhea, a medical condition of cramping pain in the lower abdomen occurring before and during menstruation, is just ascribed to acute pain and blood disease in TCM. Therefore, this research focuses on patients with primary dysmenorrhea to observe the changes in tenderness in *Diji* (SP 8) in different physiological and pathological states, so as to explore the dynamic characteristic of acupoint and to provide clinical data for the study of acupoint dynamism from the clinical perspective.

2. Materials and Methods

2.1. Setting and Participants. 30 patients with primary dysmenorrhea were recruited as the observation group between April and December of 2013 in *Dongzhimen* Hospital affiliated to Beijing University of Chinese Medicine. 30 healthy female volunteers from the Beijing University of Chinese Medicine were recruited as the control group during the same period of time.

As some patients with primary dysmenorrhea may resolve or be relieved spontaneously after giving birth, all the participants included were nulliparous, so as to reduce selection bias. In the observation group, the oldest participant was 34 years old, the youngest was 21 years old, and the mean of their age was 26 ± 5 years. In the control group, the oldest was 32 years old, the youngest was 32 years old, the smallest age was 23 years, and the mean of their age was 25 ± 2 years. After the statistical analysis of the distribution of age and disease duration between the control group and the observation group, the differences were not statistically significant. See details in Table 1.

2.2. Diagnostic Criteria, Inclusion Criteria, and Exclusion Criteria

2.2.1. Observation Group

(1) Diagnosis Criteria. Referring to the Canadian Department of Gynecology and Obstetrics Association in 2005 *primary dysmenorrhea clinical guideline* [2], standards are as follows.

(1) The first one is women with lower abdominal pain that begins somewhere between several hours before and a few hours after the onset of the menstrual bleeding, usually persisting up to 2-3 days; (2) the pain is characteristically colicky or dull and located in the midline of the lower abdomen but may extend to both lower quadrants, the lumbar area, and the thighs; (3) the pain is frequently associated with symptoms including diarrhea, nausea, vomiting, fatigue, light-headedness, headache, dizziness, and, rarely, syncope and fever; (4) the symptoms are more or less reproducible from one menstrual period to the other; (5) type B ultrasonic examination and gynecological examination exclude the organic pathological changes in the reproductive organs.

(2) Inclusion Criteria. These criteria include the following: (1) patients who fulfill the diagnostic standard of the primary dysmenorrhea; (2) patients of ages between 18 and 35 years; (3) patients who have never given birth; (4) patients who have disease duration ≥6 months; (5) patients who have regular menstrual cycle (28 ± 7) d; (6) patients who have abdominal pain which occurs 48 hrs within the onset of menstruation; (7) patients with COX Dysmenorrhea Symptom Scale (CMSS) [3] total score ≥8; (8) patients with VAS score of the abdomen pain ≥40 during the attack of dysmenorrhea; (9) patients who have no participation in any other medication or modality clinical trials; (10) patients who signed informed consent.

(3) Exclusion Criteria. These criteria include the following: (1) patients with life threatening disorders, such as cardiovascular, liver, kidney, hematopoietic system disorders, and mental diseases; (2) patients who have skin problem on and near *Diji* (SP 8), such as soft tissue damage, ulceration, scar, and skin calluses; (3) patients who received other related treatments within a month or intake of pain killers, sedatives, and hormone drugs within 2 weeks; (4) patients who are physically weak or judged not suitable to participate in this research by researchers.

2.2.2. Control Group

(1) Inclusion Criteria. These criteria include the following: (1) healthy women with no abnormalities in the physical examination within the recent semester; (2) patients of ages between 18 and 35 years; (3) patients who have never given birth; (4) patients who have no history of dysmenorrhea in the past; (5) patients who have mostly regular menstrual cycle (28 ± 7) d; (6) patients who signed informed consent.

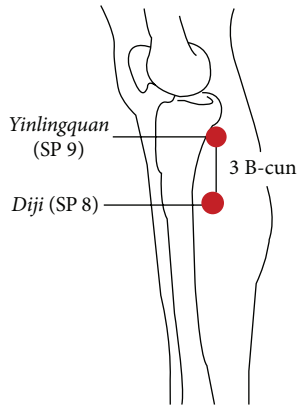

Figure 1: Standard position of *Diji* (SP 8).

(2) Exclusion Criteria. These criteria include the following: (1) patients suffering from frequent lower abdominal pain of unknown reason; (2) patients with skin problems on and near *Diji* (SP 8), such as soft tissue damage, ulceration, scar, and skin calluses; (3) patients who received other related treatments within a month or intake of pain killers, sedatives, and hormone drugs within 2 weeks; (4) patients with mental disorders or judged not suitable to participate in this research by researchers.

2.3. Tenderness Detection on Diji (SP 8). In order to guarantee the quality of the study, every segment of the operation was performed by the same researcher, who had received training over six months, to ensure the standardization and unity of the operation.

2.3.1. Detecting Point. *Diji* (SP 8) on both sides of the legs and their surrounding areas of a total of 60 participants in both the observation group and control group was detected.

The participants were instructed to lie supine with legs straightened in a relaxed manner while fully exposing the parts below the knees.

Diji (SP 8) was located referring to *WHO standard acupuncture point locations in the Western Pacific Region* [4], which is "on the tibial aspect of the leg, posterior to the medial border of the tibia, 3 B-cun inferior to SP9" (Figure 1). Mark this point as the standard position of *Diji* (SP 8).

2.3.2. Detecting Tenderness with VAS [5]. Starting from the standard position of *Diji* (SP 8), the researcher pressed spirally with the tip of the thumb pulp in a circular area 2 cm long in radius. The intensity of the pressing force was consistent and even to the level of muscle. The tenderness was recognized when pain, soreness, or distension sensation was expressed through the immediate and fleeting reactions of participants' eyes or words.

When tenderness reaction appeared, the participants were instructed to face the reverse side of the VAS card without graduation and then move the cursor to the position that best represented the pain intensity. The researcher facing the side with calibration recorded VAS scores and marked the position. The point with highest VAS scores was the tenderest point; if there is no tenderness upon pressure, the result was just recorded with no VAS detection.

Figure 2: Algometer.

2.3.3. Detecting Pain Threshold of the Tenderest Point. After the VAS assessment, the participants were instructed to rest for 10 minutes. Then, the pain threshold of the tenderest point was detected using an algometer (National Patent number: ZL200520142236.5; Product Publication number: CN2862954; Manufacturer: Institute of Orthopedics and Traumatology Affiliated to Chinese Academy of Traditional Chinese Medicine Science; Place of Production: Beijing) (Figure 2).

Firstly set the tester to zero. Then put the probe tip (0.5 cm in diameter) of the tester vertically onto the mark point. Apply pressure gradually and evenly downward (the maximum pressure should not exceed 600 kpa for avoiding tissue damage caused by excessive force). Once the participant reports pain or a radiating pain was elicited, then the algometer was removed and the data on the tester screen was recorded as the pain threshold value. Such a procedure was conducted on both sides of the *Diji* (SP 8) area, with the left one coming first.

2.3.4. Measure the Location of the Tenderest Point. The distance between the center of the tenderest point and the standard position of *Diji* (SP 8) was measured with a soft tape measure and then recorded.

2.3.5. Detecting Time Point

(1) For the observation group, detection of time point occurred during the first day or second day following the onset of dysmenorrhea and the seventh day after menstruation (nonmenstrual period).

(2) For the control group, detection of time point took place the first or second day following menstrual onset and the seventh day after menstruation (nonmenstrual period).

2.4. Statistical Analysis. SPSS17.0 statistical software was used for analysis. Count data were tested using x^2 test. One-way ANOVA was adopted for sets of normally distributed

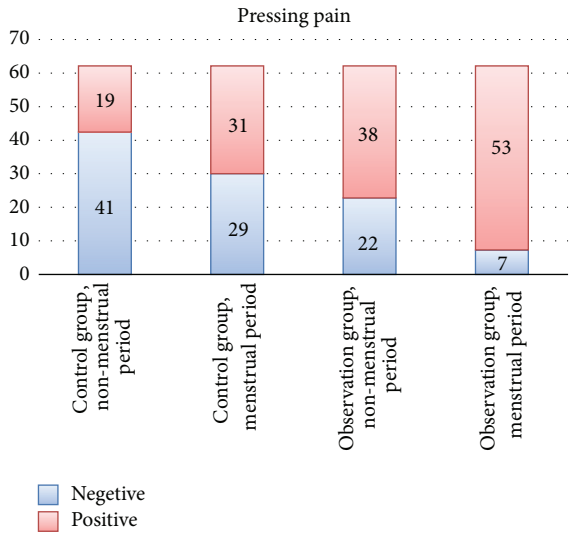

Figure 3: Comparison of the TOR of each group during menstrual and nonmenstrual period.

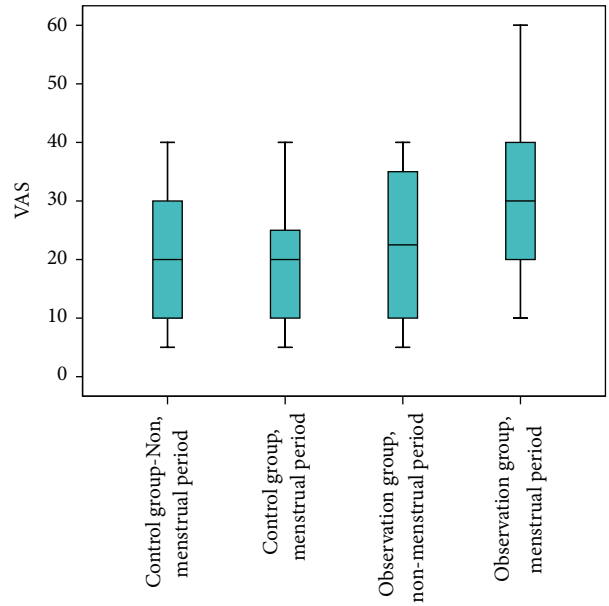

Figure 4: Comparison of VAS score between menstrual period and nonmenstrual period in two groups (mm).

data which went through paired comparison using S-L-D method. t-test was used for the data from two groups and the data were expressed by the mean plus or minus standard deviation. Skewed data were tested using the nonparametric Wilcoxon test and expressed in M (QR), that is, the median (interquartile range). All statistical tests were tested and verified using the two-sided test. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Comparisons of Tenderness Occurrence Rate in Diji (SP 8) Area. In observation group, there were 5 one-side tenderness cases and 1 pain-free case; the rest of the cases presented with tenderness on both sides in *Diji* (SP 8) area during acute onset period. Total TOR was 88.3%. During nonmenstrual period, there were 8 one-side tenderness cases and 7 pain-free cases, and the rest presented with tenderness on both sides. Total TOR was 63.3%. In control group, there were 11 one-side tenderness cases and 9 pain-free cases, and the rest of the cases presented with tenderness on both sides in *Diji* (SP 8) area during menstrual period. Total TOR was 51.7%. During nonmenstrual period, there were 5 one-side tenderness cases and 18 pain-free cases, and the rest presented with tenderness on both sides. Total TOR was 31.7%.

We used Chi-squared test to compare the TOR between two groups and two menstrual periods (corrected value $P' = 0.00833$). In the observation group, the TOR during menstrual period was significantly higher than that in the nonmenstrual period, $P < P'$. In the control group, the TOR during menstrual period was also significantly higher than that in the nonmenstrual period, $P < P'$. During nonmenstrual period, the TOR of the observation group was also significantly higher than that of the control group, $P < P'$ (Figure 3, Table 2).

3.2. Comparison of VAS Score of the Tenderest Point in Diji (SP 8) Area. Using nonparametric test, we compared VAS score between groups in menstrual period and nonmenstrual period (corrected value $P' = 0.00833$). VAS score of the observation group in the menstrual period was significantly higher than that in the nonmenstrual period, while there was no significant difference between two periods in the control group. In menstrual period, VAS score of the observation group was significantly higher than that of the control group, $P < P'$. In nonmenstrual period, VAS score of the observation group was higher than that of the control group, while there was no significant difference, $P > P'$ (Figure 4, Table 3).

3.3. Comparison of Tenderness Threshold Value of the Tenderest Point in Diji (SP 8) Area. We used one-way ANOVA to analyze two groups' tenderness threshold value of the tenderest point in *Diji* (SP 8) area in menstrual and nonmenstrual period. The results showed $F = 4.983$, $P = 0.003 < 0.05$, indicating that there was difference in threshold value between two groups in menstrual or nonmenstrual period. S-L-D method was used for further analysis. Tenderness threshold value in the menstrual period of the observation group was significantly lower than that in the nonmenstrual period, $P < P'$, while there was no significant difference between two periods in the control group. In menstrual period, tenderness threshold value of the observation group was significantly lower than that of the control group, $P < P'$. In nonmenstrual period, tenderness threshold value of the observation group was lower than that of the control group, while there was no significant difference, $P > P'$ (Figure 5, Table 4).

3.4. Location of the Tenderest Point in Diji (SP 8) Area. Our results showed overlaps between the tenderest point and

Table 2: Comparison of the TOR in *Diji* (SP 8) area between menstrual and nonmenstrual period.

Group			Menstrual period	Nonmenstrual period	x^2	P
Observation group	Tenderness	Positive	53	38		
		Negative	7	22	10.231	0.001
	TOR		88.3%	63.3%*		
Control group	Tenderness	Positive	31	19		
		Negative	29	41	4.937	0.026
	TOR		51.7%*	31.7%#		
x^2			19.206	12.063		
P			0.000	0.001		

Note: corrected value $P' = 0.00833$. *Compared with observation group in menstrual period, $P < P'$. #Compared with observation group in nonmenstrual period, $P < P'$.

Table 3: Comparison of VAS score between menstrual and nonmenstrual period in two groups (mm).

Group		Menstrual period	Nonmenstrual period	Z	P
Observation group	Number of effective values	53	38		
	Minimum value	10	5		
	Maximum value	60	40	−2.646	0.0081
	M (QR)	30 (20)	22.5 (25)*		
	Mean rank	88.76	66.12		
Control group	Number of effective values	31	19		
	Minimum value	5	5		
	Maximum value	40	40	−0.182	0.856
	M (QR)	20 (15)*	20 (20)		
	Mean rank	54.89	57.50		
Z		−3.705	−0.780		
P		0.000	0.436		

Note: corrected value $P' = 0.00833$. *Compared with observation group in menstrual period, $P < P'$.

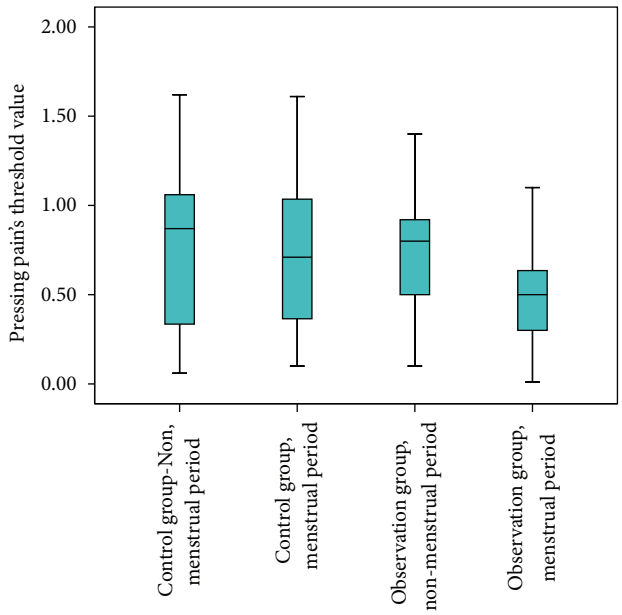

Figure 5: Tenderness threshold value in *Diji* (SP 8) area in menstrual and nonmenstrual period (kPa).

standard point in observation group during menstrual period and nonmenstrual period. Overlap rate was 22.6% and 28.9%, respectively. We measured the distance between the tenderest point and the standard point and came up with the following conclusion: if we set standard point of *Diji* (SP 8) as datum point, distribution range of the tenderest point in observation group was 0.565–0.903 cm in menstrual period and 0.515–0.974 cm in nonmenstrual period, respectively (Figure 6, Table 5).

4. Discussions

The dynamic characteristic of the acupuncture point is one of the hot topics in the acupuncture research. Previous study has shown that acupuncture points are of dimensional structure located in the interstice within the skin, vessel, muscle, sinew, bone, and even viscera, rather than fixed points. Their location may be influenced by several factors such as different physiological changes and pathological conditions of the *Zang-fu* organs and channels and the external environment and individual variety. Therefore, acupuncture points possess the individualized and dynamic characteristic [6].

Table 4: Tenderness threshold value in *Diji* (SP 8) area in menstrual and nonmenstrual period (kPa).

Group		Menstrual period	Nonmenstrual period	Mean difference	P
Observation group	Number of effective values	53	38	−0.251	0.001
	Minimum value	0.01	0.1		
	Maximum value	1.2	1.6		
	$\overline{x} \pm s$	0.497 ± 0.040	0.748 ± 0.375*		
Control group	Number of effective values	31	19	−0.027	0.801
	Minimum value	0.1	0.06		
	Maximum value	1.61	1.62		
	$\overline{x} \pm s$	0.724 ± 0.385*	0.751 ± 0.468		
Mean difference		0.227	−0.003		
P		0.006	0.979		

Note: corrected value $P' = 0.00833$. *Compared with observation group in menstrual period, $P < P'$.

Table 5: Distance between the tenderest point and standard point in *Diji* (SP 8) area (cm).

		Number of effective values	Overlap rate with standard point	Minimum value	Maximum value	M (QR)	95% CI
Observation group	Menstrual period	53	22.6%	0	3	0.7 0.6	0.565–0.903
	Nonmenstrual period	38	28.9%	0	2.6	0.7 (1)	0.515–0.974

There are four reasons for applying the pressing examination on *Diji* (SP 8) of the patients who suffered from primary dysmenorrheal to study the dynamism of acupuncture points on reflecting disease in this research. First, according to the channel and acupuncture point theory in TCM, *Diji* (SP 8) is the *Xi*-cleft point of the Spleen meridian of Foot *Taiyin*. *Xi*-cleft points are where the meridian *qi* accumulates deeply and are indicated for the acute and pain disease of the respective *Zang-fu* organs and meridians, while the *Xi*-cleft points of the Yin meridians are also indicated for blood diseases [7]. Primary dysmenorrhea, a medical condition of cramping pain in the lower abdomen occurring just before and during menstruation, is ascribed to acute pain and blood disease in TCM. Therefore, *Diji* (SP 8) is the most important and commonly used point for the treatment of dysmenorrhea [8–11]. Second, the acupuncture points relate closely to the internal *Zang-fu* organs through the pathway of the meridians; thereby the condition of the diseased *Zang-fu* organs will be reflected on the acupuncture point through the transmission of the meridians [12]. Third, though there are various examination methods and techniques for the reflection effect of acupuncture point, such as detecting the electric currency and electrical resistance of the point [13–17] and the infrared thermal imaging technique [18, 19], the most commonly used, convenient, and consensus method is detecting the tenderness and pain threshold [20–22]. Fourth, there are few researches and reports on reflecting effect of *Diji* (SP 8) on the dysmenorrhea.

Diji (SP 8) of the 30 patients with primary dysmenorrhea and 30 healthy women was palpated by hand and detected by algometer. The result of the research and the concerning issues are discussed as follows.

(1) The tenderness occurrence rates and the VAS score of *Diji* (SP 8) in menstrual period of the observation group were higher than that in nonmenstrual period and the menstrual period in the control group. The tenderness threshold of *Diji* (SP 8) in the menstrual period of observation group was lower than that in the nonmenstrual period of observation group and the menstrual period in the control group. The result showed that there exist dynamic characteristics in *Diji* (SP 8) in the tender reaction in both the physiological and pathological conditions, including the different stages in the pathological condition. The tenderness reaction was more likely to occur and more intensive and sensitive in the menstrual period of the primary dysmenorrhea patient.

(2) There is no statistical difference in the tenderness occurrence rates, VAS score, and tenderness threshold in both the menstrual period and nonmenstrual period in the control group. It indicates that there is no remarkable change in the tenderness reaction on *Diji* (SP 8) in the physiological state and the alteration of the physiological rhythm. Comparing the data in the nonmenstrual period of the test and control group, the tenderness occurrence rate of observation group is higher than that of the control group, but there is no statistical difference between the two groups in VAS value and the tenderness threshold. The results might be considered as follows: in the nonmenstrual period, the dysmenorrhea patients were still in the pathological states of blood deficiency failing to nourish the uterus or blood stasis blocking the meridian in the uterine, which makes *Diji* (SP 8) become more sensitive to pressing; however the severity was not intensive. Or it might be that the patients enrolled were not enough to show the statistical difference. From an anatomical

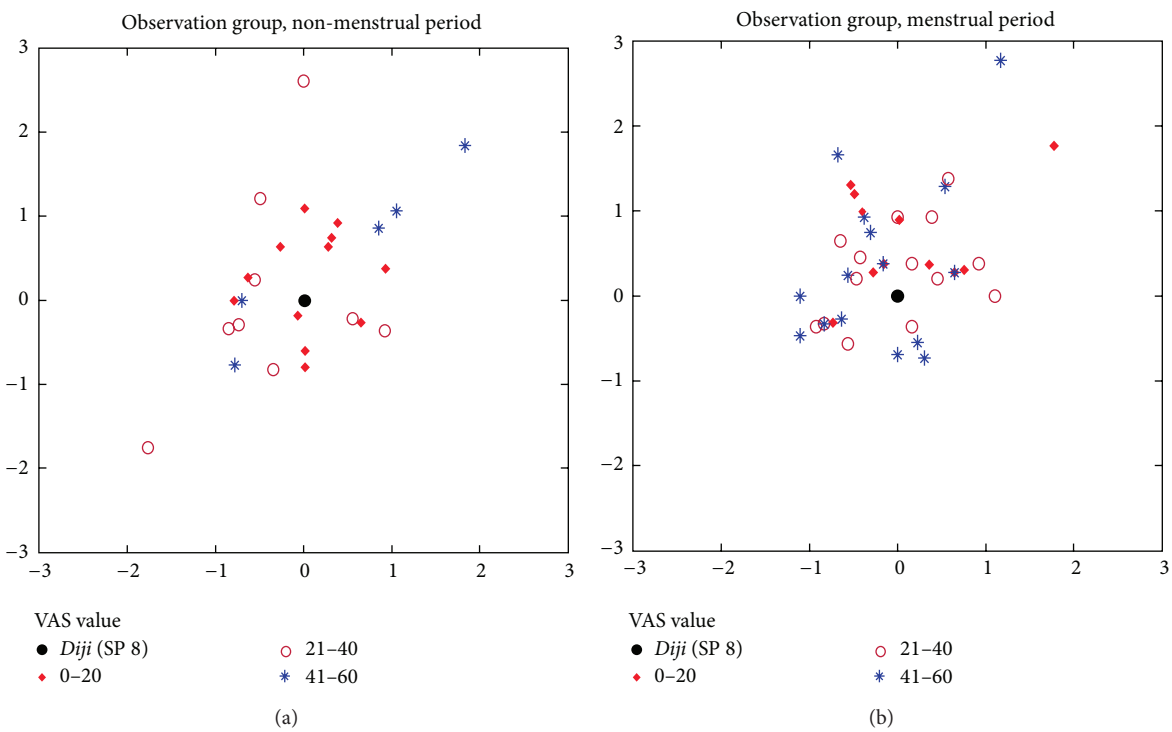

Figure 6: Location of the tenderest point in *Diji* (SP 8) area (cm).

perspective, there are parts of the saphenous nerve in shallow layer of *Diji* (SP 8) area and sympathetic nerve governing the myometrium contraction coming from the same nerve segments. Accordingly, the same situation also occurs between the tibial nerve in deep layer and parasympathetic nerve controlling the sense of uterus. It can thus be seen that *Diji* (SP 8) has a close relationship with uterus in anatomy [23]. However, the results still possibly indicate that *Diji* (SP 8) might reflect the pathological condition in the menstrual period of the dysmenorrhea patients, which also validate the viewpoint that pathological reaction in meridian and acupuncture points relates to timing [24].

(3) This research found out that, in *Diji* (SP 8) area, the tenderest points were divergent from the standardized location of *Diji* (SP 8) in the majority of dysmenorrheal patients, which indicates that the location of *Diji* (SP 8) in pathological state is different from the standardized location. Acupuncture point is both the reflective point of diseases and where the needles and moxibustion are applied for the treatment of disease in the meridian and acupuncture point theory [25]. Wang illuminates the process acupuncture reflecting disease. Acupuncture point is where both the pathological factors and meridian *qi* exist when patient suffered from a disease. If the meridian *qi* fails to dispel the pathological *qi* out of the body in time, the pathological factors accumulate in the acupuncture point; consequently, there might be pathological reactions such as pain, tenderness, and other changes on the acupuncture point [26]. Zeng et al. put forward the viewpoint that, in the pathological state, the surface reflection area of acupuncture varies with the condition of the disease; it increases when disease gets worse, decreases when disease gets better, and disappears when disease is cured [27, 28]. As to the enlargement of the surface areas around acupoint reflecting diseases, Yu et al. believe that it relates to the facilitation and sensitization of the spinal cord caused by visceral disorders, where the information coming from the body surface and viscera are assembled [29]. According to the theory of "painful locality taken as an acupoint," the tenderest point in *Diji* (SP 8) area might be the veracious point of *Diji* (SP 8) in the pathological state and it might also be the most effective point for treatment. We have found in clinic that, in the acute stage of dysmenorrhea, the part between *Sanyinjiao* (SP 6) and *Yinlingquan* (SP 9) on the pathway of Spleen meridian is the main reaction region. Tender, sore, or distending points, especially distinctive around the *Sanyinjiao* (SP 6), *Diji* (SP 8), and *Yinlingquan* (SP 9) area, will be found when palpating along the meridian. Needling on the tender point will bring instinctive effect of relieving the pain. Of course, large scale clinical trial is required to confirm our clinical observation.

(4) There have been 2 Chinese national standards of acupuncture location (1990 and 2006) and *Standard Acupuncture Nomenclature* (second edition) was

published in 1993 [30–34]. The standardization of acupuncture point location helps to standardize the needle manipulation and also promote the spreading of acupuncture worldwide. In standardized acupuncture point location, a vertical and horizontal coordinate method is adopted as much as possible to locate the acupuncture point; for example, *Zusanli* (ST 36) is "on the anterior aspect of the leg, on the line connecting ST35 with ST41, 3 B-cun (proportional bone cun) inferior to ST35." Then, *Zusanli* (ST 36) is stated as the crossing point of the two intersecting lines. Accordingly, this locating method makes some acupuncture practitioners stick to the standardized location of acupoints and ignore the dynamic character of the acupoint. However, this might result in the less satisfied effect in clinic. The description of the location of some extra acupuncture point in textbook of acupuncture and moxibustion before the standardization of acupuncture point location in China embodies the dynamic character of acupuncture point. For example, *Lanwei* (appendix) point is located around 2 cun inferior to *Zusanli* (ST 36), and *Danang* (gallbladder) is 1-2 cun inferior to *Yanglingquan* (GB 34) [35].

(5) In acupuncture clinical trials, selecting the nontraditional-acupoint site near the acupoint for treatment as the placebo control is called adjacent nonacupoint control method [36], which is the main method in the nonacupoint controlled trials [37]. The way of selecting the nonacupoint is reasonable or does not determine the validity of the control and the authenticity and veracity of the results. This research as well as others [38–41] all revealed that the location and size of the acupoints may change with the different conditions of the body, and in the pathological condition the size on the body surface may even get large, which embodies the dynamic characteristic of the acupuncture points. Therefore, the adjacent nonacupoint might just be the site of acupuncture point for treatment, and needling the adjacent nonacupoint, possibly, might produce the same or even better effect compared to that of the acupuncture point in the standardized location. Thereby, the result of clinical trials adopting the nearby nonacupoint as a control is debatable.

This research is an exploratory study on the dynamism of acupuncture points through detecting the tenderness reaction on *Diji* (SP 8) in primary dysmenorrhea patients. Further studies are required for answering questions concerning the dynamism of acupuncture points, such as what the changes of the acupuncture points in different physiological and pathological conditions as well as in different stages of the pathological conditions are and whether or not needling on the tender site of the acupuncture point might improve the treatment effect.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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