

Does Acupuncture Therapy Alter Activation of Neural Pathway for Pain Perception in Irritable Bowel Syndrome?: A Comparative Study of True and Sham Acupuncture Using Functional Magnetic Resonance Imaging

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Background/Aims

Patients with irritable bowel syndrome (IBS) are characterized by abnormal central processing with altered brain activation in response to visceral nociceptive signals. The effect of electroacupuncture (EA) on IBS patients is unclear. The study is set to study the effect of EA on brain activation during noxious rectal distension in IBS patients using a randomized sham-controlled model.

Methods

Thirty IBS-diarrhea patients were randomized to true electroacupuncture or sham acupuncture. Functional MRI was performed to evaluate cerebral activation at the following time points: (1) baseline when there was rectal distension only, (2) rectal distension during application of EA, (3) rectal distension after cessation of EA and (4) EA alone with no rectal distension. Group comparison was made under each condition using SPM5 program.

Results

Rectal distension induced significant activation of the anterior cingulate cortex, prefrontal cortex, thalamus, temporal regions and cerebellum at baseline. During and immediately after EA, increased cerebral activation from baseline was observed in the anterior cingulate cortex, bilateral prefrontal cortex, thalamus, temporal regions and right insula in both groups. However, true electroacupuncture led to significantly higher activation at right insula, as well as pulvinar and medial nucleus of the thalamus when compared to sham acupuncture.

Conclusions

We postulate that acupuncture might have the potential effect of pain modulation in IBS by 2 actions: (1) modulation of serotonin pathway at insula and (2) modulation of mood and affection in higher cortical center via ascending pathway at the pulvinar and medial nucleus of the thalamus.

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Key Words

Acupuncture; Irritable bowel syndrome; Magnetic resonance imaging

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Conflicts of interest: None.

Introduction

Irritable bowel syndrome (IBS) is a chronic relapsing functional bowel disorder that is characterized by recurrent abdominal pain and disturbance in bowel movement. Although the exact mechanism is still unclear, there is mounting evidence showing that visceral hypersensitivity plays an important role in the pathophysiology of IBS.¹⁻⁴ It has been postulated that visceral hypersensitivity is related to abnormal processing of visceral nociceptive signals in the central nervous system. Recent functional magnetic resonance imaging (fMRI) studies reported abnormal activation of specific brain functional areas that are involved in primary pain processing and regulation of affect.⁵⁻⁸ These findings suggested that visceral hypersensitivity might involve dysfunction in both pain perception as well as cognitive, affective, and motivational dimensions of pain experience.

Acupuncture is one of the treatment modalities of traditional Chinese medicine, which has been used extensively for treatment of various painful conditions and gastrointestinal diseases in China for thousands of years. The possible mechanism of acupuncture analgesia involves modulation of the activity of various neurohumoral pathways that are involved in pain perception. Electroacupuncture (EA), which refers to the application of a pulsating electrical current to acupuncture needles for acupoint stimulation, was developed in China as an extension of hand manipulation of acupuncture needles around 1934,⁹ and it has been reported to have both anti-inflammatory and anti-hyperalgesic properties in patients with fibromyalgia and osteoarthritis. Furthermore, EA may also have modulating effect on the mood with reported efficacy in treatment of depression.¹⁰ A considerable number of animal and human studies have shown that acupuncture has certain regulatory functions on gastrointestinal tract.¹¹ The therapeutic effect of acupuncture on upper gastrointestinal motility, secretion, sensation and myoelectric activity was also supported by systematic review and meta-analysis of randomized control trials, which reported efficacy in alleviation of nausea and emesis after surgery, cancer chemotherapy and pregnancy.¹²⁻¹⁶ However, the role of acupuncture in treatment of visceral pain such as IBS is less defined. Although some studies reported increased rectal sensory thresholds and improvement in bowel symptoms in IBS patients treated with acupuncture,¹⁷⁻¹⁹ other studies suggested that the apparent influence of acupuncture on rectal perception was either insignificant²⁰ or a placebo effect.²¹ It has been shown that most acupuncture trials for

IBS were of poor quality and effect of acupuncture was primarily a placebo response,^{22,23} which may imply alteration of affective and cognitive response to the pain instead of genuine analgesic effect.

We set out to evaluate the effect of EA on brain activation in patients with IBS using functional brain mapping with fMRI. The aim of this study was to discern whether EA alters activation of neural pathway for pain perception in patients with IBS during noxious mechanical distension of rectum.

Materials and Methods

Subject

The study was carried out during a 10-month period, between April 2008 to January 2009, in a university affiliated institute of digestive disease. We prospectively recruited consecutive patients who were either self-referred from a community functional gastrointestinal disorder program, or newly referred from primary care clinicians for investigations and management of recurrent abdominal pain or discomfort and altered bowel motion. These patients were invited to complete a self-administered IBS module questionnaire based on Rome III criteria and were evaluated by a gastroenterologist. Patients who met the Rome III criteria for IBS were eligible. These included the presence of recurrent abdominal pain or discomfort, which was relieved by defecation or associated with altered stool consistency or frequency for at least three months prior to study entry and was accompanied by 2 or more of the associated bowel symptoms such as altered stool form or bloating for at least 3 days per week.²⁴ Colonoscopy was then arranged within 4 weeks from initiation of assessment to exclude colonic pathology. The exclusion criteria included pregnancy, concomitant psychiatric disorders under treatment, history of acupuncture in the past six months, concomitant medications including antidiarrheal agent, antidepressant, narcotic analgesic and anti-cholinergic drugs.

These subjects were then randomly assigned to treatment with either true electroacupuncture (TA) or sham electroacupuncture (SA) group. TA group consisted of 15 subjects (8 male, 7 female; average age, 42.3 years; range, 20-63 years) and the SA group consisted of 15 subjects (7 male, 8 female; average age, 44.2 years; range, 24-65 years). All the patients were acupuncture treatment-naïve. The random allocation sequence was obtained from a computer-generated list of random numbers. Concealed allocation was achieved by an independent staff who assigned

treatments according to consecutive numbers in sealed envelopes. Ethical approval and permission to conduct the study were obtained from the hospital ethical committee. Written and verbal informed consents were obtained from all participants.

Electroacupuncture

Both TA or SA was performed by a single licensed acupuncturist. In the TA group, stainless steel needle was inserted through a 2 cm² foam rubber which hid the contact point between the needle and skin, over the respective acupoints. The acupoints chosen were ST36 (Zusanli), ST 37 (Shangjuxu) and SP6 (Sanyinjiao) (Fig. 1), which were known to strengthen and regulate alimentary function and maintain liver health. The needle was then connected with an electrical stimulation machine and stimulation was applied as continuous waveform, frequency of 10 Hz, voltage of 60 volts, and pulse width of 0.5 ms. The intensity of electrical stimulation depended on the subject's tolerance. Weak twitching of the muscle was visible to the acupuncturist when the EA was in place.

For SA group, blunted telescopic placebo needles were positioned at respective acupoints touching the skin but did not penetrate deep into the acupoints. The needle tips were hidden inside foam cubes. The needles were connected with electrical wire but no electrical stimulation was applied during the EA sessions. Both TA and SA groups were informed about possible therapy delivered to them via the devices on their lower limbs as potential treatment for IBS but the assigned treatment arm had not been

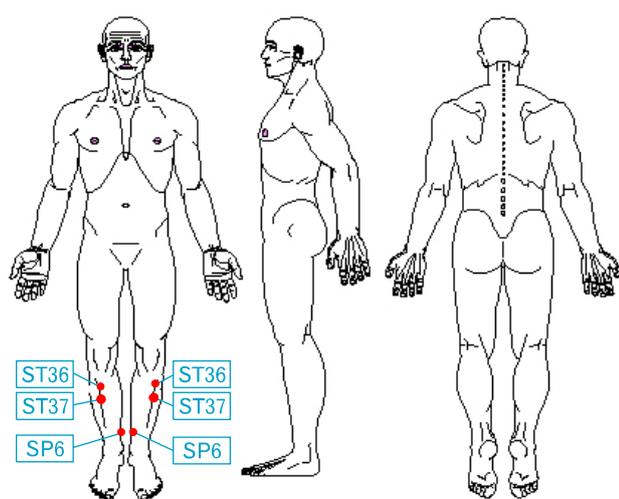


Figure 1. Position of acupoints in bilateral lower limbs: ST36 (Zusanli), ST 37 (Shangjuxu) and SP6 (Sanyinjiao), are known to strengthen and regulate alimentary function and maintain liver health.

disclosed to them.

Visceral Distension Protocols

Distension of the rectum was accomplished using a computer driven barostat while patient was positioned on the MRI table. All patients were tested under fasting conditions, 4 hours after a cleansing Fleet enema. The maximum tolerable threshold pressure was pre-determined before the actual MRI scanning, which was defined as a pressure above minimal distension pressure that elicited a “pain” response or maximum tolerable sensation. Patients were requested to squeeze the bulb repeatedly to signify request for termination of procedure. The same maximum tolerable threshold rectal pressure was used for each subject throughout the subsequent fMRI scanning.

Magnetic Resonance Imaging Scanning

Blood-oxygen-level-dependent imaging was performed in a 3 Tesla MRI scanner (Philips Achieva 3.0T X series, Quasar Dual MRI System; Philips Healthcare, Best, Netherlands). Patients were lying supine on the MRI scanning table. In each study, structural and functional images were acquired using an 8-channel receive-only head coil in the transverse orientation from the same section of the brain. Details of the sequence are given in the appendix.

Four sessions of functional scans were acquired for each subject at different stages of noxious rectal distension using the same pre-determined maximum tolerable threshold pressure as aforementioned:

(1) The first fMRI was performed according to the rectal distension protocol as aforementioned after insertion of acupuncture needles but no electrical stimulation was applied. This served as the baseline brain activation.

(2) The second fMRI was performed while there was on-going EA (either TA or SA) in addition to the rectal distension protocol. The fMRI started after subjects had received TA/SA stimulation for 15 minutes.

(3) The third fMRI was performed immediately after termination of a 30 minute session of EA with rectal distension paradigm.

(4) The fourth fMRI was performed when the subject was receiving EA without any rectal distension.

The original stimulus train and the one after intermediate time points removed during the functional scanning are shown in Figure 2. The statistical methods used in fMRI analysis have been reported in other studies.^{25,26}

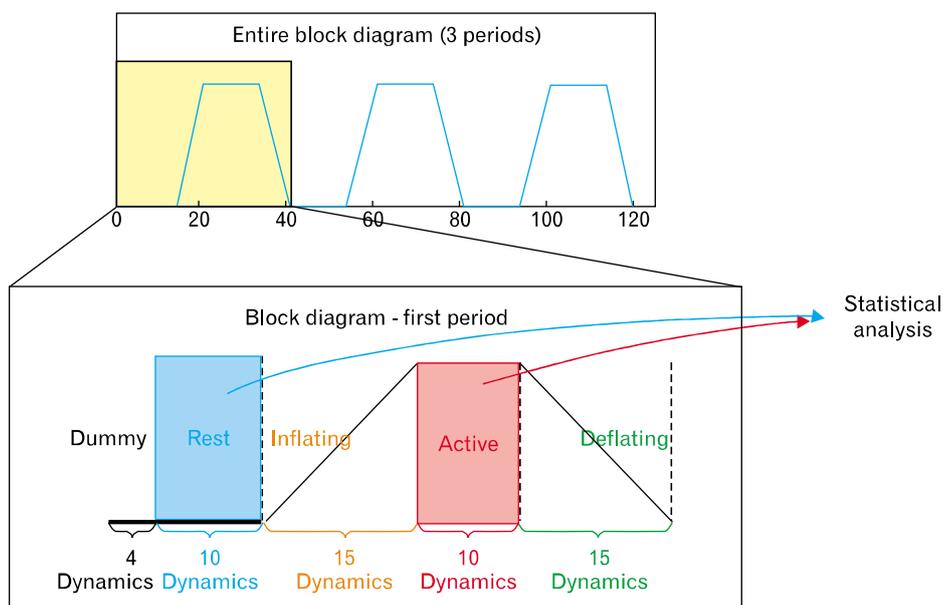


Figure 2. As there are 3 intermediate time points for balloon inflation and deflation respectively, the stimulus train is not in an ideal boxcar form. In order to restore the boxcar stimulus, the intermediate time points in the paradigm and the functional scans are removed. The original stimulus train and the one after intermediate time points removed are shown.

Rectal Sensation Rating

The subjects were asked to give verbal ratings on the degree of rectal sensation when the maximum tolerable threshold was reached during the balloon distension using a 0-10 point Likert scale (0: non-painful; 10: the most severe). The rectal sensation was rated at the following different time points: (1) during determination of maximum tolerable threshold, (2) before commencement of TA/SA treatment after insertion of needles, (3) during acupuncture treatment and (4) immediately after acupuncture treatment.

Statistical Methods

The baseline demographic characteristics of the subjects were compared using unpaired *t* test and Chi-square test for continuous variables and categorical variables, respectively. The symptom score and stool scale were compared using Mann Whitney U test. A two-sided *P*-value of less than 0.05 was considered significant.

All the functional and anatomical MRI data were transferred to an offline workstation for image analysis. The non-commercial SPM5 toolbox (<http://www.fil.ion.ucl.ac.uk/spm/>) was used, which was developed using MATLAB by the Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London. Details of the analysis are given in the Appendix.

Table 1. Demographic Data of All Subjects With True and Sham Acupuncture

	True acupuncture (n = 15)	Sham acupuncture (n = 15)	<i>P</i> -value
Mean age (yr [SD])	42.3 (12.2)	44.2 (14.5)	0.567
Male (n [%])	8 (53)	7 (47)	0.775
Smoker (n [%])	0 (0)	2 (13)	0.412
Drinker (n [%])	0 (0)	1 (7)	0.967
Duration of IBS (n [%])			
1-5 yr	5 (33)	7 (47)	0.935
> 5 yr	10 (67)	8 (53)	NS
Median score (n [range])			
Bristol stool scale	5 (3-6)	6 (4-7)	0.106
Pain/discomfort	2 (1-3)	2 (1-3)	0.325
Bloating	0 (0-4)	1 (0-2)	0.367
Sense of incomplete evacuation	2 (0-4)	1 (0-3)	0.624
Baseline maximum tolerable threshold (mmHg [SD])	40.0 (9.3)	41.3 (7.4)	0.539

IBS, irritable bowel syndrome.

Results

Thirty patients with IBS-diarrhea (15 male, 15 female; average age 43.3 years; range 20-65 years) were recruited. There was no significant difference in baseline characteristics between the 2 groups of patients (Table 1).

Baseline Brain Activation Before Electroacupuncture (Session 1)

During noxious rectal distension, significant brain activation ($P < 0.001$, uncorrected) was observed in the anterior cingulate cortex (ACC), perigenual cingulate cortex, bilateral prefrontal cortex, thalamus, cerebellum, temporal lobes and right insula cortex of the subjects. There was no regional difference in brain activation between TA and SA during baseline.

Brain Activation During Electroacupuncture (Session 2)

The effects of TA and SA on brain activation by rectal pain were assessed during session 2. Increased activation from baseline was observed in both TA and SA.

In TA group, increased brain activation from baseline was observed in perigenual cingulate cortex, bilateral prefrontal cor-

tex and temporal lobes, right insula and bilateral somatosensory cortex during session 2. In SA group, increased brain activation from baseline was observed in ACC, bilateral prefrontal cortex and left somatosensory cortex. Details of z-scores and peak voxel co-ordinates for regions activated in session 2 are presented in Table 2.

The differential effect of TA was determined using the serial subtraction method. In this study, we applied ([TA session 2 > TA session 1] > [SA session 2 > SA session 1]). Right insula and thalamus were the only 2 sites which showed significant higher brain activation in TA when compared with SA (Fig. 3).

Analogously, the non-specific needling effect of SA was assessed using the serial subtraction ([SA session 2 > SA session 1] > [TA session 2 > TA session 1]), and no definite brain region of significant difference was found.

Table 2. Details of Z-scores and Peak Voxel Co-ordinates for Regions Activated in Session 2 (Rectal Distension During True/Sham Electroacupuncture)

	Activated region	Laterality	BA	Talairach coordinates				
				z-score	x	y	z	
TA	Frontal_Superior	R	10	5.406	18	51	12	
	Temporal_Pole_Superior	R	38	4.816	54	12	-9	
	Temporal_Middle	L	21	4.803	-60	-30	-3	
	Cuneus	R	0	4.463	12	-72	27	
	Precuneus	L	0	4.395	-9	-51	45	
	Postcentral	R	2	4.142	48	-30	48	
	Temporal_Superior	R	48	4.034	54	-15	3	
	Insula	R	48	3.812	30	21	15	
	Postcentral	L	48	3.810	-51	-9	24	
	Postcentral	R	43	3.788	63	-6	18	
	Occipital_Superior	R	7	3.773	27	-75	42	
	SA	Occipital_Inferior	L	19	5.266	-48	-75	-3
		Paracentral_Lobule	L	4	4.766	-3	-21	57
		Angular	L	39	4.439	-42	-60	42
Occipital_Middle		L	19	4.260	-27	-78	36	
Fusiform		R	19	4.191	30	-69	0	
Frontal_Inferior_Opercular		L	48	3.908	-45	15	18	
Cingulum_Anterior		L	0	3.630	0	24	18	
Fusiform		L	37	3.529	-48	-57	-18	
Precentral		R	6	3.457	45	6	42	
Occipital_Middle		R	19	3.411	33	-78	33	
Frontal_Inferior_Triangular	R	48	3.375	42	24	24		

BA, brain activation; TA, true electroacupuncture; SA, sham electroacupuncture; R, right; L, left.

In TA group, increased brain activation from baseline was observed in perigenual cingulate cortex (L mid cingulum), bilateral prefrontal cortex and temporal lobes, right insula and bilateral somatosensory cortex during session 2. In SA group, increased brain activation from baseline was observed in anterior cingulate cortex, bilateral prefrontal cortex and left somatosensory cortex (post central).

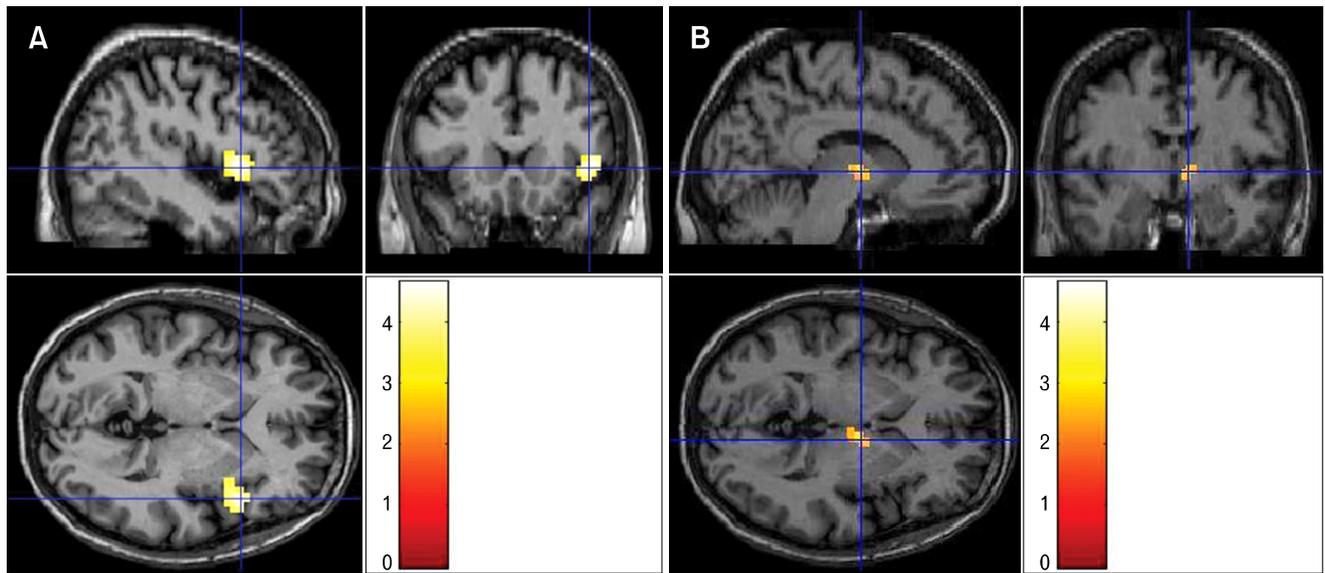


Figure 3. Statistical parametric map showing the differential effect of true electroacupuncture (TA), which is determined using the serial subtraction ([TA session 2 > TA session 1] > [sham electroacupuncture (SA) session 2 > SA session 1]). Right insula (A) and thalamus (B) are the only two sites which show significant higher brain activation in TA when compared with SA.

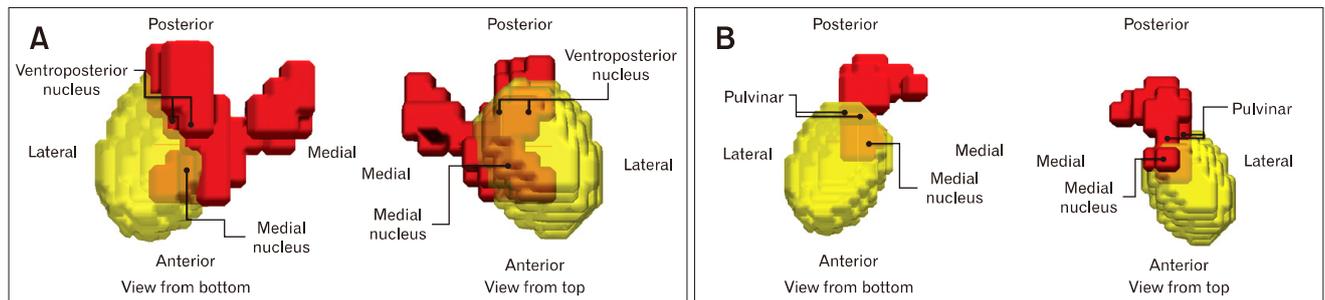


Figure 4. Thalamic activation. (A) Thalamic activation (indicated by red) during baseline (session 1). The yellow portion indicated the rest of thalamus without significant activation. (B) Thalamic activation (indicated by red) showing the differential effect of true acupuncture (TA) using serial subtraction ([TA session 2 > TA session 1] > [sham electroacupuncture (SA) session 2 > SA session 1]). The yellow portion indicated the rest of thalamus without significant activation.

Detailed Voxel Analysis of Activation at Thalamus Between Baseline and Differential Effect of True Electroacupuncture

Further 3D voxel based analysis showed that during baseline (session 1), the activation of right thalamus predominantly involved medial nucleus and ventroposterior (both ventroposterior lateral and ventroposterior medial) nucleus (Fig. 4A). For the differential effect of TA using serial subtraction ([TA session 2 > TA session 1] > [SA session 2 > SA session 1]), the activation of thalamus mainly involved the pulvinar and medial nucleus while there was no significant difference in the ven-

tro-posterior nucleus (Fig. 4B).

Brain Activation After Cessation of Electroacupuncture (Session 3)

In TA, increased brain activation from baseline was observed in bilateral prefrontal cortex, temporal regions and somatosensory cortex. In SA, increased brain activation from baseline was observed in right prefrontal cortex, left temporal region and cerebellum. Details of z-scores and peak voxel co-ordinates for regions activated in session 3 are given in Table 3.

When comparing the brain activation of TA between session 2 and 3, the brain activation in all regions (including ACC, pre-

Table 3. Details of Z-scores and Peak Voxel Co-ordinates for Regions Activated in Session 3 (Rectal Distension After Cessation of True/Sham Electroacupuncture)

	Activated Region	Laterality	BA	Talairach Coordinates				
				z-score	x	y	z	
TA	Precentral	R	6	5.353	39	-3	51	
	Frontal_Superior_Medial	L	32	5.144	-9	42	24	
	Parietal_Inferior	L	40	5.051	-30	-45	45	
	Temporal_Middle	L	21	4.944	-60	-3	-15	
	Frontal_Superior_Medial	L	10	4.779	-12	54	6	
	Parietal_Inferior	R	40	4.200	45	-51	45	
	Fusiform	L	37	4.172	-27	-33	-24	
	Calcarine	L	17	3.953	-3	-63	9	
	Rolandic_Opercular	R	48	3.852	63	-3	15	
	Occipital_Superior	R	19	3.836	27	-78	39	
	Temporal_Superior	R	48	3.832	51	-15	3	
	Temporal_Middle	L	37	3.705	-63	-54	9	
	Paracentral_Lobule	R	4	3.687	12	-30	54	
	Parietal_Inferior	R	40	3.661	33	-36	51	
	SA	Cerebelum_9	R	0	4.226	15	-54	-42
		Temporal_Inferior	L	20	4.104	-45	-9	-36
		Cerebelum_8	L	0	4.010	-21	-57	-45
Brain_stem		R	35	4.000	12	-15	-21	
Cerebelum_Crus2		L	0	3.986	-27	-75	-39	
Frontal_Middle		R	45	3.911	45	36	21	
Temporal_Superior		R	42	3.810	51	-36	12	
Frontal_Middle		R	46	3.593	33	45	12	
Frontal_Inferior_Opercular		R	44	3.541	57	15	27	
Angular		L	40	3.382	-36	-57	36	

BA, brain activation; TA, true electroacupuncture; SA, sham electroacupuncture; R, right; L, left.

In TA, increased brain activation from baseline was observed in bilateral prefrontal cortex, temporal regions and somatosensory cortex. In SA, increased brain activation from baseline was observed in right prefrontal cortex, left temporal region and cerebellum.

frontal cortex, thalamus, insula, temporal lobes) on session 2 was significantly greater than those observed in session 3. In SA, ACC and temporal region showed higher activation in session 2 while the prefrontal cortex showed higher activation in session 3.

The differential effect of TA was determined using the serial subtraction ($[TA \text{ session } 3 > TA \text{ session } 1] > [SA \text{ session } 3 > SA \text{ session } 1]$), no focal region of significant difference was found. Analogously, the non-specific needling effect of SA was assessed using the serial subtraction ($[SA \text{ session } 3 > SA \text{ session } 1] > [TA \text{ session } 3 > TA \text{ session } 1]$), no definite brain region of significant difference was found.

Brain Activation During True Electroacupuncture/Sham Acupuncture Alone (Session 4)

There was no significant difference in brain activation between TA and SA without rectal distension.

Table 4. Comparison of Median Rectal Rating at Different Sessions in Subjects Receiving True and Shame Acupuncture

Mean rectal pain rating (n [SD])	True acupuncture (n = 15)	Sham acupuncture (n = 15)	P-value
Baseline	7.4 (1.3)	7.3 (1.4)	0.852
Before acupuncture treatment	7.5 (1.2)	7.2 (1.3)	0.717
During acupuncture treatment	7.8 (1.4)	7.3 (1.1)	0.440
Immediately after acupuncture treatment	7.6 (1.4)	7.7 (1.3)	0.717
P-value (within group comparison)	$P = 0.842$	$P = 0.403$	

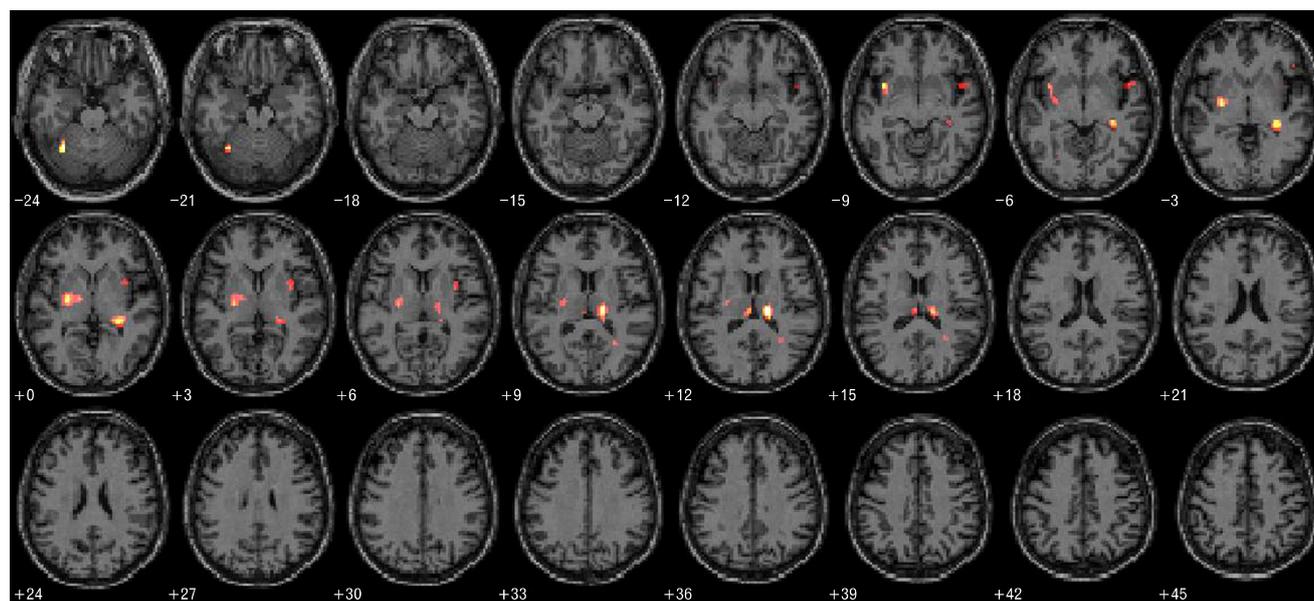


Figure 5. Significant correlation ($P < 0.005$, uncorrected) between subjective rectal pain rating and brain activation was observed in hypothalamus (z-score, 3.455), bilateral thalami (z-score, 3.207 and 2.912) and bilateral insula (z-score, 2.820 and 3.030).

Rectal Pain Rating and Its Correlation With Brain Activation

There was no significant change in rectal pain ratings at different phases of acupuncture treatment in both groups of patients (Table 4). And there was also no difference in rectal pain ratings at each phase of acupuncture treatment between the 2 groups. However, a significant correlation ($P < 0.005$, uncorrected) between subjective rectal pain rating and brain activation was observed in hypothalamus (z-score, 3.455), bilateral thalami (z-score, 3.207 and 2.912) and bilateral insula (z-score, 2.820 and 3.030) (Fig. 5).

Discussion

We set out to evaluate the effect of EA on brain activation during noxious rectal distension in IBS patients in this randomized sham-controlled pilot study. Although there was no significant difference in rectal sensation in response to noxious mechanical distension, we observed differential activation at right insula as well as pulvinar and medial nucleus of the thalamus with EA treatment.

The strengths of this study include the following:

- (1) One of the few studies that focused on the effects of acupuncture on brain activation in response to visceral pain.

- (2) All patients were acupuncture treatment naïve which allowed proper blinding.
- (3) Stringent diagnostic criteria of IBS and the presence of visceral hyperalgesia in most subjects using barostat.
- (4) Specially designed acupuncture needles allowed real-time evaluation of the effects of acupuncture.

The limitations of the study include the following:

- (1) Small sample size
- (2) Acute provoked mechanical visceral pain rather than spontaneous pain caused by a variety of physiological (chemical, heat, pH and etc) stimuli
- (3) Single session rather than repeated treatment sessions
- (4) Not evaluated the effects of “individualized” treatment
- (5) Unable to clearly distinguish between non-specific needling effect of SA from genuine placebo effect.

Numerous neuropeptides are involved in the visceral sensory pathway. Among these, serotonin (5-hydroxytryptamine, 5-HT) has been postulated to be the most important neurotransmitter involved in the pathogenesis of IBS. It is highly abundant in the gastrointestinal tract.²⁷ A 5-HT also plays a major role in the central processing of visceral sensory signals. Central processing of visceral nociceptive signals involves an integrated network of various structures in the brain, which include sensory cortex, thalamus, hypothalamus and midbrain.²⁸ These centers further modulate processing of the nociceptive signals at the spinal cord level.

Disturbances in 5-HT signaling may contribute to gastrointestinal dysfunction and hypersensitivity in IBS. On the other hand, the effect of acupuncture on visceral hyperalgesia might also be mediated via its modulation of central processing mechanisms via the 5-HT pathway.

Previous study showed that there were abundant 5-HT_{2A} receptors within the insula.²⁹ Activation of this region by TA might indicate that there was pain modulation effect associated with acupuncture.

In the thalamus, it is well documented that pulvinar has extensive connections with various parts of the cortex.^{30,31} The pulvinar has been associated with the function of attention,^{32,33} and is intimately connected with the amygdale and the cingulate cortex, both of which are involved in the processing of emotional stimuli and these processes are related to 5-HT mechanism.³⁴ In addition, the pulvinar is involved in both facilitation and inhibition pathways.³⁵ The medial and dorsomedial nuclei of the thalamus project to the frontal cortex^{30,31} and are associated with the pathway responsible for regulation and modulation of memory and emotion,³⁶ as well as the functions of executive nature. Activation of these regions by TA might indicate potential effect of acupuncture on mood and affection regulation. Furthermore, the ventroposterior nucleus (ventroposterior lateral and ventroposterior medial), a known sensory center which showed activation during baseline (session 1), was not significantly activated or deactivated by TA, which indicated that the activation of this region by noxious stimuli (rectal distension) was not significantly different between the TA and SA group.

In this study, there was significant fMRI differences between the TA and SA groups, which may reflect the interaction between the acupuncture evoked sensation and the noxious sensation evoked by rectal distension at central nervous system. This finding leads to our postulation that acupuncture might have the potential modulating effect of central pain processing in IBS by 2 actions: (1) modulation of 5-HT pathway at insula and (2) modulation of mood and affection in higher cortical center via ascending pathway at the pulvinar and medial nucleus of the thalamus. Both (1) and (2) might then contribute to descending inhibitory control pathways (5-HT-mediated) from the periaqueductal grey matter through the raphe nucleus magnus to the spinal cord.^{36,37} The raphe nucleus magnus is thought to be the main supraspinal center regulating the negative feedback mechanism for modulating pain³⁸ and EA-mediated inhibitory effect is attenuated by lesion at raphe nucleus magnus.³⁹ The above has therefore been suggested as one of the important pathways for acupuncture

analgesia.^{40,41} Our observation also echoes with an earlier animal study which suggested that EA attenuated visceral hyperalgesia through down-regulation of central serotonergic activities in the brain-gut axis.⁴²

Like other placebo controlled drug trials⁴³ in treatment of IBS, SA also has placebo effects^{22,23} on the central processing pathway of visceral pain. Similar activation from TA and SA can be seen in ACC, temporal regions and prefrontal cortex. However, in TA, all involved brain regions had persistently greater activation in session 2 when there was real-time electrical stimulation, while there was inconsistency in activation pattern between the sessions 2 and 3 in SA. Since both temporal and prefrontal cortices are associated with emotional control, the observed brain activation in the above regions is highly dependent on patient's profile, such as coping capacity.²³ Another possible explanation is that, since subjects in SA anticipated treatment during session 2, they might have responded with higher activation in secondary associative sensory processing regions at prefrontal and temporal regions, similar to the observation of the previous studies in which IBS patients had higher brain activation in the above regions to luminal/anticipatory rectal stimulation.⁴⁴

A major limitation of this study is the single session of EA, which might not reflect the real life practice of acupuncture, which comprises of repeated treatment sessions. This might also explain why there was no significant difference in pain rating by our subjects. However, the lack of subjective pain relief in the presence of significant changes in brain activation may be accounted by several explanations: Firstly, acupuncture may act primarily on the affective response to pain rather than direct anti-hyperalgesic effect. Secondly, the changes in brain activation induced by acupuncture may be more relevant to chronic pain rather than acute pain. Thirdly, a single session of acupuncture may not be sufficient to accomplish clinical analgesic effect despite changes in brain activation. Fourthly, the sample size of our study may not be sufficient to detect clinical difference in pain reduction. Whether repeated treatment sessions can transform these subliminal effects to clinical anti-hyperalgesia requires further elucidation in future studies.

Persistent greater activation from baseline during session 3 suggested that the effect of EA probably lasted beyond the actual stimulation although the activation was much weaker than during the electrical stimulation. However, how long would it last and whether the effect could be extended with longer or multiple stimulations remained unanswered and further investigation is required for better understanding.

In conclusion, our preliminary result supports our hypothesis that acupuncture can alter the activation of neural pathway for pain perception in IBS. We postulated that the potential effect of acupuncture on pain modulation involves (1) modulation of 5-HT pathway at insula and (2) modulation of mood and affection in higher cortical center via ascending pathway at the pulvinar and medial nucleus of the thalamus. Further study is required to study the full effect of acupuncture on IBS after a complete therapeutic course of therapy has been given to the patients.

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Appendix

Sequences for Magnetic Resonance Imaging Scanning

A whole brain anatomical data set was acquired with T1-weighted sequence (repetition time (TR)/echo time (TE): 7.6 ms/3.5 ms, field of view 230 mm, 250 contiguous slices, 0.6 mm thickness, reconstruction matrix 224×224) for functional to anatomical image co-registration purposes. The functional scans were then performed using PRESTO (principles of echo shifting with a train of observation) sequence (TR 28 ms, TE (shifted) 40 ms, flip angle 7° , slab thickness 125 mm, field of view $230 \text{ mm} \times 184 \text{ mm}$, acquisition matrix 80×51) for acquisition of 120 dynamic scans in each period with a nominal in-plane resolution of $2.8 \text{ mm} \times 3.6 \text{ mm}$ and a temporal resolution of 2.7 sec per dynamic scan. A block paradigm (composed of alternating rectal tube inflation and deflation) was used. Each paradigm consisted of 3 periods. As there were 3 intermediate time points for balloon inflation and deflation respectively, the stimulus train was not in an ideal boxcar form. In order to restore the boxcar stimulus, the intermediate time points in the paradigm and the functional scans were removed.

Functional Magnetic Resonance Imaging Analysis

Firstly, all fMRI images were realigned with the first scan, and then co-registered with the anatomical MRI of each patient. Subsequently, to enable group-based analysis, the spatially aligned MRI and fMRI data of the same subject was reoriented into the standardized Talairach space by registering with the Montreal Neurological Institute (MNI) brain template. The spatially nor-

malized volumes were re-sliced and resulted in a voxel size of $2 \text{ mm}^3 \times 2 \text{ mm}^3 \times 2 \text{ mm}^3$. The Gaussian smoothing with a kernel of 8 mm was applied to increase the signal to noise ratio. To comprehensively search for significant brain activation under different conditions, the following voxel-wise whole-brain analyses were performed.

First-level analysis

This analysis was based on brain activation detection of each single subject. Brain voxels activated by the box-car stimulus were detected voxel-wisely based on the standard general linear model. The statistical z-value for each voxel estimated from general linear model was produced. The statistical z map was constructed at the threshold of $P < 0.001$ (uncorrected). Clusters containing more than 10 voxels were considered as valid.

Second-level analysis

This analysis was conducted to calculate the group-based statistical maps using the results produced in the first-level analysis. Firstly, brain activations in subjects of the 2 groups, TA and SA, were compared for the four sessions independently. Secondly, to specifically assess the change in brain activation of IBS during EA, the inter-session comparison was performed by comparing the first-level analysis results from session 1 and session 2, independently in TA and SA groups. Thirdly, to assess the post EA effect on IBS, we compared the results between session 1 and session 3. For all the resulted statistical maps, the threshold of P-value was set at 0.001 (uncorrected) and spatial threshold was set at 10 voxels per cluster.

Third-level analysis

To further explicitly locate the significant difference in brain activation between TA and SA, we compared the inter-session difference of TA and SA between session 1 and 2 and between session 1 and 3, which resulted in the second-level analysis.