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Cerebral responses to puncturing at different acupoints for treating meal-related functional dyspepsia

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Key Messages

- This study aims to investigate the similarities and differences in cerebral responses to puncturing at different acupoints for treating meal-related functional dyspepsia (FD) so as to explore the acupoint specificity of acupuncture treating for FD.
- 20 FD patients were enrolled and randomized divided into two groups. PET-CT scans were performed on the FD patients at baseline and after receiving different acupuncture treatment.
- Different acupoints have similar clinical efficacy but relatively different cerebral responses.

Abstract

Background *To investigate the similarities and differences in cerebral responses to puncturing at different acupoints for treating meal-related functional dyspepsia (FD).* ***Methods*** *Twenty right-handed FD patients were enrolled and randomized divided into two groups. Each patient received 20 sessions' electroacupuncture treatment. The acupoints used in Group A were four acupoints on the Stomach Meridian, and the acupoints used in Group B were four acupoints on the Gallbladder Meridian. PET-CT scans were performed before and after acupuncture treatment to record the changes of cerebral glycometabolism.* ***Key Results*** *After treatment, the dyspepsia symptoms and the quality of life (QOL) of the patients in each group were significantly improved ($p < 0.05$) and there was insignificant difference in efficacy between the two groups ($p > 0.05$). In Group A, deactivation in brainstem, bilateral anterior cingulate cortex (ACC) and cerebellum, left superior medial frontal gyrus, orbital frontal cortex (OFC), and thalamus, etc., and activation in bilateral middle cingulate cortex (MCC), precuneus and lingual gyrus, etc. were observed. In Group B, deactivation in brainstem, bilateral thalamus, putamen, ACC, postterior cingulate cortex, hippocampus and cerebellum, etc., and activation in bilateral MCC, precuneus, left OFC, etc. were observed ($p < 0.05$, Family-wise error corrected).* ***Conclusions & Inferences*** *Different acupoints have similar clinical efficacy but relatively different cerebral responses. The influence on the sensory transduction regions (brainstem and thalamus) and visceral modulation regions might be the common mechanism of different acupoints treating for FD, and the modulation on some emotion/cognition-related areas (e.g., prefrontal cortex) is the potential difference between the different acupoints.*

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INTRODUCTION

Functional dyspepsia (FD) is one of the important functional gastrointestinal disorders (FGID). According to the Rome III consensus, FD has been defined as dyspepsia symptoms originating from the gastroduodenal region in the absence of any organic, systemic, or metabolic disease that readily explains the symptoms, and further subdivided into two diagnostic categories: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS).[1] Among these two subgroups, PDS has higher prevalence than EPS. It is marked by the non-painful symptoms including abdominal fullness, early satiety and is also referred to as meal-related FD.[2] Functional dyspepsia is currently considered to be a biopsychosocial disorder with disturbances of gastroduodenal motor function, heightened visceral sensitivity and a central nervous system disturbance.[3] Psychological disturbances had been proposed as one of the possible risk factors of FD.[4] It was reported that FD patients had significantly higher levels of psychiatric illness than the healthy subjects (HS)[5] and the patients with organic dyspepsia,[6] and that both anxiety and depression were associated with symptoms of FD,[7,8] Although FD is not a life-threatening disease, its treatment remains a major challenge[9] for its high prevalence,[10] unclear pathology[11], and significant impact on the quality of life (QOL).[12] Thus, the search for an effective alternative therapy attracts both patients and practitioners.

Acupuncture, as a natural therapy originated from China, has been proved to be effective for relieving gastric symptoms such as belch, abdominal distension, stomachache, and promoting appetite with an abundance of clinic and experimental data.[13–16] Our previous randomized clinical trial (RCT) [17] also demonstrated that puncturing at real acupoints significantly improved the dyspepsia symptoms and the QOL of FD patients, and was superior to puncturing at sham acupoints and taking itopride orally. Furthermore, we found that acupuncturing at different acupoints (the acupoints on the Stomach Meridian, the acupoints on the Gallbladder Meridian, and the alarm and transport acupoints) has relative different efficacy. The results suggested that the benefit of acupuncture for treating FD might associate with acupoint specificity.

Acupoint specificity is one of the key issues in acupuncture researches. In the light of the theory of traditional Chinese acupuncture, the acupoint specificity has at least two aspects: the effect of acupoint differs from that of sham acupoint, and different acupoints have different effects. With the development of the neuroimaging techniques, using functional brain imaging, such as positron emission tomography (PET) and functional magnetic resonance imaging to explore acupoint specificity has been an active area of research recently.[18] However, the majority of these studies focused on the comparison between real acupoint and sham acupoint. Little work has been addressed on the comparison between different acupoints.

To explore whether the cerebral response to acupuncture treating for FD has the acupoint specificity, we have carried out two PET studies in the last 5 years. First, we investigate the influence of puncturing at real acupoints on cerebral glycometabolism of FD patients. We found that even short-term manual acupuncture (once daily for 5 days) could affect the cerebral activities of FD patients.[19] Second, we compared the differences in cerebral responses between puncturing at real acupoints and puncturing at sham acupoints. We found that real acupoints and sham acupoints elicited relatively different brain responses, and that real acupoints had more remarkable modulation on the homeostatic afferent network.[20]

In this current study, we attempt to investigate the cerebral activity changes evoked by different acupoints (the acupoints on the Stomach Meridian *vs* the acupoints on the Gallbladder Meridian) on FD patients by Fluorine-18 Fluorodeoxyglucose (18F-FDG) PET-CT, and to evaluate the influence of puncturing at different acupoints on the symptom, QOL and emotional state using the Nepean Dyspepsia Index (NDI), the Zung Self-Rating Anxiety Scale (SAS) and the Zung Self-Rating Depression Scale (SDS), respectively. We hold that the different acupoints have both similarities and differences in modulating cerebral activity of FD patients, and hypothesized that (i) as a non-specific, mechanical stimulation and an effective therapy for FGIDs, acupuncture manipulation at different acupoints might activate some brain regions which participate in sensory transduction and visceral modulation. The regions such as brain stem and thalamus might be the common regions responding to different acupoints; and (ii) affective factors are considered as one of the possible risk of FD, and the cognitive and affective regions are the common regions involved in the modulation of acupuncture on FGIDs. So some regions which associate with emotion and cognition process including regions in prefrontal cortex and/or cingulate cortex might show different responses to different acupoints.

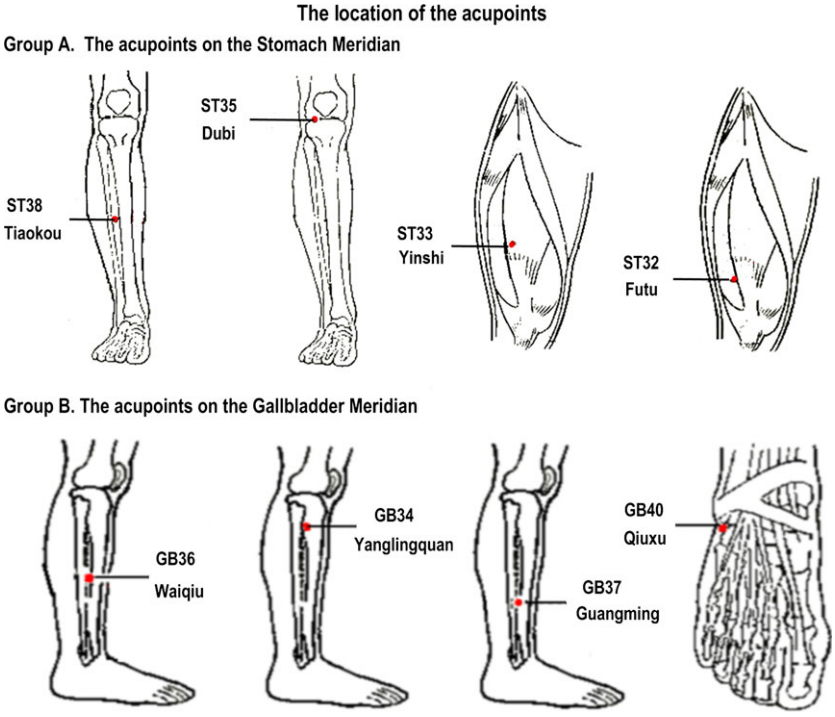

Figure 1 The location of the acupoints. The acupoints used in Group A include ST32 (Futu), ST33 (Yinbai), ST35 (Dubi), and ST38 (Tiaokou). The acupoints used in Group B include GB34 (Yanglingquan), GB36 (Waiqiu), GB37 (Guangming), and GB40 (Qiuxu).

MATERIALS AND METHODS

Participants

The sample size in each group was estimated based on the PET-CT data analysis requirement and reference of previous study. Twenty FD patients were enrolled in this study and separately signed written informed consent forms. These patients were recruited at the outpatient department in the Third Teaching Hospital of Chengdu University of Traditional Chinese Medicine.

After evaluation by 2 gastroenterologists and a psychologist and undergoing careful laboratory examinations, participants were enrolled if they fulfilled all of the following inclusion criteria: (i) were right-handed and aged 20 to 30, (ii) matched the Rome III diagnosis criteria on FD and the diagnosis criteria on PDS, (iii) were acupuncture-naive. Participants were excluded if they (i) were pregnant or lactating, (ii) had a history of psychiatric and neurological disorders, or head trauma with loss of consciousness, (iii) were currently in use of gastrointestinal dynamic promoting drugs, (iv) were suffering from serious cardiovascular, respiratory or renal illnesses, (v) participated currently in other clinical trials, and (vi) had any contraindications to acupuncture (e.g., anticoagulation therapy).

After a 2-weeks baseline period, the included patients were randomly assigned to either Group A or Group B using a computer generated randomization sequence. The sequence was concealed from the care providers through the use of sequentially numbered, opaque, sealed envelopes. Patients were blinded to group assignment.

This study was performed according to the principles of the Declaration of Helsinki (Version Edinburgh 2000). The study protocol was approved by the Ethics Committee of the Teaching Hospital of Chengdu University of Traditional Chinese Medicine. The number of our trial registration is: ChiCTR-TRC-11003028 (http://www.chictr.org/cn/).

Acupuncture interventions

The treatment of each group consisted of 20 sessions of 30 min duration, each administered over a period of 4 weeks (five sessions per week). The acupoints used in the study included: Group A: four acupoints on the Stomach Meridian including ST32 (Futu), ST33 (Yinshi), ST35 (Dubi), and ST38 (Tiaokou); Group B: four acupoints on the Gallbladder Meridian including GB34 (Yanglingquan), GB36 (Waiqiu), GB37 (Guangming), and GB40 (Qiuxu) (Fig. 1). According to the acupuncture theory and clinical application, all of these acupoints can be used to treat gastrointestinal disorders. To avoid excessive stimulus, all acupoints were punctured unilaterally and alternated between left side and right side. It meant that in the first time, acupuncture stimulation was performed at the points on the left side, and in the second time, acupuncture stimulation was performed at the points on the right side. After the overlying skin was cleaned with tincture iodine and alcohol, the sterile acupuncture needles (0.25 mm in diameter, 25 or 40 mm in length, Hwatuo, Suzhou, China) were inserted perpendicularly into the points for 15–25 mm. and gently twisted, lifted, and thrusted in an even amplitude, force and speed for 4–6 times till a *deqi* response (including soreness, numbness, distention, and heaviness) was obtained. Furthermore, an auxiliary needle (13 mm in length and 0.18 mm in diameter) was inserted into the points which were lateral (proximal limbs or trunk) to each acupoint at a depth of 2 mm without manual stimulation. Each acupuncture needle and its auxiliary needle connected to the electrical lead of the HAN's acupoint nerve stimulator (model LH 200A TENS; HANS, Nanjing, China) for 30 min with a stimulation frequency of 2/100 Hz and stimulation intensity varied from 0.1 to 1.0 mA. The stimulation intensity was controlled by the patient's tolerance degree.

During treatment, patients were asked not to take antacids (such as PPIs and H2 blockers), prokinetics, antidepressants,

anxiolytics, or TCM herbs which can relieve FD symptoms. If the symptoms were intolerable, patients could use Domperidone Tablets temporarily with the dosage documented.

Outcome measurement

The NDI,[21] including symptom index and QOL index, was used to assess the clinical effects at the baseline and at the end of 4-weeks treatment. The Nepean Dyspepsia Symptom Index (NDSI) is based on 15 dyspepsia-related physical signs rated for frequency (0–4), intensity (0–5), and bothersomeness (0–4). The number 0 represented no symptoms, and higher numbers paralleled worsening of the symptoms. The Nepean Dyspepsia QOL Index (NDQLI) includes four domains, namely interference (13 items), know/control (7 items), eat/drink (3 items), and sleep/disturb (2 items). Higher scores indicate better QOL. The translated version of NDI was found, by our prior research, to be reliable, and valid for measuring symptom severity and QOL in Chinese patients with FD.[22]

As the emotional state was closely related to the symptoms of FD, the Zung SAS[23], and the Zung SDS[24] were used in this study to quantify the anxiety/depression-related symptoms of the participants at the baseline and the end of 4-weeks treatment.

PET-CT Scan

All patients received a PET-CT scan at the end of baseline and the end of 4-weeks treatment. After an overnight fast, all patients went through the following sequential procedure: (i) examinations of blood pressure and blood sugar, (ii) a 20-minute rest in a darkroom, (iii) a tracer injection (18F-FDG, synthesized with Mini Tracer accelerator. 0.11 mci/kg dosage) at the back of the right hand, (iv) a 40-minute rest, and (v) a PET-CT scan. Participants were instructed to remain relaxed during the whole study with eyes blindfolded and ears plugged.

PET-CT images covered the whole brain and were paralleled to the AC-PC line. Image acquisition was started after a 40 min uptake period (bed: 1; collection mode: 3D; slice thickness: 3 mm; slice interval: 1.5 mm; matrix size: 256 × 256; total counts: 3×10^9). Upon the completion of data acquisition, the images were reconstructed using ordered subset expectation maximization with six iterations and 16 subsets.

Statistical analysis

Clinical data All the physiological and psychological measures were analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). All the numerical variables in this article are presented as mean (SD). Independent-samples *t*-test (for between-group analysis) and paired-samples *t*-test (for within-group analysis) were used on numerical variables. Chi-squared test was used on categorical variables and two-sided test was applied on all available data. A *p*-value <0.05 was considered statistically significant.

PET-CT data The PET-CT data were processed with Statistical parametrical maps 5.0 (SPM5.0). All PET images from each subject were subjected to coregistration onto their corresponding CT images to improve the accuracy of the spatial normalization, then spatially normalized to the standard SPM-PET template and resliced to 2-mm isotropic resolution in Montreal Neurological Institute space. The normalized data set was then spatially smoothed with a 6 mm full width at half maximum Gaussian kernel. A two sample *t*-test was performed to examine the differences in cerebral glycometabolism between the two groups at the baseline. A paired *t*-test which was defined as FD patients after treatment minus FD patients at baseline was performed to detect the cerebral activity changes in each group after treatment. For visualization, all the results were transformed into the Talairach stereotactic space and overlaid on MRIcro (available: http://www.sph.s.c.edu/comd/rorden/mricro.html) for presentation.

RESULTS

The data of one FD patient were excluded from our analysis due to his relatively significant head motions during the scan. During the acupuncture treatment, none of the FD patients had taken any gastrointestinal dynamic promoting medicine including Domperidone Tablet.

The baseline comparisons between the two groups

There were no significant differences in the demographic data including age, sex, weight, and height, and

Table 1 Baseline demographic and clinical characteristics of FD patients in two groups

Characteristic	Group A	Group B	Statistic value	*p*-value
Demographic data				
No. of patients (*n*)	9	10	0.090	0.764
No. of women, *n* (%)	6 (66.700%)	6 (60.000%)		
Age (years), mean ± SD	22.222 ± 2.333	21.800 ± 0.632	0.552	0.588
Height (cm), mean ± SD	164.778 ± 7.293	164.000 ± 7.438	0.230	0.821
Weight (kg), mean ± SD	56.000 ± 8.170	52.800 ± 3.966	1.105	0.285
Status of disease				
Duration of disease (month), mean ± SD	25.000 ± 10.966	25.400 ± 13.656	−0.070	0.945
NDSI score, mean ± SD	44.444 ± 14.883	46.000 ± 19.465	−0.194	0.849
NDQLI score, mean ± SD	76.479 ± 8.295	75.446 ± 17.523	0.159	0.875
Emotional status				
SAS score, mean ± SD	41.806 ± 9.825	40.875 ± 9.466	0.210	0.836
SDS score, mean ± SD	42.778 ± 7.571	40.375 ± 9.072	0.623	0.542

SD, Standard deviation; NDSI, Nepean Dyspepsia Symptom Index; NDQLI, Nepean Dyspepsia QOL Index; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.

Table 2 Clinical outcome measurements in each group before and after treatment

Items	Group A	Group B
NDSI scores		
Baseline, mean ± SD	44.444 ± 14.883	46.000 ± 19.465
End of treatment, mean ± SD	18.111 ± 8.810	16.800 ± 12.891
Statistic value	3.795	5.627
p-value	0.005	0.000
NDQLI scores		
Baseline, mean ± SD	76.469 ± 8.295	75.446 ± 17.523
End of treatment, mean ± SD	91.513 ± 6.650	89.568 ± 11.590
Statistic value	−6.520	−.3.315
p-value	0.000	0.009
SAS scores		
Baseline, mean ± SD	41.806 ± 9.825	40.875 ± 9.466
End of treatment, mean ± SD	32.917 ± 7.421	33.255 ± 6.458
Statistic value	4.215	3.327
p-value	0.003	0.009
SDS scores		
Baseline, mean ± SD	42.778 ± 7.572	40.375 ± 9.072
End of treatment, mean ± SD	34.028 ± 7.256	34.565 ± 7.605
Statistic value	5.209	1.740
p-value	0.001	0.116

SD, Standard deviation; NDSI, Nepean Dyspepsia Symptom Index; NDQLI, Nepean Dyspepsia QOL Index; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.

the status of disease including the duration of disease, NDSI scores and NDQLI scores, and the emotional status including the SAS scores and SDS scores between the two groups ($p > 0.05$; Table 1).

There were insignificant differences in the cerebral glycometabolism between the two groups ($p < 0.05$, family-wise error corrected with a minimal cluster size of 50 voxels).

The curative effects of FD patients in the two groups after treatment

The within-group analyses showed that, compare to those at the baseline, the NDSI scores, and the SAS scores in the two groups were significantly decreased ($p < 0.05$) and the NDQLI scores in the two groups were significantly increased at the end of treatment ($p < 0.05$). The significantly decreased of SDS scores were observed in Group A ($p < 0.05$), not in Group B ($p > 0.05$; Table 2).

The between-group analyses showed that, at the end of treatment, the change in the NDSI scores (baseline minus end of treatment), the change in the NDQLI scores (baseline minus end of treatment), the change in the SAS scores (baseline minus end of treatment), and the change in the SDS scores (baseline minus end of treatment) between the two groups show insignificantly difference ($p > 0.05$; Table 3).

The cerebral glycometabolism changes of FD patients in the two groups after treatment

After treatment, the FD patients in Group A demonstrated a decreased cerebral glycometabolism in brainstem, bilateral anterior cingulate cortex (ACC; BA25) and cerebellum, left superior medial frontal gyrus (BA9, BA10), orbital frontal cortex (OFC, BA11), thalamus, and superior temporal frontal gyrus (BA38); and an increased cerebral glycometabolism in bilateral middle cingulate cortex (MCC), precuneus (BA7) and lingual gyrus (BA18, BA17), left inferior frontal gyrus (BA48) and right putamen ($p < 0.05$, family-wise error corrected with a minimal cluster size of 50 voxels; Table 4, Fig. 2A).

The FD patients in Group B demonstrated a significantly decreased cerebral glycometabolism in brainstem, bilateral thalamus, putamen, ACC (BA24), posterior cingulate cortex (PCC, BA29, BA26), hippocampus (BA20, BA37), cerebellum, and superior temporal frontal gyrus (BA48, BA47); and a significantly increased cerebral glycometabolism in bilateral MCC

Table 3 Comparison on the therapeutic effects between Group A and Group B

Items	Group A	Group B	Statistic value	*p*-value
No. of patients	9	10	0.090	0.764
NDSI score				
End of treatment, mean ± SD	18.111 ± 8.810	16.800 ± 12.891	0.256	0.801
End of treatment—baseline, mean ± SD	−26.333 ± 20.815	−29.200 ± 16.410	0.335	0.742
NDQLI score				
End of treatment, mean ± SD	91.513 ± 6.651	89.568 ± 11.590	0.669	0.513
End of treatment—baseline, mean ± SD	15.044 ± 6.922	13.122 ± 12.516	0.407	0.689
SAS score				
End of treatment, mean ± SD	32.917 ± 7.421	33.255 ± 6.458	−0.106	0.917
End of treatment—baseline, mean ± SD	−6.667 ± 8.883	−7.620 ± 7.242	0.258	0.800
SDS score				
End of treatment, mean ± SD	34.028 ± 7.256	34.565 ± 7.605	−0.157	0.877
End of treatment—baseline, mean ± SD	−8.750 ± 5.039	−5.810 ± 10.558	−0.760	0.458

SD, Standard deviation; NDSI, Nepean Dyspepsia Symptom Index; NDQLI, Nepean Dyspepsia QOL Index; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.

Table 4 The cerebral glycometabolism changes in FD patients after acupuncture treatment (End of treatment minus baseline)

		Group A							Group B						
		Talairach							Talairach						
Region	Side	X	Y	Z	*t*-value	BA	Sign	Cluster size	X	Y	Z	*t*-value	BA	Sign	Cluster size
Inferior Frontal Gyrus	L	46	26	21	6.758	BA48	↑	64	/	/	/	/	/	/	/
Superior Medial	L	−1	45	49	−10.88	BA9	↓	65	−4	50	35	6.758	BA9	↑	50
Frontal Gyrus	L	−1	54	33	−7.148	BA10	↓	56	−1	62	11	6.172	BA10	↑	53
OFC	L	−1	67	5	−7.688	BA11	↓	58	−2	49	−7	5.586	BA11	↑	62
Brainstem		9	−17	−39	−5.391	/	↓	59	3	−19	−27	−10.47	/	↓	78
Thalamus	L	−10	−17	19	−6.367	/	↓	57	−11	−9	−1	−7.344	/	↓	94
	R	/	/	/	/	/	/	/	9	−7	0	−10.66	/	↓	51
Putamen	L	/	/	/	/	/	/	/	−29	4	−5	−6.758	/	↓	61
	R	34	−3	−1	6.367	/	↑	53	30	−2	−4	−5.586	/	↓	86
Cerebellum	L	−42	−72	−37	−7.734	/	↓	421	−3	−47	1	−8.906	/	↓	271
	R	45	−62	−37	−6.172	/	↓	598	13	−35	−13	−5.586	/	↓	182
ACC	L	−2	36	3	−9.188	BA25	↓	60	−5	23	23	−17.11	BA24	↓	58
	R	2	32	2	−6.172	BA25	↓	62	3	36	14	−6.578	BA24	↓	75
MCC	L	−2	−40	−47	9.562	/	↑	51	−3	18	38	13.2	BA23	↑	60
	R	4	−35	48	10.47	/	↑	67	1	−17	37	14.18	BA23	↑	57
PCC	L	/	/	/	/	/	/	/	−4	−42	16	−8.906	BA29	↓	59
	R	/	/	/	/	/	/	/	1	39	21	−7.148	BA26	↓	55
Precuneus	L	0	59	53	11.81	BA7	↑	53	−2	−59	56	9.297	BA7	↑	61
	R	2	−58	52	8.125	BA7	↑	143	3	−60	54	6.953	BA7	↑	55
Hippocampus	L	/	/	/	/	/	/	/	−34	−32	−3	−5.781	BA37	↓	51
	R	/	/	/	/	/	/	/	33	−29	−3	−7.344	BA20	↓	59
Superior temporal Gyrus	L	−54	5	−6	−10.08	BA38	↓	57	−51	−32	12	−9.688	BA48	↓	69
	R	/	/	/	/	/	/	/	51	−20	−4	−5.586	BA47	↓	70
Lingual Gyrus	L	16	−59	−1	6.953	BA18	↑	103	/	/	/	/	/	/	/
	R	−2	−73	−1	6.012	BA17	↑	132	/	/	/	/	/	/	/

'Sign' indicates whether the structure showed a signal increase or decrease. (↑/↓) increase/decrease. $p < 0.05$, family-wise error corrected with a minimal cluster size of 50 voxels. R, right; L, left; BA, Brodmann area; OFC, Orbital Frontal Cortex; ACC, Anterior Cingulate Cortex; MCC, Middle Cingulate Cortex; PCC, Posterior Cingulate Cortex.

(BA23) and precuneus (BA7), left superior medial frontal gyrus (BA9, BA10) and OFC (BA11) ($p < 0.05$, family-wise error corrected with a minimal cluster size of 50 voxels; Table 4, Fig. 2B).

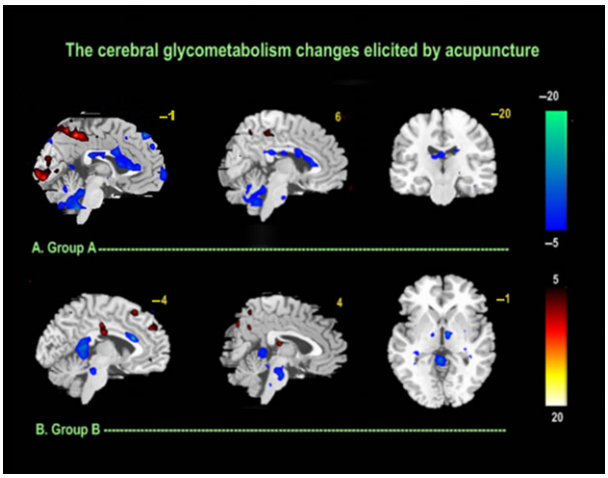

Figure 2 The cerebral glycometabolism changes elicited by acupuncture. $p < 0.05$, family-wise error corrected with a minimal cluster size of 50 voxels.

DISCUSSION

Here, we explored the cerebral glycometabolism changes in meal-related FD patients evoked by puncturing at different acupoints. The results indicated that both the acupoints on the Stomach Meridian and the acupoints on the Gallbladder Meridian can elicit extensive cerebral glycometabolism changes. The different acupoints had similarities and differences in cerebral activity change and symptom improvement.

Clinical effects

In this study, puncturing at the acupoints on the Stomach Meridian and puncturing at the acupoints on the Gallbladder Meridian decreased the NDSI scores and the SAS scores, and increased the NDQLI scores. The results were consistent with acupuncture theory and our previous study.[17] According to the acupuncture theory, both the Stomach meridian and the Gallbladder meridian connect with stomach, so the acupoints on these two meridian can be used to treat gastrointestinal disorders. Our previous multiple centers RCT[17] indicated that both the acupoints on the

Stomach Meridian and the acupoints on the Gallbladder Meridian could significantly relieve the symptoms and improve the QOL of FD patients, and their effects were superior to that of the sham acupoints. So we hold that these two acupuncture treatments were effective for regulating gastrointestinal function of FD patients.

Cerebral activity changes

Even through significant changes in cerebral activity were observed in multiple regions in both groups after acupuncture treatment, we limit our discussion to the regions implicated in our two main priori hypotheses and compare them to some published results.

Some brain regions which participate in sensory transduction and visceral modulation might be the common regions responding to different acupoints stimulation This hypothesis was based on the reasons (i) acupuncture manipulation is performed by inserting a thin needle into the skin and the underlying muscle layer, then rotating and twisting the needle until a *deqi* sensation (including soreness, numbness, distention, and heaviness) is obtained. This unspecific procedure is bound to stimulate the somatic afferent nerves of the skin and muscles, and influence the activities of some regions in sensory transduction pathway. Sato, *et al.*[25] found that gastric motility was often excited when the acupoints on the limbs were stimulated, and the afferent nerve pathway of the excitatory gastric response is composed of hindpaw cutaneous and muscle afferent nerves, the efferent nerve pathway is the gastric vagal efferent nerve, and its reflex center requires the presence of the brain. In this study, all these acupoints locate in the lower limb, and they should have similar efferent and afferent nerve pathways, and have common effect on some brain regions; (ii) this study and our previous RCT[17] demonstrated that both puncturing at the acupoints on the Stomach Meridian and puncturing at the acupoints on the Gallbladder Meridian were effective for FD patients. For the central integration is the key to acupuncture efficacy, the results suggested that these two acupuncture treatments have similar central mechanism. For example, they might influence the activities of some brain regions involved in visceral modulation. In this study, the common regions responding to the different acupoints stimulation included brainstem, thalamus, ACC, cerebellum, MCC, superior temporal frontal gyrus, and precuneus.

The role of brainstem and thalamus in sensory transduction—Brainstem is the pathway for all fiber tracts passing up and down from peripheral nerves and spinal cord to the highest parts of the brain. Thalamus may be thought of as a kind of switchboard of information. Its function includes relaying sensation, special sense and motor signals to the cerebral cortex, etc. Thus, we hold that brainstem and thalamus play important roles in gastrointestinal sensory process and the acupuncture signal transduction. On the one hand, the dysfunction of brainstem and thalamus of FD patients had been reported.[26,27] For example, our previous PET-CT studies[26]on FD patients demonstrated that, compared to the HS, the FD patients showed a significantly increased glycometabolism in brainstem and thalamus, and the signal increase in thalamus, showed a very significant correlation with symptom severity. On the other hand, the somatic sensory information produced by acupuncture stimulation from the body is projected to the brainstem and thalamus. Our previous PET-CT study showed that both puncturing at real acupoints and puncturing at sham acupoints all decreased the hyperactivities of brainstem, thalamus.[20]Other neuroimaging studies on healthy adults also demonstrate that acupuncture stimulation at either real or sham acupoints leads to activity changes within thalamus,[28] and puncturing at different acupoints (PC6, PC7, and GB37) elicits activity changes in the brainstem.[29]

The role of ACC and cerebellum in central pathology of FD—Anterior cingulate cortex, considered as a key region of 'gut–brain communication',[30] has a close interconnection with the insula, prefrontal, limbic, and other subcortical structures, and plays an important role in processing pain and gastrointestinal sensory signals. Activation in ACC can be found in nearly all reported FGIDs studies, regardless of study paradigms and analysis methods.[31] Cerebellum, with its complicated afferent and efferent connections with the cerebrum and adjacent midbrain regions, provides a crucial role in regulating viscera activities, high-order cognitive functions, and affective behaviors.[32] Although this study did not investigate the differences in cerebral activity between FD patients and HS, multiple neuroimaging studies[26,27,33,34] indicated that FD patients had functional and structural abnormalities in ACC and cerebellum. For example, By $H_2$15O-PET, Van Oudenhove, *et al.*[27] demonstrated that activity in ACC, and cerebellum of FD patients differed from those of HS. Our previous 18F-FDG PET-CT study[26] demonstrated that the glycometabolism of ACC, MCC and cerebellum in FD patients were significantly increased, and the signal increase in ACC, and cerebellum showed a very significant

correlation with symptoms. Furthermore, our structural MRI study[33] showed that, compared to HS, FD patients showed a decreased gray matter density (GMD) in the ACC and cerebellum, and our diffusion tensor imaging study[34] found that the FD patients showed microstructural damage in multiple white matter tracts which connected with cingulate cortex. In this study, after different acupuncture treatments, the cerebral glycometablism in ACC and cerebellum were significantly decreased. Although the different acupoints appeared to alter different parts of the ACC and cerebellum, the majority of the altered parts in ACC and cerebellum participate in regulating viscera activities. So the results suggest that the activity changes in ACC and cerebellum were a common mechanism of these two acupuncture treatments for FD.

It is worth mentioning that, as an important integration cortex, ACC not only involves in the process of gastrointestinal sensory signals but also participates in the emotion modulation.[35] For example, Drevets, *et al.*[36] found decrease in glucose metabolism of the subgenual ACC was related to depressive mood. In this study, the decreased cerebral glucose metabolism in the ACC was found in both groups after acupuncture therapy. It remained to be determined whether the activity decrease in ACC was mainly relevant to emotional symptoms improvement (depression and anxiety), because using 18F-FDG PET-CT, we did not found significant difference in glucose metabolism in ACC between the FD patients with depression and anxiety and the FD patients without depression and anxiety.[37]

In summary, we confirm our hypothesis that some brain regions which participate in sensory transduction and visceral modulation including brainstem, thalamus, ACC, and cerebellum are the common regions responding to different acupoints.

Some regions which associate with emotion and cognition process might show different responses to different acupoints stimulation

This hypothesis was based on the reasons that (i) emotional factors are considered as one of the possible risk of FD.[4] Studies have demonstrated that anxiety seems to be related abnormal antral retention of food,[7] and that depression is related to the abdominal fullness severity.[8] (ii) The central pathogenesis of FD involves in the functional and structural abnormalities in emotion and cognition-related brain regions. Recent neuroimaging studies demonstrated the influence of psychiatric factors on brain activities of FD patients. For example, some researchers found that, in FD patients, anxiety correlated negatively with cingulate cortex activity, and positively with dorsal pons activity,[27] and that abuse history was associated with differences in insula, prefrontal, and hippocampus/amygdala activity.[38] Our previous structural MRI study[33] found that many regions in emotional arousal circuitry of FD patients showed a significant reduction in GMD. However, after regressing SAS/SDS, the regional GMD changes in some emotion-related regions including the OFC and medial prefrontal cortex (MPFC) were not found. The result indicated that psychological factors might be one of the essential factors significantly affecting the regional brain structure of FD patients. (iii) The activity changes in the affective and cognitive related regions were commonly seen in acupuncture-neuroimaging studies,[18–20,28,29,39,40] while the results were heterogeneous (activated by acupuncture or deactivated by acupuncture) in terms of the study questions, methodology and quality.[41]

In this study, the within-group analysis showed that several prefrontal regions (MPFC and OFC, etc.), PCC, hippocampus, lingual gyrus, and putamen demonstrated different responses to different acupoints stimulation. Among these regions, left MPFC and OFC showed deactivations in Group A and activations in Group B, and PCC and hippocampus only shown deactivation in Group B after treatment. These regions mentioned above all involve in the emotion and cognition modulation. This result demonstrated that both of the acupoints on the Stomach Meridian and the acupoints on the Gallbladder Meridian elicited the activity changes in some affective and cognitive related areas, but the specific regions and the trend of functional change (increase or decrease) were relatively different. Furthermore, we also found that although the between-group analysis showed that the difference in the changes SDS scores between Group A and B were not significant, the within-group analysis demonstrated that the decreased SDS scores only found in Group A ($p < 0.05$), not in Group B ($p > 0.05$). We speculated that the differences in the neural activity pattern of the affective and cognitive related areas (the specific regions and the trend of functional change) might contribute to the tiny difference in depression improvement between the two groups.

Furthermore, the current results indicated that the different acupoints had relatively different modulating mechanism. This result was consisted with other neuroimaging studies focused on the comparison between different acupionts.[39–41] For example, Feng, *et al.*[40] using functional connectivity analysis found

that, compared to GB37, increased correlations for PC6 were primarily between the prefrontal regions and somatosensory regions, whereas decreased correlations were mainly related with the occipital regions. They hold that acupuncture at different acupoints may exert heterogeneous modulatory effects on the poststimulus resting brain. Our previous study[42] also found that acupuncture stimulation at different points on similar body regions in migraine patients induced different patterns of cerebral glucose metabolism. In this study, we hypothesized that the different response of these regions to different acupoints stimulation might relate to the acupoints specificity.

In summary, this study found that the different responses in some affection/cognition-related regions were the potential difference between the acupoints of Gallbladder meridian and the acupoints of Stomach Meridian treating for FD, and might associate with acupoint specificity. However, of particular note is that the neural specificity of acupoint do not all involve in the affection/cognition-related regions, and the neural specificity of acupoint has relative function-orientation and disease-orientation. In another words, in different conditions (disease *vs* health, or different pathological conditions), the neural specificity of acupoint might has different manifestations.

CONCLUSION

In conclusion, although both the acuppoints on Stomach Meridian and the acupoints on the Gallblaader Meridian improved the symptoms and QOL of FD patients, different acupoints elicited relatively different cerebral responses. The modulation on the sensory transduction regions and disease-related (visceral modulation) regions including brainstem, thalamus, cerebellum, and ACC might be the common mechanism of different acupoints for treating FD, and the influence on some emotion/cognition-related areas including PFC, PCC, and hippocampus, etc., is the potential difference between different acupoints stimulation.

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DISCLOSURE

The authors have no competing interests.

AUTHOR CONTRIBUTION

FL, XL, YL, YT, FZ, SY, and JT designed the study; FZ, WS, LL, ML, XL, and YT performed the study; WQ, JS, and XG analyzed the data; FZ, LL, YT drafted the manuscript; FL, YL, ML, XG, JT, XL, SY, and WS revised the manuscript.

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