The Salient Characteristics of the Central Effects of Acupuncture Needling: Limbic-Paralimbic-Neocortical Network Modulation

Jiliang Fang,[1,2*] Zhen Jin,[3] Yin Wang,[4] Ke Li,[3] Jian Kong,[2] Erika E. Nixon,[2] Yawei Zeng,[3] Yanshuang Ren,[1] Haibin Tong,[1] Yinghui Wang,[4] Ping Wang,[1] and Kathleen Kin-Sang Hui[2]

[1]Department of Radiology, Guang An Men Hospital, China Academy of Chinese Medical Sciences, Beijing, China
[2]Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts
[3]MRI Division, Department of Radiology, Beijing 306 Hospital, Beijing, China
[4]Department of Acupuncture, Guang An Men Hospital, China Academy of Chinese Medical Sciences, Beijing, China

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Abstract: Human and animal studies suggest that acupuncture produces many beneficial effects through the central nervous system. However, the neural substrates of acupuncture actions are not completely clear to date. fMRI studies at *Hegu* (LI4) and *Zusanl*i (ST36) indicated that the limbic system may play an important role for acupuncture effects. To test if this finding applies to other major classical acupoints, fMRI was performed on 10 healthy adults during manual acupuncture at *Taichong* (LV3), *Xingjia*n (LV2), *Neiting* (ST44), and a sham point on the dorsum of the left foot. Although certain differences could be observed between real and sham points, the hemodynamic response (BOLD signal changes) and psychophysical response (sensory experience) to acupuncture were generally similar for all four points. Acupuncture produced extensive deactivation of the limbic-paralimbic-neocortical system. Clusters of deactivated regions were seen in the medial prefrontal cortex (frontal pole, pregenual cingulate), the temporal lobe (amygdala, hippocampus, and parahippocampus) and the posterior medial cortex (precuneus, posterior cingulate). The sensorimotor cortices (somatosensory cortices, supplementary motor cortex), thalamus and occasional paralimbic structures such as the insula and anterior middle cingulate cortex showed activation. Our results provide additional evidence in support of previous reports that acupuncture modulates the limbic-paralimbic-neocortical network. We hypothesize that acupuncture may mediate its antipain, antianxiety, and other therapeutic effects via this intrinsic neural circuit that plays a central role in the affective and cognitive dimensions of pain as well as in the regulation and integration of emotion, memory processing, autonomic, endocrine, immunological, and sensorimotor functions. *Hum Brain Mapp 30:1196–1206, 2009.*   © 2008 **Wiley-Liss, Inc.**

Key words: acupuncture; fMRI; limbic-paralimbic-neocortical network; acupoint specificity; needling; *deqi*

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INTRODUCTION

Acupuncture is becoming one of the most widely used treatments in alternative medicine [NIH, 1998]. Clinical and experimental studies indicate that most of the anti-pain, antianxiety, and other modulatory effects of acupuncture are mediated via the central nervous system [Han, et al., 1982; Pomeranz, 1995; Zhu, 1997]. However, the pathways of acupuncture actions on the brain have not been well characterized to date. Recently, fMRI has been used successfully to monitor the dynamic response of the brain to acupuncture in human subjects [Cho et al., 1998, 2000, 2006b; Fang et al., 2004, 2006, 2007; Gareus et al., 2002; Hui et al., 1997a,b, 1998, 2000, 2005; Kong et al., 2002, 2007a,b; Li et al., 2003a,b; Liu et al., 2004; Napadow et al., 2005, 2007; Parrish et al., 2005; Sun et al., 2006; Wang et al., 2007; Wu et al., 1999, 2002; Yan et al., 2005; Yoo et al., 2004; Zhang et al., 2003, 2004]. These studies show that acupuncture stimulation produces extensive hemodynamic response within the subcortical and cortical regions. Many acupuncture fMRI studies have focused on acupoints that are most widely used in clinical practice, such as LI4, ST36, and GB34. The overall response pattern showed significant overlap between them in either manual or electrical acupuncture. Besides activation of the somatosensory and association cortices that is to be expected of somatosensory stimulation, widespread deactivation occurs in the limbic system [Fang et al., 2006; Hui et al., 2000, 2005; Kong et al., 2002; Napadow et al., 2005; Wang et al., 2007; Wu et al., 1999, 2002]. A major subset of these brain areas is closely associated with the pain matrix, such as: the cingulate cortex, amygdala, hippocampus, thalamus, insula, and periaqueductal gray. Other acupoints have also been investigated, including BL57, BL60, BL67, K3, LV3, GB40, SP6, SJ8, DU15, PC6, GB37 [Cho et al., 1998, 2000; Fang et al., 2004, 2007; Kong et al., 2007a,b; Li et al., 2003a,b; Parrish et al., 2005; Yan et al., 2005; Yoo et al., 2004; Zhang et al., 2004]. Many of the authors interpret the differences between acupoints or the differences between acupoints and sham points as acupoint specificity in support of the traditional Chinese medicine (TCM) theory. However, the evidence is as yet unconvincing. Considerable discrepancy exists in the literature. Cho et al. were the first to report that highly specific effects were associated with different acupoints, that acupuncture at "vision-related" *Kunlun* (BL60), *Zhiyin* (BL67) activated the visual cortex while acupuncture at "auditory-related" *Taixi* (K3) activated the auditory cortex [Cho et al., 1998, 2000; Li et al., 2003a; Parrish et al., 2005]. However, the results could not be replicated [Gareus et al., 2002]. It has been shown by multiple studies that acupuncture at many "nonvision" or "nonauditory" related acupoints also elicited BOLD signal response in the visual or auditory cortex [Fang et al., 2006, 2007; Hui et al., 2005; Kong et al., 2007a,b; Li et al., 2003b; Napadow et al., 2005, 2007; Sun et al., 2006; Wang et al., 2007; Wu et al., 2002; Yan et al., 2005; Yoo et al., 2004]. Cho has recently replaced his "acupoint-brain-organ" specific pathway theory with "the broad sense hypothalamus-pituitary-adrenal axis" hypothesis [Cho et al., 2006a,b]. On the other hand, Hui hypothesizes that acupuncture recruits intrinsic brain networks involved in the regulation and integration of multiple brain functions to mediate its effects, and that the limbic system may play a major role. Functional MRI studies of acupuncture at *Hegu* (LI4), *Zusanli* (ST36) and *Taichong* (LV3) have provided evidence in support of the hypothesis [Fang et al., 2007; Hui et al., 1997b, 1998, 2000, 2005; Sun et al., 2006]. The present study compares the fMRI response of the brain to acupuncture at multiple acupoints of different meridian origins on the same individuals in order to explore the effects that may be specific or common to the different acupoints. Acupuncture was performed at *Taichong* (LV3) and *Xingjian* (LV2) of the liver meridian, *Neiting* (ST44) of the stomach meridian and a sham point located between the III and IV metatarsals on the foot. These acupoints of meridian origin are commonly used for treating pain, hypertension, gastrointestinal, and other physiological dysfunctions [Chen, 1995; Cheng, 2000]. The results of the study provided additional evidence for the modulation of the limbic system by acupuncture.

MATERIALS AND METHODS

Subject Recruitment and Evaluation

Thirty healthy adult volunteers were recruited by advertisements approved by the Institutional Review Board at Guang An Men Hospital, China Academy of Chinese Medical Sciences. A qualified staff member in the study group explained the purpose of the investigation, its procedure, its potential discomforts and risks, and obtained a signed informed consent from the candidate prior to commencement of the study procedures. The subjects were enrolled and pretested for response to acupuncture analgesia with thermal stimulation on the first toe. Subjects whose pain threshold was elevated 10% or more by acupuncture were enrolled into the imaging test. The successful candidates included 5 male and 5 female, 22 to 28 years old, right handed and no history of mental and psychological disorders. All of them had experience of acupuncture before our study and had a positive view about acupuncture.

Acupuncture Protocol

Manual acupuncture was administered by the same licensed acupuncturist throughout the study. Three classical acupoints (LV3, LV2, and ST44) and a sham point between metatarsal III and IV on the dorsum of the left foot were tested in randomized order, using disposable sterile stainless steel needles, diameter 0.25 mm, length 30 mm (Huatuo, Suzhou Medical Appliance Company). Each experimental run lasted 6 min [Fig. 1]. The needle was inserted perpendicular to the skin surface to a depth of
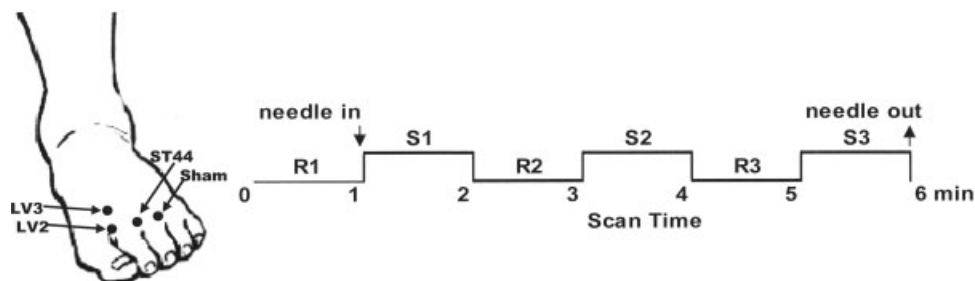
Figure 1.

Acupuncture: LV2, LV3 of liver meridian, ST44 of stomach meridian, sham point between meta-tarsals III and IV. Needle inserted and pretested for sensations prior to scanning. Total scan time 6 min. Three stimulation periods (S1,2,3) were interleaved with rest periods (R1) without needle and (R2, 3) with needle in place, each lasting 1 min.

2–4 mm depending on the acupoint. It was rotated backward and forward with even motion at a rate of 160/min with an amplitude around 180°. Epochs of needle manipulation were interleaved with epochs of needle at rest, each lasting 1 min. The different *deqi* sensations experienced by the subject during needle manipulation including aching, soreness, numbness, and distension were scored by the subject on a scale of 0–5 after every procedure. It was the acupuncturist's aim to generate *deqi* or acupuncture sensations without provoking undue discomfort or sharp pain [Hui et al., 2000, 2005].

Imaging

All MRI experiments were performed at a 1.9 T whole body scanner (Prestige, GE/Elscint, Haifa, Israel) equipped with a volumetric head coil. For the fMRI images, a gradient echo planar imaging (EPI) T2*-weighted sequence-based on blood oxygenation level dependent (BOLD) effect was employed [Kwong et al., 1992], using TR/TE at 3,000 ms/45 ms, flip angle 90°, field of view (FOV) 373 × 212 mm^2, matrix 128 × 72, in-plane resolution 2.9 × 2.9 mm^2. Nineteen continuous axial sections, each 6.0 mm thick, were collected to encompass the whole brain. Another set of 19 contiguous axial spin-echo T1-weighted images (TR/TE 750/12 ms, FOV 220 × 220 mm^2, 6.0 mm thick resolution 1 × 1 mm^2) was acquired for image registration.

fMRI Data Analysis

Individual and group analysis of functional imaging data were performed using SPM99. The data acquired in the first minute were excluded from the analysis to discard the unstable BOLD signals. Functional images were realigned for head motion (none of the subjects had head movements exceeding 2 mm). All functional images were normalized to MNI (Montreal Neurological Institute) stereotactic space and spatially smoothed by an 8 mm full-width half-maximum Gaussian kernel.

The fixed effects analysis was used for the individual data of each acupoint or the sham point from every subject, based on the general linear model with box car function as the reference waveform convolved with Poisson HRF (Haemodynamic Response Function). Regional activation and deactivation of brain regions during acupuncture was thresholded at $P \leq 0.001$ (uncorrected) with a minimum cluster size of 10 contiguous voxels.

The analysis of random effects (one-sample t test on a voxel-by-voxel basis) was performed for group data of each acupoint or the sham point from 10 subjects, thresholded at the same P value and cluster size as the individual analysis. Furthermore, one-way ANOVA (analysis of variance) was used to explore the differences among the classical points and sham point ($P \leq 0.005$, uncorrected, minimal cluster size 10 voxels, the degrees of freedom 9). Their time-courses were checked to ensure correlation with the experimental paradigm. The locations of the regions of interest are presented in MNI coordinates.

Acupuncture Sensations Analysis

The sum of the scores for the sensations reported by the subject provided a total acupuncture sensations score. The *deqi* response was thresholded at a minimal total score of 5. The Fisher's exact test and the student's t test were used to compare the frequency and intensity of sensations between points respectively.

RESULTS

Psychophysical Response

Most of the subjects reported *deqi* during acupuncture at all four points, 8 of 9 subjects for LV2, 8 of 10 for the sham point, 7 of 10 for ST44 and 6 of 9 for LV3 respectively ($P > 0.05$). One subject with acupuncture at LV3 and LV2 did not have sensations recorded. Aching was significantly more intense at LV3 compared with LV2 ($P < 0.05$).
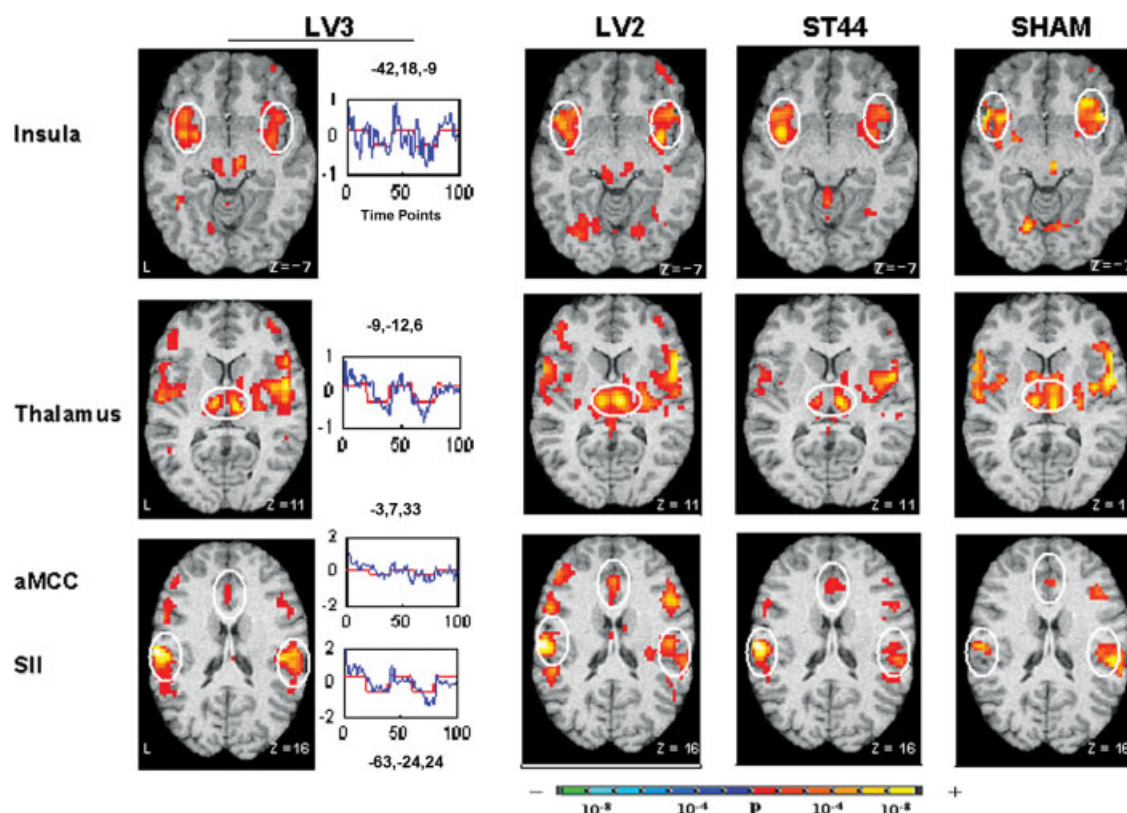
Figure 2.
Activation network: bilateral insula, thalamus, anterior middle cingulate cortex (aMCC), somatosensory cortex (SII: BA40, 43). ($n = 10$), $P \leq 0.001$, minimal cluster size of 10 voxels, uncorrected.

No significant difference was observed in the intensity and frequency of other sensations between the points. No subjects experienced sharp pain.

Hemodynamic Response

Overall, the group data of the hemodynamic response to acupuncture at the three acupoints and the sham point shared many common features in the patterns of brain activation or deactivation, even though there were differences in preferential locations and in the magnitude of signal change between the points [Figs. 2 and 3, Tables I and II].
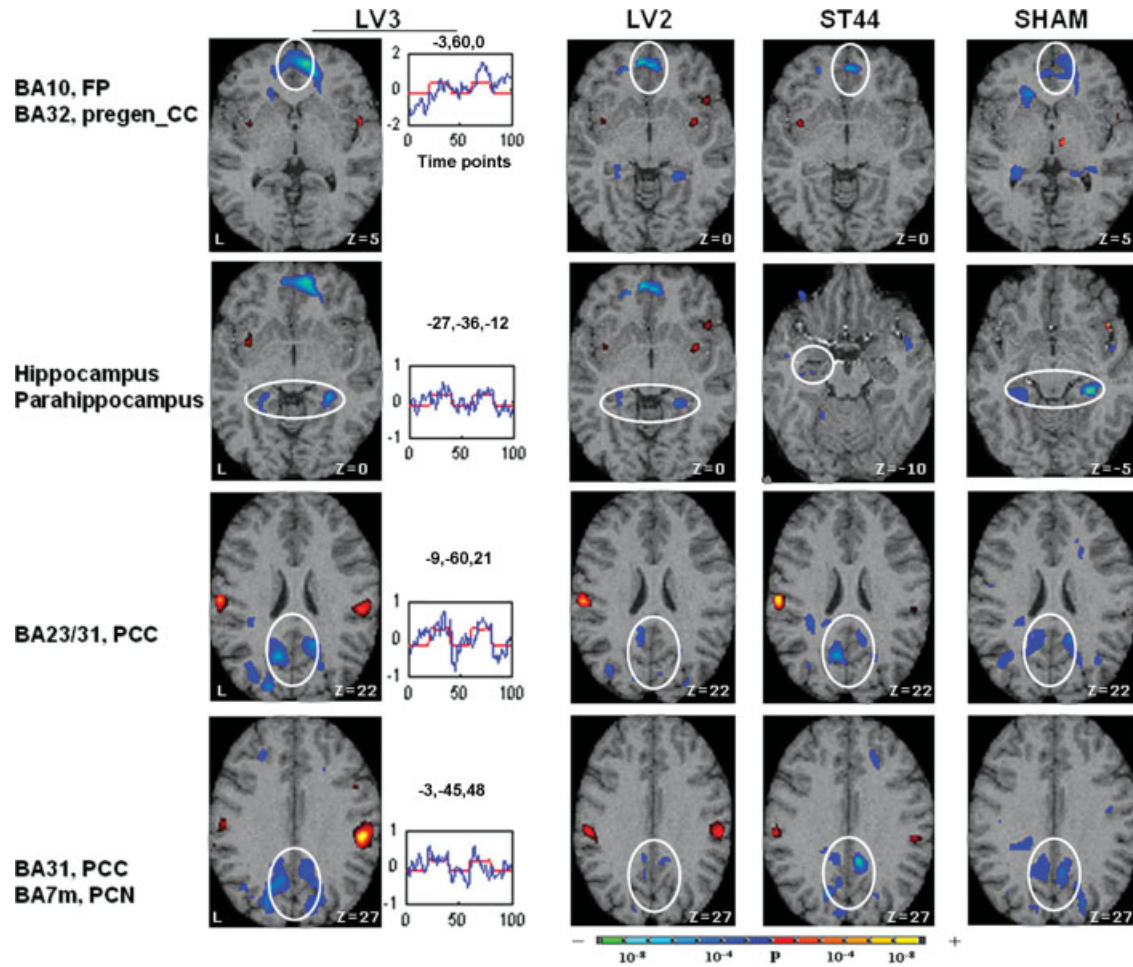
Activation Network [Fig. 2, Table I]

The spatial distribution and the intensity of the signal increases showed significant similarity across the points. The secondary somatosensory cortex (SII_BA40/43), supplementary motor cortex (SMC_BA6), insula, thalamus, and anterior middle cingulate cortex (aMCC_BA24/32) showed strong bilateral activation for all the points. Signal increases in the thalamus and SMC_BA6 were more pro-

minent on the right (contralateral) hemisphere. Variation between acupoints was observed in the primary somatosensory cortex (BA3/1/2), superior temporal gyrus (BA22), superior frontal gyrus (BA46) and posterior cingulate cortex (BA23_dorsal).

Deactivation in the Limbic-Paralimbic-Neocortical Network [Fig. 3, Table II]

The decrease in signal intensity was concentrated in three brain regions: the medial prefrontal cortex (frontal pole, pregenual cingulate), the temporal lobe (amygdala, hippocampus, parahippocampus, and temporal pole) and the posterior medial cortex (PMC) (precuneus, posterior cingulate). Most of the structures either belonged to or were intimately related to the limbic system. The response was generally bilateral. The frontal pole (BA10_medial) was deactivated for all the points. However, deactivation of the pregenual cingulate (BA32) was seen only with LV2 and the sham point. Interestingly, the response at the sham point was similar to LV3, but more intensive and extensive than LV2 and ST44. In the temporal lobe, signal decreases were detected in the hippocampus and parahip-

Figure 3.
Deactivation network: frontal pole (BA10_medial), pregenual cingulate cortex (BA32), hippocampus, parahippocampus, posterior cingulate cortex (BA23,31), precuneus (BA7_ medial). (n = 10), $P \leq 0.001$, minimal cluster size of 10 voxels, uncorrected.

TABLE I. Regions of fMRI signal Increase during acupuncture (n =10)

Structure	Hemis-phere[a]	LV3 MNI (mm) $x\ y\ z$	LV3 Peak T-value	LV2 MNI (mm) $x\ y\ z$	LV2 Peak T-value	ST40 MNI (mm) $x\ y\ z$	ST40 Peak T-value	Sham MNI (mm) $x\ y\ z$	Sham Peak T-value
aMCC(BA24/32)	L	−3,7,33	4.85	−3,−3,36	5.6	−3,30,27	4.52	−3,27,27	4.81
	R	3,9,33	6.07	6,6,33	6.49	6,24,30	5.09	3,33,24	4.96
SMC(BA6)	L	−3,21,45	4.53	−3,12,42	3.23	−3,12,48	4.08	−3,6,45	5.01
	R	3,12,48	5.99	6,12,57	5.61	3,12,48	5.64	3,6,42	5.27
Thalamus	L	−9,−12,6	6.62	−9,−14,3	6.95	−9,−15,3	6.01	−9,−12,0	5.26
(Dorsomedial)	R	12,−15,6	8.2	9,−14,6	7.9	9,−14,2	7.91	9,−12,0	6.98
Insula	L	−42,18,−9	5.65	−42,15,−9	5.8	−34,24,−7	5.81	−36,18,−9	4.99
	R	36,12,−9	6.17	36,21,−9	5.27	39,24,−3	5.07	33,21,−9	5
SII(BA43)	L	−59,−14,17	10.15	−59,−14,17	9.06	−60,−12,15	6.63	−59,−9,6	5.11
	R	60,−15,15	6.96	54,−12,15	5.47	54,−12,12	4.29	51,−9,12	5.37
SII(BA40)	L	−63,−24,24	6.97	−60,−18,18	8.35	−60,−18,15	8.55	−51,−18,15	5.03
	R	53,−25,29	9.78	63,−27,21	5.68	63,−24,24	6.35	63,−24,24	6.4
SI(BA3,1,2)	L	−54,−18,30	7.23	−57,−21,24	8.13	–	–	–	–

BA, Brodmann area; aMCC, anterior middle cingulate cortex; SMC, supplementary motor cortex; SII, secondary somatosensory cortex; SI, primary somatosensory cortex.
$P \leq 0.001\ T \geq 3.11$ uncorrected.
The activations of somatosensory cortices, thalamus and Insula during acupuncture were similar in the general pattern for the four points, Activation of SI was not observed with ST40 and the Sham point. The MNI coordinates and the peak T value were taken from the voxel with maximal signal change for each structure.
[a] Left: Ipsilateral.

TABLE II. Regions of fMRI signal decrease during acupuncture (*n* = 10)

Structure	Hemisphere[a]	LV3 MNI (mm) x y z	LV3 Peak *T* value	LV2 MNI (mm) x y z	LV2 Peak *T* value	ST40 MNI (mm) x y z	ST40 Peak *T* value	Sham MNI (mm) x y z	Sham Peak *T* value
FP(BA10)	L	−3,60,0	−5,03	−6,54,−9	−5.23	−9,54,0	−4.77	−6,45,−6	−5.35
	R	6,57,−3	−6.51	6,51,−12	−4.94	6,54,−12	−5.28	15,57,−3	−5.16
pregenual CC(BA32)	L	–	–	−6,36,0	−4.68	–	–	−3,39,0	−3.9
	R	–	–	9,36,−3	−4.34	–	–	6,36,0	−4.03
Amygdala	L	–	–	−24,−9,−15	−3.77	−33,−6,−30	−4.67	−30,−3,−27	−4.5
	R	–	–	–	–	–	–	–	–
Hippocampus	L	−27,−36,−12	−4.78	−24,−33,−9	−3.48	−39,−21,−21	−4.58	−35,−12,25	−5.41
	R	30,−39,−9	−6.42	27,−39,−9	−3.66	–	–	33,−33,−15	−7.46
Parahyppocampus	L	−24,−42,−15	−4.08	−24,−45,−6	−3.33	–	–	−27,−36,−18	−4.96
	R	30,−45,−12	−5.26	24,−45,−9	−3.74	–	–	30,−36,−18	−6.8
TP(BA38)	L	−45,12,−30	−3.87	–	–	–	–	−48,12,−30	−4.37
	R	48,6,−30	−3.57	–	–	48,9,−24	−3.83	45,9,−30	−3.99
PCN(BA7)	L	−3,−45,48	−3.61	–	–	−6,−60,33	−3.73	−9,−36,60	−4.4
	R	6,−39,51	−4.18	–	–	–	–	9,−36,54	−4.23
PCC(BA31)	L	−9,−60,27	−5.4	−6,−58,30	−3.29	−6,57,24	−3.9	−6,−33,45	−5.04
	R	6,−54,39	−5.08	6,−48,30	−3.58	9,−42,33	−4.64	6,−54,30	−5.14
RCS(BA29/30)	L	−6,−51,9	−4.5	−12,−50,10	−3.57	–	–	−9,−48,9	−3.82
	R	12,−51,9	−4.98	9,−45,9	−3.37	–	–	12,−48,3	−3.82
CN(BA18/19)	L	–	–	−9,−84,33	−4.25	−6,−84,27	−4.44	−9,−75,30	−3.6
	R	6,−78,33	−3.54	9,−78,21	−3.56	6,−84,27	−3.43	9,−72,33	−4.11

BA Brodmann area; FP, frontal pole; Pregenual CC, anterior cingulate cortex; TP, temporal pole; PCN, Precuneus; PCC, posterior cingulate cortex; RCS, retrosplenial cortex; CN, cuneus.
$P \leq 0.001\ T \geq 3.11$ uncorrected.
The deactivations in the limbic-paralimbic-neurocortical system for four points were similar in the general pattern. The MNI coordinates and the peak T values were taken from the voxel with maximal signal change for each structure.
[a] Left: ipsilateral.

pocampus for LV2, LV3, and the sham point. In contrast, no signal changes were found in parahippocampus and right hippocampus for ST44. The right amygdala was deactivated with LV2, ST44, and the sham point but not with LV3. The temporal pole showed signal decrease with LV3, ST44, and the sham point. The PMC demonstrated widespread bilateral deactivation in the precuneus (BA7_medial), posterior cingulate (BA31, BA23_ventral) and retrosplenial cortex (BA29/30) for most of the points, with the exception of BA29/30 for ST44, and BA7_medial

for LV2. The extrastriate visual cortex (BA18/19) showed deactivation for all points.

The results of ANOVA showed that differences in fMRI signal response between the real acupoints (LV2, ST44) and the sham point were limited to a small number of spatial distributions with small changes in signal intensity (thresholded at $P \leq 0.005$) [Table III]. The left temporal pole was activated for LV2 but deactivated for the sham point. The posterior cingulate cortex (BA31) revealed deactivation with the sham point but not with LV2 and ST44. The right supra-

TABLE III. fMRI Signal changes—acupoint *vs* sham point (one-way ANOVA)

	Structure	Hemisphere	MNI (mm) x y z	Peak *T*-value	*T* (LV2)	*T* (sham)	*T* (ST44)
LV2 vs Sham	TP(BA38)	L	−39,6,−24	4.68	3.68	−4.1	
	PCC(BA31)	L	−9,30,45	3.35	0	−5.47	
	PCC(BA31)	R	9,−36,39	3.00	0	−4.62	
	SMG(BA40)	R	51,−33,42	3.49	3.42	0	
ST44 vs Sham	PCC(BA31)	L	−9,−30,45	3.35		−5.47	0
	RSC(BA30)	L	−9,−33,−3	3.56		0	4.66
LV3 vs Sham			No voxel above the threshold				

BA, Broadmann area; TP, temporal pole; PCC, posterior cingulate cortex; RSC, retrosplenial cortex; SMG, supramarginal gyrus.
$P \leq 0.005/T \geq 2.72$.
Differences in BOLD fMRI signal response were detected: In the TP, PCC, SMG between LV2 and sham; and In the PCC, RSC between ST44 and sham point. No significant difference was noted between LV3 and the sham point. The MNI co-ordinates and the peak *T* value were taken from the voxel with maximal signal change for each structure.

marginal gyrus (BA40) showed activation for LV2, and the left retrosplenial cortex (BA30) deactivation for ST44; these changes were not observed for the sham point. No difference was detected between LV3 and the sham point.

DISCUSSION

In our study we compared the central effects of manual acupuncture at three classical acupoints on two meridians and a non-meridian point in the vicinity. All four sites are located in muscle/connective tissue layers supplied with similar segmental innervations (peroneal nerve, L4-S1). The overall hemodynamic response demonstrated many common features in the activation and deactivation networks, though variations in preferential spatial distributions and magnitude of response were observed.

Deactivation in the Limbic-Paralimbic-Neocortical Network

Prominent fMRI signal decreases were distributed in the frontal pole, medial prefrontal cortex, anterior cingulate cortex; amygdala, hippocampus, parahippocampus, temporal pole; precuneus, posterior cingulate, and retrosplenial cortex to form a general pattern of LPNN deactivation for all the points. The results were consistent with previous reports of fMRI signal response of acupuncture at different acupoints. Manual acupuncture at LI4, ST36, and LV3 showed signal decreases in different limbic, paralimbic and neocortical regions such as the amygdala, hippocampus, parahippocampus, anterior cingulate cortex, precuneus, medial prefrontal cortex, and temporal pole [Fang et al., 2006, 2007; Hui et al., 1997b, 2000, 2005; Hui, 2007; Kong et al., 2002; Napadow et al., 2005; Sun et al., 2006; Wu et al.,1999; Yan et al., 2005]. Electroacupuncture at LI4, ST36, SP6, GB34, GB37, and UB60 demonstrated similar signal decreases in the amygdala, hippocampus, parahippocampus, anterior cingulated cortex, medial orbital prefrontal cortex, hypothalamus, and precuneus [Kong et al., 2002, 2007a; Napadow et al., 2005; Wang et al., 2007; Wu et al., 2002]. Transcutaneous electrical nerve stimulation at ST36 and SP6, or GB34 and BL57 also revealed deactivation in hippocampus and amygdale. Moreover, deactivation of the amygdala and hippocampus was found to correlate with the elevation of pain threshold in the subjects [Zhang et al., 2003]. Thus, the in vivo evidence of acupuncture action on humans as revealed by modern neuroimaging strongly suggests that the deactivation of the LPNN is characteristic of acupuncture needling.

However, considerable controversy is found in the acupuncture neuroimaging literature [Dhond et al., 2007]. Like most fMRI/PET studies in other fields most investigators have focused on the activation effects of acupuncture stimulation. Until recently, reports of activation far outnumber those of deactivation. Signal increases have been reported in the visual cortex, auditory cortex, primary, and second-ary somatosensory cortices, supplementary motor cortex, cingulate cortex, thalamus, insula, hypothalamus, periaqueductal gray, and cerebellum [Biella et al., 2001; Cho et al., 1998, 2000; Fang et al., 2004; Hsieh et al., 1998; Li et al., 2003a,b; Liu et al., 2004; Parrish et al., 2005; Yan et al., 2005; Yoo et al., 2004]. While the finding of activation in most of these regions is valid, the interpretation that the response was specific to the acupoints employed is unconvincing. Contrary to the high specificity reported for the visual and auditory acupoints as mentioned above, acupuncture at many "nonvision" or "nonauditory" related acupoints also elicited BOLD signal response in the visual or auditory cortices.

While the different results in the literature may be attributed to the acupoints employed, it is likely that the intensity of stimulation, needling techniques, data acquisition, and the confounding effect of sharp pain may be important factors. We have shown in repeated studies that the hemodynamic response was markedly influenced by the sensory experience. With gentle needling that induced deqi absent sharp pain, extensive deactivation was observed in the limbic-paralimbic-neocortical network as described earlier. In the concomitant presence of inadvertent sharp pain, the signal decreases in a large subset of these regions were attenuated or reverted to signal increases [Fang et al., 2007; Hui et al., 2000, 2005, 2007; Napadow et al., 2005; Sun et al., 2006]. Inadvertent noxious stimulation could occur when the intensity of stimulation exceeds the subject's pain threshold. The pain that results will obscure the effect of deqi on the LPNN, the salient feature of acupuncture needling. It is, therefore, highly recommended to separate the datasets according to the psychophysical response in the data analysis, a practice that is as yet uncommon in the acupuncture imaging literature.

The Neuroanatomical and Functional Connectivities in the Limbic-Paralimbic-Neocortical Network

The limbic-paralimbic-neocortical circuit plays a major role in modulating the cognitive and affective dimensions of pain processing, and is central to the regulation and integration of emotion, cognition, conciousness, sensorimotor, autonomic, endocrine, and immunological functions. Extensive and dense interconnections in the limbic-paralimbic-neocortical system are demonstrated by neural tracer's technique in monkeys. For example, all PMC areas are interconnected with the anterior cingulate, the medial prefrontal cortex, amygdale, and hippocampus; while the orbital and medial prefrontal cortex (OMPFC) are extensively linked with the medial temporal lobe and cingulate limbic structures [Carmichael and Price, 1995; Parvizi et al., 2006; Vogt et al., 2006]. Such intimate anatomical connections support a close functional relationship among limbic-paralimbic-neocortical structures. It is widely recognized that the amygdala, hippocampus, parahippocampus, and anterior cingulate cortex play a central role in the regulation of

emotions, motivation, memory, and affective dimension of pain [LeDoux, 2000; Rainville et al., 1997; Zubieta et al., 2001]. The PMC is critical in a wide spectrum of highly integrated tasks such as visuo-spatial imagery, episodic memory retrieval and self-processing operations [Cavanna and Trimble, 2006; Vincent et al., 2006]; the OMPFC may be involved in sensory integration, decision making and expectation [Gusnard et al., 2001; Mill and Wallis, 2002].

The large subset of the deactivation regions involved such as the medial prefrontal cortex, anterior cingulate cortex, amygdala, hippocampus, parahippocampus, and PMC is known to be activated by pain, anxiety, and psychostimulants [Becerra et al., 1999; Bingel et al., 2002; Kufahl et al., 2007; Simpson et al., 2001]. This phenomenon was also observed in acupuncture that provoked sharp pain [Fang et al., 2007; Hui et al., 2000, 2005; Sun et al., 2006]. Moreover, it was reported that the deactivation of the amygdala and hippocampus correlated with the elevation of pain threshold in the subjects [Zhang et al., 2003]. The interpretation of decreases in BOLD signals is not completely clear, but increasing evidence indicates that it is a marker of neuronal deactivation [Shmuel et al., 2006; Stefanovic et al., 2004]. Acupuncture may mediate its antipain, antianxiety, and other diverse modulatory effects via deactivation of this limbic-paralimbic-neocortical circuit.

Defaut Mode Network and the Limbic-Paralimbic-Neocortical Network

Interestingly, the so-called "default mode network" (DMN) of the brain overlaps with the LPNN deactivated by acupuncture [Fang et al., 2007; Hui et al., 2000, 2005, 2007]. These regions demonstrate highest metabolic rate when a subject is at rest, but become deactivated during goal-directed tasks [Buckner et al., 2008; Fransson, 2006; Raichle et al., 2001; Shulman et al., 1997]. It is suggested that the important function of this network is to enter a mode of preparedness and alertness for possible changes in the internal or external milieu. We postulate that this intrinsic organization may participate as a core of the LPNN network that response to acupuncture stimulation.

Activation Network in Acupuncture Studies

The activation in somatosensory cortex together with its associated cortices and thalamus is to be expected from somatosensory stimulation at somatic points [Davis et al., 1998]. However, a couple of paralimbic regions are also involved. The anterior middle cingulate cortex is related to cognitive attention, opioidergic pain modulation as well as placebo analgesia [Benedetti et al., 1999; Han et al., 1982; Schnitzler and Ploner, 2000; Zubieta et al., 2001]. The insula processes convergent information to produce an emotionally relevant context for sensory experience, including olfactory, gustatory, and viscera's stimulations [Caria et al., 2007; Davis et al., 1998]. Overall, this study showed more prominent activation in intensity and in

extent compared to some earlier studies in manual acupuncture [Fang et al., 2004, 2006, 2007; Hui et al., 2000, 2005; Wu et al., 1999]. This could contribute to the stronger stimulation of the somatosensory network: the speed of needle manipulation of 160/min was faster than other fMRI studies (60–120/min) in the literature.

fMRI BOLD Signal Change Patterns: Acupoints versus Sham Point

The main purpose of comparing a meridian point (acupoint) and a nonmeridian point (sham point) is to examine if the central effects elicited at the acupoint are similar to those elicited at the sham point. This may help clarify whether acupoint specificity exists [Lao et al., 2001]. However, to find a real sham point on the human body is difficult. The designation of a real acupoint is empirical: the 354 acupoints employed in the ninth century have grown to over 2,000 today [Pomeranz, 1995; Shanghai College of Traditional Chinese Medicine, 1974; Zhu, 1997]. Many of the acupoints in use today were unknown in earlier days. The potential effect of acupuncture at any somatic point is unknown until it is tested. Therefore, what is selected as a nonpoint or sham point cannot be assumed to be inert or inactive just because it is not known to be active today. Moreover, clinical data showed acupuncture analgesia at sham points was effective in about 33–50% of patients with chronic pain, while true points worked in about 55–85% [Richardson and Vincent, 1986; Vincent and Richardson, 1989]. Sham points can be used for comparison with the real points to detect possible similarities and/or differences, but they should not be considered to be inert control [Lund and Lundeberg, 2006]. This may at least partly explain the remarkable overlap of brain response among the sham point and the classical acupoints in our study. The overlap phenomenon was also observed in previous acupuncture studies [Fang et al., 2004; Kong et al., 2007a; Wu et al., 2002].

Central Effects of Acupoint, Segmental Innervations, Tissue Type, and Meridian Origin

In our study the three acupoints are located on two different meridians and the sham point on a nonmeridian site. All of them are supplied by similar segmental innervations (peroneal nerve, L4–S1) [Chen, 1995; Cheng, 2000; Williams, 1995]. Hence, the similarity in segmental innervations could contribute to the similarities of central effects on the brain in our findings. However, significant overlap was also demonstrated between LI4 on the hand and ST36 on the leg that have very different segmental innervations. It is proposed that the tissue type of the acupoint site may be an important factor on the central effects of acupuncture [Fang et al., 2007; Hui et al., 2000, 2005]. The four points in this study are located in muscle/fibrous connective tissue layers, with differences in proportions of the components. It is plausible that the general similarity in tissue type might contribute to similar central effects with

minor variations, an interesting factor that deserves exploration. As for the meridian origin, there was no enough evidence of a significant role. The issue of acupoint specificity of central effects requires further investigation.

Limitations

This study failed to provide definitive answers on the subject of acupoint specificity. Although powerful for monitoring dynamic changes in the brain, our fMRI study only tested for the acute hemodynamic response during the short period of acupuncture needling, not for any delayed response that could occur with prolonged or repeated stimulations. We did not assess physiological responses, such as autonomic, endocrine, and immunological functions that may differ between acupoints. Moreover, brain imaging does not address the changes that may be involved at the local level or at the spinal segmental level in acupuncture action [Gollub et al., 1999; Langevin et al., 2006; Li et al., 2004]. Importantly, the effects of acupuncture on patients may differ from that on healthy subjects [Napadow et al., 2007]. The subjects in this study were preselected to be responders to acupuncture with an elevation of >10% in their pain thresholds in a presession acupuncture intervention. Their hyper-sensitivity to needle manipulation could be a factor for the failure to show significant differences of the BOLD response between real acupoint and sham point. The study design is exploratory and the sample size is limited. Larger sample studies with greater statistical power, on acupoints belonging to more meridians, located in different types of tissues and with different innervations as well as a broader spectrum of subjects in health and in disease are required for the clarification of acupoint specificity, given its importance in TCM.

CONCLUSIONS

We found that the patterns of hemodynamic and psychophysical responses to acupuncture stimulation at three classical acupoints and a sham point were generally similar, with variations in the preferential spatial distributions at the global level. The characteristic pattern of the hemodynamic response is extensive deactivation of a limbic-paralimbic-neocortical network that shows functional and structural connectivity. The antipain, antianxiety, and other modulatory effects of acupuncture may be mediated by this intrinsic neural system. The specificity of acupoint effects requires further investigation.

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