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Magnetic Resonance Imaging 30 (2012) 672 – 682
MAGNETIC RESONANCE IMAGING

FMRI connectivity analysis of acupuncture effects on the whole brain network in mild cognitive impairment patients

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Received 24 June 2011; revised 23 October 2011; accepted 31 January 2012

Abstract

The increased risk for the elderly with mild cognitive impairment (MCI) to progress to Alzheimer's disease makes it an appropriate condition for investigation. While the use of acupuncture as a complementary therapeutic method for treating MCI is popular in certain parts of the world, the underlying mechanism is still elusive. We sought to investigate the acupuncture effects on the functional connectivity throughout the entire brain in MCI patients compared to healthy controls (HC). The functional magnetic resonance imaging experiment was performed with two different paradigms, namely, deep acupuncture (DA) and superficial acupuncture (SA), at acupoint KI3. We first identified regions showing abnormal functional connectivity in the MCI group compared to HC during the resting state and subsequently tested whether these regions could be modulated by acupuncture. Then, we made the comparison of MCI vs. HC to test whether there were any specific modulatory patterns in the poststimulus resting brain between the two groups. Finally, we made the comparisons of DA vs. SA in each group to test the effect of acupuncture with different needling depths. We found the temporal regions (hippocampus, thalamus, fusiform gyrus) showing abnormal functional connectivity during the resting state. These regions are implicated in memory encoding and retrieving. Furthermore, we found significant changes in functional connectivity related with the abnormal regions in MCI patients following acupuncture. Compared to HC, the correlations related with the temporal regions were enhanced in the poststimulus resting brain in MCI patients. Compared to SA, significantly increased correlations related with the temporal regions were found for the DA condition. The enhanced correlations in the memory-related brain regions following acupuncture may be related to the purported therapeutically beneficial effects of acupuncture for the treatment of MCI. The heterogeneous modulatory patterns between DA and SA may suggest that deep muscle insertion of acupuncture is necessary to achieve the appreciable clinical effect.

Keywords: Mild cognitive impairment (MCI); fMRI; Acupuncture; Functional connectivity

1. Introduction

Mild cognitive impairment (MCI) is considered to be an intermediate state between normal aging and dementia [1]. The most prominent feature is an isolated mild decline in memory, whereas other cognitive functions remain intact. MCI has become a hot issue in dementia studies in recent years because MCI turns into Alzheimer's disease (AD) at a high rate of approximately 10% to 15% per year [2]. AD, the most common form of dementia, is characterized by significant impairments in multiple cognitive domains including memory, attention, reasoning, language and executive functions. The increased risk for elderly populations that suffer from MCI to progress to AD makes it an appropriate condition for investigation.

The use of acupuncture as a complementary therapeutic method for treating a variety of neurologic diseases,

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0730-725X/$ – see front matter
doi:10.1016/j.mri.2012.01.003

including MCI and AD, is popular in certain parts of the world [3]. In spite of its public acceptance, the underlying neural mechanism is still elusive. In the past decades, noninvasive functional magnetic resonance imaging (fMRI) technique has provided new insights into the central physiological functions involved in acupuncture. Neuroimaging studies of acupuncture have indicated that the primary acupuncture effects are mediated by the central nervous system [4–14]. However, the majority of these studies have been performed on healthy subjects. It is generally agreed that acupuncture plays a homeostatic role and thus may have a greater effect on patients with a pathological imbalance compared to healthy controls (HC) [15,16]. Hence, imaging its effect on the brain networks in patients may further help to elucidate the mechanisms by which acupuncture achieves its therapeutic effects. One previous study adopted fMRI to study a total of 26 AD patients who underwent acupuncture, and found that acupuncture could activate the temporal lobe (such as hippocampus, insula), some regions of the parietal lobe and cerebellum in AD patients [17]. These regions are consistent with impaired brain areas in AD patients, which are closely correlated with the cognitive function (memory, reason, language, executive, etc.). This study provides the preliminary neurophysiological evidence for the potential efficacy effect of acupuncture on AD. But to the best of our knowledge, no fMRI studies have been published on neural correlations in response to acupuncture in patients with MCI. The underlying neural mechanism may illustrate the functional substrate of the purported therapeutically beneficial effect of acupuncture on MCI.

Acupuncture is practiced in many different ways, with one of the fundamental differences in approach being the depth that acupuncture needles are inserted. According to the theory of the Traditional Chinese Medicine, the depth of needling is integral to the putative therapeutic gain [18]. A primary interest in this area therefore is whether these different depths of needling elicit similar or different responses. However, few have evaluated the functional correlations in the poststimulus resting brain modulated by deep acupuncture (DA, with the needling depths usually 1–2 cm depending on the local musculature) and superficial acupuncture (SA, with the needling depths perhaps 1–2 mm just below the skin). One recent report demonstrates that deep muscle acupuncture may better overlap with its proximity to ascending nerve tracks than to the density of cutaneous afferences [19]. In addition, SA has been assumed to minimize the therapeutic effect while triggering most of the nonspecific effects of needling [20]. Therefore, comparing functional connectivity patterns modulated by DA to those modulated by SA may provide precise and specific modulatory patterns related to the therapeutic effect of acupuncture.

Previous neuroimaging studies have shown that MCI patients may have widespread alterations involving frontal, parietal, temporal, occipital and subcortical regions [21–23]. Studying functional connectivity from the perspective of the whole brain will be helpful for a better understanding of the pathophysiology of MCI. In the present study, we sought to investigate the acupuncture effects on the functional connectivity throughout the entire brain in MCI patients compared to HC. This method has been demonstrated to be helpful for investigation of the large-scale functional brain networks modulated by verum acupuncture compared to sham acupuncture in our previous study [24]. The fMRI experiment was performed with two different paradigms employed, namely, DA and SA, at acupoint KI3. We first identified regions showing abnormal functional connectivity in MCI group compared to HC during the resting state and subsequently tested whether these regions could be modulated by acupuncture in the poststimulus resting brain. After that, we made the comparison of MCI vs. HC to test whether there were any specific modulatory patterns in the poststimulus resting brain between the two groups. Finally, we made the comparisons of DA vs. SA in each group to explore and test the effect of acupuncture with different needling depths.

2. Materials and methods

2.1. Subjects

MCI patients were recruited at the rehabilitation department of the Bao'an People's Hospital of Shenzhen. MCI patients were diagnosed using the criteria for amnestic MCI [25], with Mini-Mental State Examination (MMSE) scores >25 [26] and Clinical Dementia Rating (CDR) scale scores of 0.5 [27]. Twelve MCI patients and 12 age-matched HC subjects were included (see Table 1 for subjects' characteristics). All subjects were right-handed and acupuncture naive according to the Edinburgh Handedness Inventory [28]. Subjects were excluded if they had any significant medical, neurological or psychiatric illness, or if they were taking medication or other substances known to influence cerebral function. After being given a complete description of the study, all subjects signed the informed consent form. All protocols were approved by a local subcommittee on human studies.

2.2. Experimental paradigm

Previous studies generally adopted a multiblock paradigm, which implicitly presumes the temporal intensity

Table 1
Subject characteristics

	HC	MCI
N	12	12
Age (years)	60.6±5.8	59.3±3.3
Education	2.4±0.5	2.3±0.4
Sex (male/female)	4/8	1/11
CDR	0	0.5
MMSE	29.8±0.4 *	26.4±0.9 *

Education level was determined on a discrete scale with three levels: low=1, middle=2, high=3. Data are presented as mean±S.D.

* Statistically significant difference at the $P<.0001$ level.

profiles of the certain event conforming to the "on–off" specifications. Due to the sustained effect of acupuncture, the temporal aspects of the blood-oxygen-level-dependent response to acupuncture may violate the assumptions of the block-designed estimates [5]. In this study, we adopted a new experimental paradigm, namely, the nonrepeated event-related fMRI design to investigate the prolonged effects after acupuncture administration [10].

For each group, the experiment consisted of three functional runs. For a baseline control, a resting state (REST) scan was conducted for 6 min without any stimulation (Fig. 1A). We then employed the new experimental paradigm, in which two functional runs, DA and SA, were conducted and only one single stimulation period was given during each of these two runs (Fig. 1B, C). For both the DA and SA runs, an acupuncture needle was inserted from the beginning, and after resting for 1 min, the needle was manipulated for 2 min; then, another REST scan was conducted for 6 min without any stimulation. All participants were not informed of the order in which these three runs would be performed and were asked to remain relaxed without engaging in any mental tasks. To facilitate blinding, they were also instructed to keep their eyes closed to prevent them from actually observing the procedures. According to participants' reports after the scanning, they affirmed keeping awake during the whole process. The presentation sequence of two acupuncture runs was randomized and balanced throughout the population, and every participant performed only one run each day in order to eliminate potential long-lasting effect following acupuncture administration. At the end of each acupuncture scan, the subjects adopted a 10-point scale to self-rate the intensities about the deqi sensations they had felt during the stimulation (0=no sensation, 1–3=mild, 4–6=moderate, 7–8=strong, 9=severe and 10=unbearable sensation) [29].

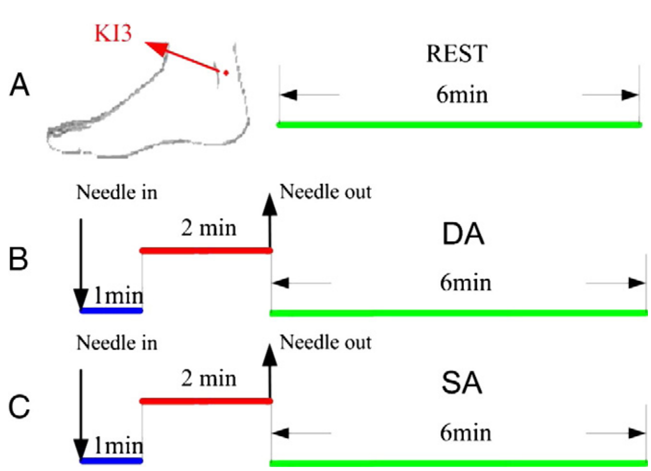

Fig. 1. Experimental paradigm. (A) The paradigm for a REST run lasting for 6 min. (B) The paradigm for a DA run totally lasting for 9.0 min. (C) The paradigm for an SA run totally lasting for 9.0 min. The images acquired during the time points labeled by green color were used for functional connectivity analysis.

DA was performed at acupoint KI3 (Taixi, located in a depression between the medial malleolus and heel tendon). This is one of the most frequently used acupoint and is proven to have various efficacies in the treatment of dementia [17]. Acupuncture stimulation was delivered using a sterile disposable 38-ga stainless steel acupuncture needle 0.2 mm in diameter and 40 mm in length. The needle was inserted vertically to a depth of 1–2 cm, and administration was delivered by a balanced "tonifying and reducing" technique [9]. Stimulation consisted of rotating the needle clockwise and counterclockwise for 1 min at a rate of 60 times per min. The procedure was performed by the same experienced and licensed acupuncturist on all participants. Considering that SA can effectively reduce the subjects' bias toward the stimulation, we adopted the SA as a control model. SA was initially devised by an experienced acupuncturist, with needling at acupoint KI3 (1–2 mm) with stimulation intensity, and manipulation methods were all identical to those used in the DA.

2.3. Data acquisition and preprocessing

Magnetic resonance imaging data were acquired using a 3.0-T Signa (GE) MR scanner. Head movements were prevented by a custom-built head holder. The images were parallel to the anterior commissure (AC)–posterior commissure (PC) plane and covered the whole brain. Thirty axial slices were obtained using a T2*-weighted single-shot, gradient-recalled echo planar imaging sequence [field of view (FOV)=220 mm×220 mm, matrix=64×64, thickness=4 mm, repetition time (TR)=2000 ms, echo time (TE)=30 ms, flip angle=77°]. After the functional run, high-resolution structural information on each subject was also acquired using three-dimensional (3D) MRI sequences with a voxel size of 1 mm^3 for anatomical localization (TR=2.1 s, TE=4.6 ms, matrix=256×256, FOV=230 mm×230 mm, flip angle=8°, slice thickness=1 mm).

For the REST run, the data were preprocessed by removing the first five time points to eliminate nonequilibrium effects of magnetization. For both the DA and SA runs, only the datasets after manipulation were selected (total of 180 time points, the same time points as in the REST run), and the first five time points were discarded in order to obtain a stable resting state. The remaining time points (labeled by green color in Fig. 1) were used for functional connectivity analyses. All images were preprocessed using statistical parametric mapping (SPM5, http://www.fil.ion.ucl.ac.uk/spm/). First, the image data underwent slice-timing correction and realignment for head motions using least squares minimization. Then, the standard MNI template provided by SPM5 was used in spatial normalization with resampling at 2 mm×2 mm×2 mm. After that, the functional images were spatially smoothed with a 3D Gaussian kernel [full width at half maximum (FWHM)=6 mm]. After that, the functional images were spatially smoothed with a 6-mm FWHM Gaussian kernel. Several procedures were adopted to remove possible spurious variances from the data through linear regression [30,31]: (1) six motion parameters, (2) whole brain signal averaged over the entire brain, (3) signal from a region in cerebrospinal fluid, (4) signal from a region centered in the white matter and (5) linear drift. Finally, the fMRI waveform of each voxel was temporally band-pass filtered (0.01 Hz$<f<$0.08 Hz).

2.4. Whole brain functional connectivity analysis

The preprocessed datasets were firstly parcellated into 90 cortical and subcortical regions using anatomical templates defined by Tzourio-Mazoyer et al. (shown in Table 2) [32]. We obtained the mean time series of each of the 90 regions by averaging the fMRI time series over all voxels in the region. The Pearson correlation coefficients were computed between each pair of brain regions for each subject in three conditions (REST, DA, SA) [22,31]. Fisher's r to z transformation was applied to improve the normality of the correlation coefficients. For each condition, we firstly performed an analysis of variance (ANOVA) with factors subject, region of interest (ROI) pair and MCI status in each group. For the REST condition, we compared the correlation coefficients of each pair between MCI and HC by performing two-sample two-tailed t test (P<.05, multiple comparisons corrected) to find the regions showing abnormal functional connectivity in MCI patients. Then, we compared the correlations coefficients of each pair between postacupuncture and rest (DA vs. REST or SA vs. REST) by performing paired t test (P<.05, multiple comparisons corrected) in each group to test whether these abnormal regions could be modulated by acupuncture. After that, we compared the correlation coefficients of each pair between MCI and HC in postacupuncture condition (DA or SA) by performing two-sample two-tailed t test (P<.05, multiple comparisons corrected) to test whether there is any difference of modulatory patterns modulated by acupuncture between the groups. Finally, we compared the correlation coefficients of each pair between DA and SA in each group by performing paired t test (P<.05, multiple comparisons corrected) to test the effect of acupuncture with different needling depths on the resting brain. To account for multiple comparisons, the Benjamini and Hochberg false discovery rate was applied [33].

Table 2
Ninety cortical and subcortical regions of the whole brain (45 in each hemisphere)

Region	Abbreviation	Region	Abbreviation
Superior frontal gyrus	SFG	Insula	INS
Superior frontal gyrus, orbital	SFGO	Thalamus	THA
Superior frontal gyrus, medial	SFGM	Superior temporal gyrus	STG
Superior frontal gyrus, medial orbital	SFGMO	Superior temporal pole	STP
		Middle temporal gyrus	MTG
Middle frontal gyrus	MFG	Middle temporal pole	MTP
Middle frontal gyrus, orbital	MFGO	Inferior temporal gyrus	ITG
Inferior frontal gyrus, opercular	IFGOP	Heschl gyrus	HES
Inferior frontal gyrus, triangular	IFGT	Hippocampus	HIPP
Inferior frontal gyrus, orbital	IFGO	Parahippocampal gyrus	PHIP
Rectus gyrus	REG	Amygdala	AMYG
Anterior cingulate gyrus	ACC	Fusiform gyrus	FG
Olfactory cortex	OLF	Caudate nucleus	CAU
Precentral gyrus	PreCG	putamen	PUTA
Supplementary motor area	SMA	pallidum	PAL
Middle cingulate gyrus	MCC	Postcentral gyrus	PoCG
Rolandic operculum	ROL	Superior parietal gyrus	SPG
Calcarine cortex	CAL	Inferior parietal gyrus	IPG
Cuneus	CUN	Supramarginal gyrus	SMG
Lingual gyrus	LING	Angular gyrus	ANG
Superior occipital gyrus	SOG	Paracentral lobule	PCL
Middle occipital gyrus	MOG	Precuneus	PCNU
Inferior occipital gyrus	IOG	Posterior cingulate gyrus	PCC

3. Results

3.1. Psychophysical response

The prevalence of subjective "deqi" sensations was expressed as the percentage of individuals in the group that reported the given sensations (Figs. 2A and 3A). For both groups, no difference was found with regard to the prevalence of the listed sensations elicited by DA and SA (P>.05). However, differences did exist with respect to the type of sensations. In the MCI group, soreness (DA: 100%, SA: 58%), numbness (DA: 100%, SA: 75%), fullness (DA: 100%, SA: 91%), warmth (DA: 50%, SA: 17%) and heaviness (DA: 8%, SA: 0%) were found to be greater for the DA condition. In the HC group, soreness (DA: 100%, SA: 58%), numbness (DA: 100%, SA: 75%), fullness (DA: 100%, SA: 91%), warmth (DA: 50%, SA: 8%) and heaviness (DA: 8%, SA: 0%) were found to be greater for the DA condition. For both conditions, no difference was found with regard to the prevalence of the listed sensations between MCI and HC (P>.05). However, differences did exist with respect to the type of sensations. For the DA condition, warmth (MCI: 58%, HC: 50%) and tingling (MCI: 16%, HC: 8%) were found to be greater in the MCI group. For the SA condition, warmth (MCI: 16%, HC: 8%) and tingling (MCI: 16%, HC: 8%) were found to be greater in the MCI group.

The intensity of sensations was expressed as the average score±S.E. (Figs. 2B and 3B). In both groups,

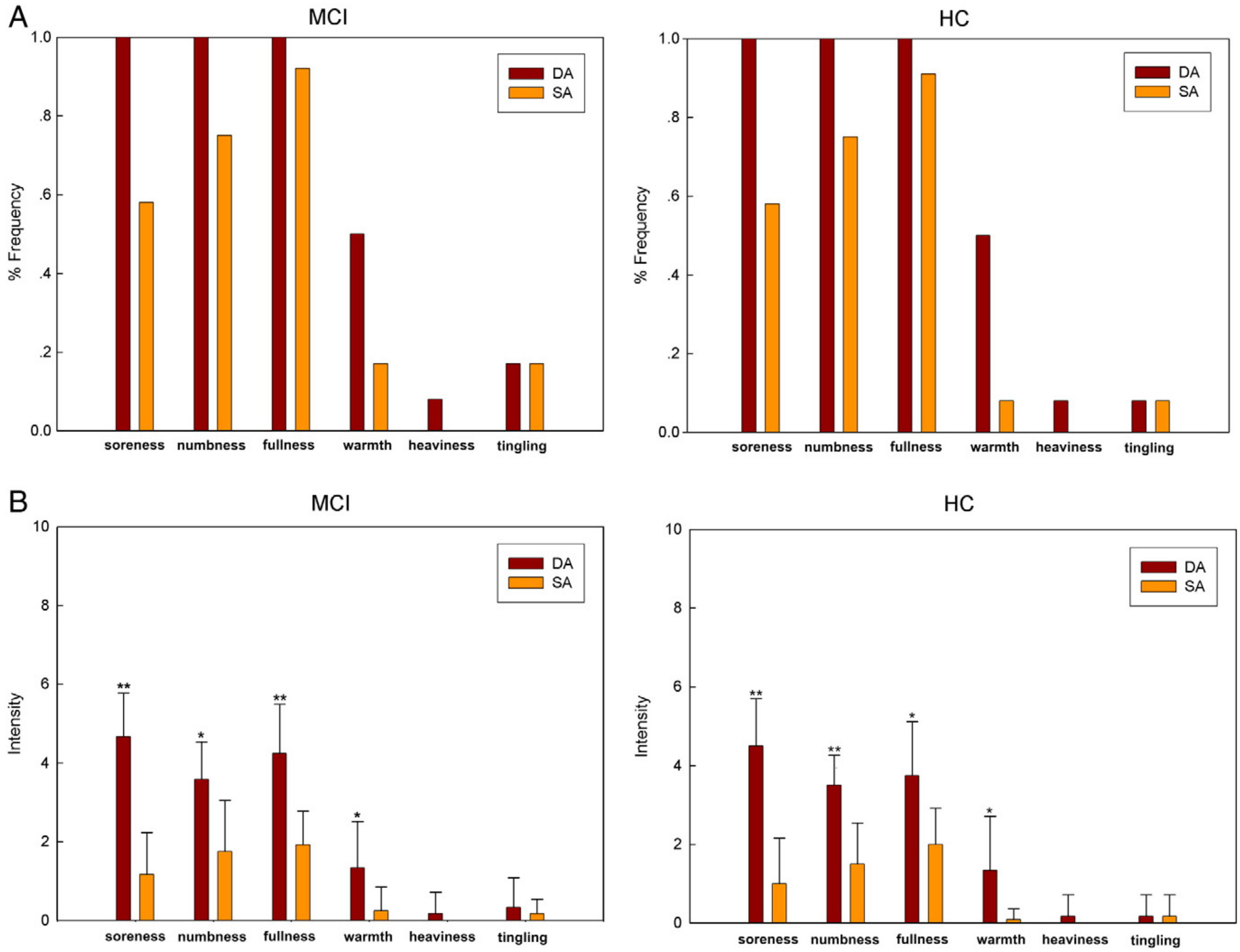

Fig. 2. Averaged psychophysical response (*N*=12) in the MCI and HC groups. (A) The percentage of subjects who reported having experienced the given sensation (at least one subject experienced the seven sensations listed). (B) The intensity of reported sensations measured by an average score (with standard error bars) on a scale from 0 denoting no sensation to 10 denoting an unbearable sensation. The intensity of numbness, fullness and soreness was found to be greater for the DA than the SA under Fisher's Exact Test (*P<.01; **P<.001).

no difference was found with regard to the averaged intensities of the listed sensations elicited by DA and SA (P>.05). These results indicated that DA and SA could effectively reduce the subjects' bias toward the stimulation. However, differences did exist with respect to the type of sensations. In the MCI group, soreness was more intense for DA than SA (P<.001), and numbness was more intense for DA than SA (P<.01); fullness was stronger for DA than SA (P<.001), and warmth was more intense for DA than SA (P<.01). In the HC group, soreness was more intense for DA than SA (P<.001), and numbness was more intense for DA than SA (P<.001); fullness was stronger for DA than SA (P<.01), and warmth was more intense for DA than SA (P<.01). For both conditions, the averaged intensities were approximately similar in the MCI and HC groups (P>.05). Considering a little difference in psychophysical response between MCI and HC, the neuroimaging findings were likely not the results of differences induced by the sensations.

3.2. Direct comparisons for MCI vs. HC in REST

During the resting state, we made comparison of MCI vs. HC to find the regions showing abnormal functional connectivity (shown in Table 3 and Fig. 4). The ANOVA result demonstrated a significant effect of ROI pair (HC: F=9.73, P=3.5E-6; MCI: F=14.6, P=1.3E-7). No statistically significant effect of the factors subject (HC: F=1.53, P=0.15; MCI, F=0.78, P=0.64) and MCI status (HC: F=0.43, P=0.51; MCI: F=2.75, P=0.06) was observed. The significantly decreased correlations (P<.05, multiple comparisons corrected) between MCI and HC were mainly related with the temporal regions (hippocampus, thalamus, fusiform gyrus) and the prefrontal cortex (inferior frontal gyrus, orbital; olfactory cortex; superior frontal gyrus, medial; middle frontal gyrus). These findings suggested these regions showing abnormal functional connectivity in MCI patients. On the other hand, significantly increased correlations (P<.05, multiple comparisons corrected) were

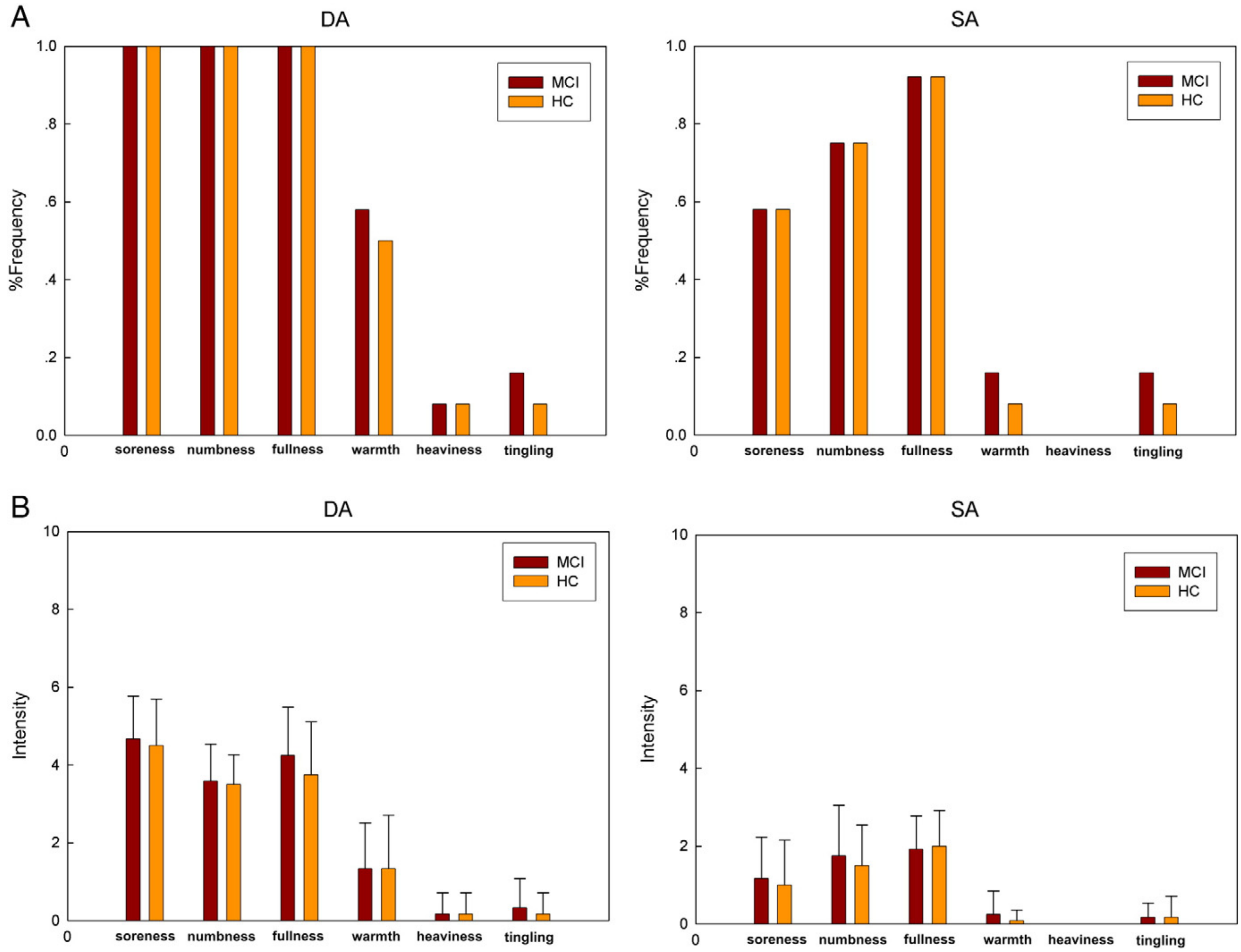

Fig. 3. Averaged psychophysical response (N=12) for the DA and SA conditions. (A) The percentage of subjects who reported having experienced the given sensation (at least one subject experienced the seven sensations listed). (B) The intensity of reported sensations measured by an average score (with standard error bars) on a scale from 0 denoting no sensation to 10 denoting an unbearable sensation.

Table 3
The significantly different interregional correlations between MCI and HC (P<.05, multiple comparisons corrected) in REST

Region 1	Classification	Region 2	Classification	P value
REST(MCI>HC)				
L_PHIP	Temporal	R_PHIP	Temporal	.0024
L_THA	Temporal	R_THA	Temporal	.0077
R_SFG	Frontal	R_FG	Temporal	.0091
R_IFGO	Frontal	R_FG	Temporal	.0031
L_ROL	Frontal	L_IPG	Parietal	.0066
R_SMG	Parietal	L_STG	Temporal	.0094
R_PCL	Parietal	R_PAL	Corpus striatum	.0091
REST(MCI<HC)				
R_IFGO	Frontal	L_HIPP	Temporal	.0072
R_IFGO	Frontal	L_FG	Temporal	.0055
L_OLF	Frontal	L_THA	Temporal	.0066
R_SFGM	Frontal	R_PoCG	Parietal	.0088
L_MFG	Frontal	L_OLF	Frontal	.0092
L_IFGO	Frontal	L_OLF	Frontal	.0047
L_PoCG	Parietal	R_CAU	Corpus striatum	.0020

For the abbreviations of the regions, see Table 2. L, left; R, right.

found mainly between interhemispheric symmetric regions [parahippocampal gyrus (PHIP), thalamus] and within the ipsilateral regions.

3.3. Direct comparisons for MCI vs. HC for acupuncture condition

We then performed comparisons for DA vs. REST and SA vs. REST to explore acupuncture effects on the resting brain in each group (shown in Tables 4 and 5, and Fig. 5).

For the DA condition, the significantly increased correlations (P<.05, multiple comparisons corrected) were related with the abnormal regions (hippocampus; olfactory cortex; superior frontal gyrus, medial) in the MCI group. This result suggests that acupuncture could modulate the abnormal regions in MCI patients. In addition, the significantly increased correlations (P<.05, multiple comparisons corrected) were also related with the other regions such as the insula, PHIP and cingulate cortex. We also found

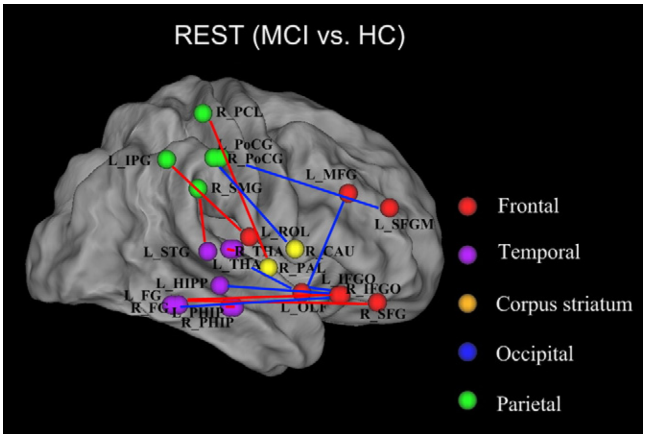

Fig. 4. Significant differences in functional correlations between the MCI and HC groups in REST. The red and blue lines indicate the significantly increased and decreased functional correlations between the corresponding regions (P<.05, multiple comparisons corrected), respectively. For the abbreviations of the regions, see Table 2. L, left; R, right.

that the significantly increased correlations (P<.05, multiple comparisons corrected) related with the temporal regions such as the hippocampus and middle temporal gyrus for the DA condition in the HC group. When compared to HC, the correlation related with the temporal regions (hippocampus, amygdala, PHIP), insula and cingulate cortex were significantly enhanced (P<.05, multiple comparisons corrected) in the MCI group (shown in Table 6 and Fig. 6). Similar patterns were also found for the SA condition but with little extent in temporal regions.

3.4. Direct comparisons for DA vs. SA in each group

Finally, we made the comparisons of DA vs. SA in each group to test the effect of acupuncture with different needling depths on the resting brain (shown in Table 7 and Fig. 7). For the DA condition, the ANOVA analysis revealed a significant effect of ROI pair (HC: F=11.19, P=8E-7; MCI: F=12.55, P=7.6E-7). No statistically significant effect of the factors subject (HC: F=0.87, P=0.52; MCI: F=0.49, P=0.89) and MCI status (HC: F=0.52, P=0.46; MCI: F=1.94, P=0.14) was observed. For the SA condition, the ANOVA result also showed a significant effect of ROI pair (HC: F=11.22, P=1.9E-6; MCI: F=13.05, P=5.7E-7). No statistically significant effect of the factors subject (HC: F=1.83, P=0.07; MCI, F=1.56, P=0.12) and MCI status (HC: F=0.19, P=0.66; MCI: F=0.15, P=0.86) was observed. In the MCI group, significantly increased correlations (P<.05, multiple comparisons corrected) were mainly related with the insula, amygdala and frontal regions (olfactory cortex; superior frontal gyrus, medial; middle frontal gyrus, orbital). In the HC group, significantly increased correlations (P<.05, multiple comparisons corrected) were mainly related with the temporal regions (hippocampus; amygdala) and frontal regions (middle frontal gyrus, orbital; olfactory cortex).

4. Discussions

Functional correlations in the poststimulus resting brain may underlie the neural mechanism of acupuncture for the treatment of MCI. However, very few studies have yet investigated the functional organization of the whole brain modulated by acupuncture in MCI patients. The present study explored the whole brain functional connectivity in the resting brain modulated by DA in MCI patients compared to HC, controlling for SA. Results indicated significant changes in the functional connectivity related with the abnormal regions such as the temporal regions (hippocampus,

Table 4
The significantly different interregional correlations between DA and REST, and SA and REST in the MCI group (P<.05, multiple comparisons corrected)

Region 1	Classification	Region 2	Classification	P value
DA>REST				
R_HIPP	Temporal	R_HES	Temporal	.0057
R_PHIP	Temporal	L_MTP	Temporal	.007
L_PHIP	Temporal	R_PHIP	Temporal	.0086
R_STG	Temporal	L_HES	Temporal	.0043
R_STG	Temporal	R_MFGO	Frontal	.0027
L_INS	INS	L_SFG	Frontal	.0006
R_INS	INS	L_ITG	Temporal	.0049
R_INS	INS	R_STP	Temporal	.0061
R_ACC	Frontal	L_MCC	Frontal	.0001
L_ACC	Frontal	L_MCC	Frontal	.0005
R_ACC	Frontal	R_MCC	Frontal	.0009
R_ACC	Frontal	L_IFGT	Frontal	.0032
L_ACC	Frontal	R_IFGT	Frontal	.0053
L_ACC	Frontal	L_IFGT	Frontal	.007
L_PCC	Parietal	R_SFGM	Frontal	.0026
L_PCC	Parietal	L_SFGM	Frontal	.0031
R_OLF	Frontal	L_IFGOP	Frontal	.0071
L_SFG	Frontal	R_IFGOP	Frontal	.0021
DA<REST				
L_IFGT	Frontal	R_CAL	Occipital	.0001
L_IFGT	Frontal	L_LING	Occipital	.0004
L_IFGT	Frontal	R_LING	Occipital	.0000
L_IFGOP	Frontal	L_MOG	Occipital	.0001
R_IFGOP	Frontal	R_LING	Occipital	.0005
L_SFGO	Frontal	R_IOG	Occipital	.0009
R_CAL	Occipital	L_CUN	Occipital	.0005
R_CAL	Occipital	L_MOG	Occipital	.0003
R_SFGM	Frontal	R_FG	Temporal	.0006
SA>REST				
R_IFGO	Frontal	L_SFGO	Frontal	.007
R_IFGO	Frontal	L_SFGM	Frontal	.0028
R_IFGO	Frontal	R_FG	Temporal	.0057
R_IPG	Parietal	R_ANG	Parietal	.0029
L_PCC	Parietal	R_PUTA	Corpus	.0029
SA<REST				
L_IFGOP	Frontal	L_IFGO	Frontal	.0029
L_IFGOP	Frontal	L_MFGO	Frontal	.0014
L_IFGT	Frontal	L_MFGO	Frontal	.0025
R_IFGT	Frontal	L_IOG	Occipital	.0043
L_IFGO	Frontal	L_PCNU	Parietal	.0048
R_AMYG	Temporal	L_PAL	Corpus	.0027
R_SFGM	Frontal	L_REG	Frontal	.0024

For the abbreviations of the regions, see Table 2.

Table 5
The significantly different interregional correlations between DA and REST, and SA and REST in the HC group (P<.05, multiple comparisons corrected)

Region 1	Classification	Region 2	Classification	P value
DA>REST				
L_HIPP	Temporal	L_HES	Temporal	.0057
R_HIPP	Temporal	L_MTG	Temporal	.0019
R_HIPP	Temporal	R_MTG	Temporal	.0074
R_MTP	Temporal	R_MOG	Occipital	.0059
R_PCNU	Parietal	L_SMA	Frontal	.0020
DA<REST				
L_PHIP	Temporal	L_FG	Temporal	.0049
L_PHIP	Temporal	R_FG	Temporal	.0024
L_MFG	Frontal	L_PAL	Corpus	.0045
L_ROL	Frontal	L_PCNU	Parietal	.0037
R_ROL	Frontal	R_PCL	Parietal	.0016
L_SFGO	Frontal	L_STP	Temporal	.0014
SA>REST				
R_PCNU	Parietal	L_MFGO	Frontal	.0045
R_PCNU	Parietal	L_IFGOP	Frontal	.0056
R_PCNU	Parietal	L_IFGT	Frontal	.0002
R_PCNU	Parietal	R_MCC	Frontal	.0026
SA<REST				
L_SFGO	Frontal	R_OLF	Frontal	.0012
R_IFGT	Frontal	L_HIPP	Temporal	.0011
L_MFG	Frontal	R_IOG	Occipital	.0015
R_PCL	Parietal	L_IOG	Occipital	.0008

For the abbreviations of the regions, see Table 2.

amygdala) following acupuncture in MCI patients. These regions are implicated in memory encoding and retrieving. Furthermore, DA could enhance the correlations related with these regions compared to SA.

During the resting state, we found abnormal functional connectivity related with the temporal regions (hippocampus, thalamus, fusiform gyrus) and the prefrontal cortex (inferior frontal gyrus, orbital; olfactory cortex; superior frontal gyrus, medial; middle frontal gyrus) in MCI patients compared to HC. These regions are implicated in memory encoding and retrieving. This result is compatible with previous studies that have suggested the dysfunction of these regions in MCI patients [34–36]. On the other hand, significantly increased correlations were found mainly between interhemispheric symmetric regions (PHIP, thalamus) and within the ipsilateral regions. Although there is little previous information concerning increased connectivity in MCI, previous studies have reported increased functional connectivity within lobes in AD patients [22]. This evidence of increased connectivity has been interpreted as a recruitment of additional neural resources in these regions to compensate for losses of cognitive functionality in other brain regions. The present study extended previous findings concerning brain functional abnormalities into MCI patients.

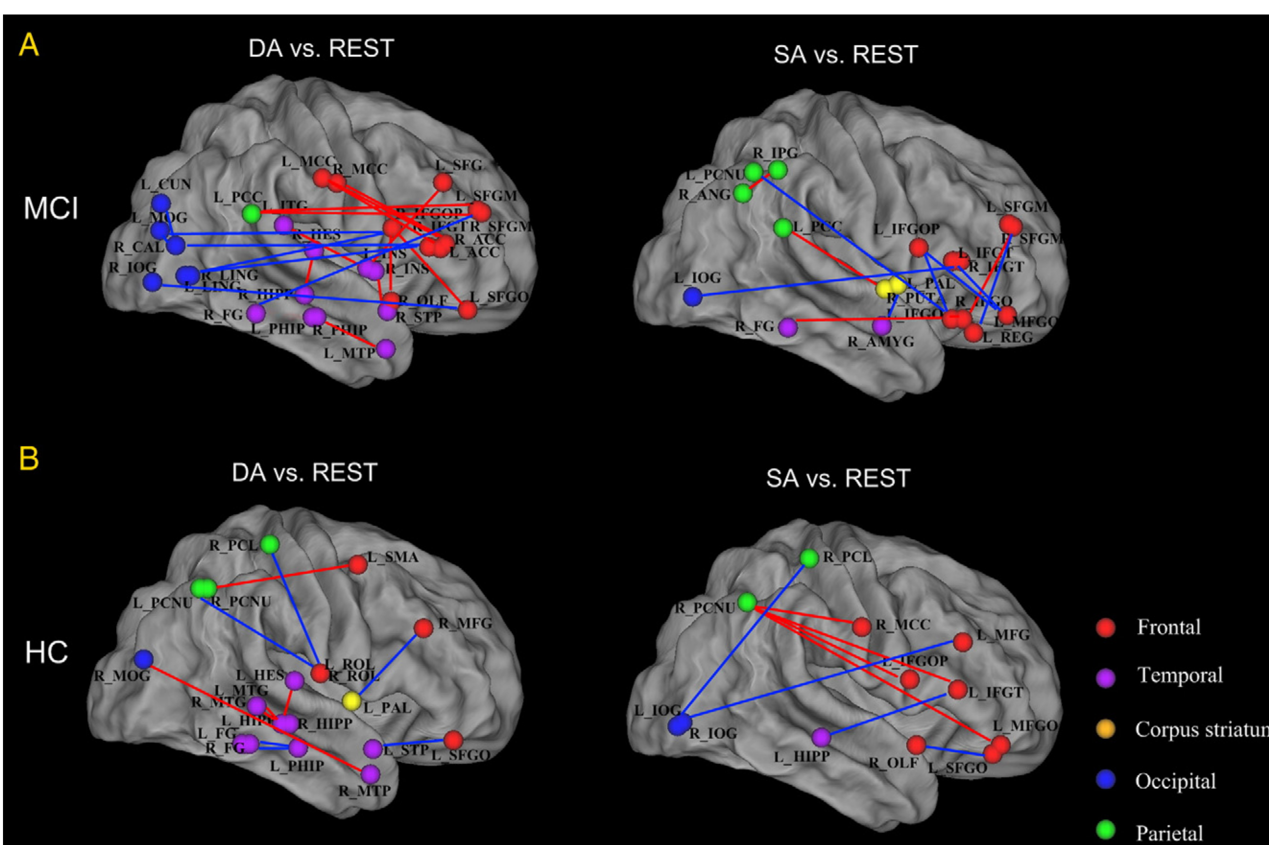

Fig. 5. Significant differences in functional correlations following DA or SA compared to REST in MCI and HC groups. The red and blue lines indicate the significantly increased and decreased functional correlations between the corresponding regions (P<.05, FDR-corrected), respectively.

Table 6
The significantly different interregional correlations between MCI and HC ($P<.05$, multiple comparisons corrected) for each condition (DA, SA)

Region 1	Classification	Region 2	Classification	*P* value
DA (MCI>HC)				
L_ACC	Frontal	L_MCC	Frontal	.0039
R_ACC	Frontal	L_MCC	Frontal	.0004
R_ACC	Frontal	R_MCC	Frontal	.0052
L_ACC	Frontal	R_IFGT	Frontal	.0004
R_MCC	Frontal	R_STP	Temporal	.0022
R_HIPP	Temporal	L_AMYG	Temporal	.0089
L_PHIP	Temporal	R_PHIP	Temporal	.0006
L_PHIP	Temporal	L_AMYG	Temporal	.0004
L_AMYG	Temporal	L_SMG	Parietal	.0050
L_STGp	Temporal	R_SMG	Parietal	.0094
L_SFG	Frontal	L_INS	INS	.0003
DA (MCI<HC)				
L_IFGT	Frontal	R_CAL	Occipital	.0017
L_IFGT	Frontal	L_MOG	Occipital	.0006
L_OLF	Frontal	L_CAL	Occipital	.0011
L_OLF	Frontal	R_LING	Occipital	.0033
L_ACC	Frontal	L_CAU	Corpus striatum	.0026
L_IOG	Occipital	R_PUTA	Corpus striatum	.0043
SA (MCI>HC)				
L_SFGO	Frontal	R_OLF	Frontal	.0076
L_MFG	Frontal	R_IFGT	Frontal	.0020
R_MFG	Frontal	L_PoCG	Parietal	.0054
R_IFGT	Frontal	L_AMYG	Temporal	.0067
R_IFGO	Frontal	L_SFGM	Frontal	.0053
R_IFGO	Frontal	R_SFGM	Frontal	.0017
L_PHIP	Temporal	R_PHIP	Temporal	.0050
L_FG	Temporal	R_PCL	Parietal	.0088
R_PoCG	Parietal	R_ANG	Parietal	.0003
R_ANG	Parietal	L_PCL	Parietal	0006
SA (MCI<HC)				
L_IFGOP	Frontal	R_PCNU	Parietal	.0091
L_IFGT	Frontal	L_PCNU	Parietal	.0013
L_IFGT	Frontal	R_PCNU	Parietal	.0009
L_SFGM	Frontal	R_SMG	Parietal	.0045

For the abbreviations of the regions, see Table 2.

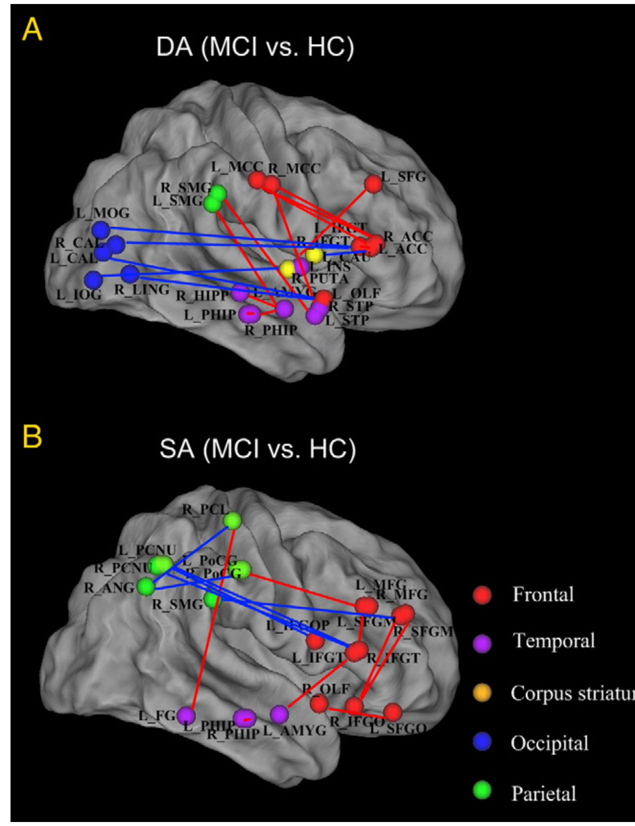

Fig. 6. Significant differences in functional correlations between the MCI and HC groups. (A) DA. (B) SA. The red and blue lines indicate the significantly increased and decreased functional correlations between the corresponding regions ($P<.05$, multiple comparisons corrected), respectively. For the abbreviations of the regions, see Table 2.

For the DA condition, we found that acupuncture could enhance the correlations related with the abnormal regions (hippocampus; olfactory cortex; superior frontal gyrus, medial) in MCI patients. The enhanced correlations in the abnormal regions between MCI and HC may relate to the specific modulatory effect of acupuncture on MCI. Abnormalities in the connectivity associated with the hippocampus in MCI have been reported by previous studies [37]. The hippocampus plays a key role in a distributed network supporting memory encoding and retrieval [38]. The significantly enhanced correlations associated with hippocampus in patients may relate to the acupuncture improvement of the impairment of memory in MCI patients. In addition, the significantly increased correlations were also related with the other regions such as the insula, PHIP and cingulate cortex. The insula is involved in diverse functions usually linked to emotion or the regulation of the body's homeostasis including perception and cognitive functioning [39]. In practice, the well-identified physical effects of acupuncture needling and its purported clinical efficacy also suggest that acupuncture acts in maintaining a homeostatic balance of the internal state within and across multiple brain systems such as the limbic regions [4,40]. The limbic regions receive both direct and indirect signals from the internal milieu, construct composite and dynamic representations of the body's state and generate regulatory signals necessary to maintain the body's homeostasis [41,42]. Thus, we inferred that the insula may also engage in monitoring the ongoing modulation of acupuncture effects on the internal homoeostasis states of the organism in MCI patients. In addition, the cingulate cortex also has a key role in affective motivation and autonomic drive of bodily responses [43]. We speculated that the increased correlations related with these emotion-related regions may relate to the behavior improvement effects of acupuncture on MCI reported by previous studies [44]. For the DA condition, the correlations related with the temporal regions (hippocampus, amygdala, PHIP), insula and cingulate cortex were also enhanced when comparing MCI to HC. These regions, which were closely correlated with the memory function, may relate to the therapeutic effect of DA on MCI. Similar patterns were found following SA but with little extent in temporal regions. These findings may provide

Table 7
The significantly different interregional correlations between DA and SA (P<.05, multiple comparisons corrected) in the MCI and HC groups

Region 1	Classification	Region 2	Classification	P value
MCI (DA>SA)				
L_MFGO	Frontal	L_IFGOP	Frontal	.0032
R_OLF	Frontal	R_SFGM	Frontal	.0020
L_SFGM	Frontal	R_SMG	Parietal	.0086
R_INS	INS	R_STP	Temporal	.0060
L_PAL	Corpus striatum	R_AMYG	Temporal	.0006
MCI (DA<SA)				
L_IFGT	Frontal	L_LING	Occipital	.0002
L_IFGT	Frontal	L_SOG	Occipital	.0014
L_IFGT	Frontal	R_CAL	Occipital	.0035
R_IFGO	Frontal	L_SFGM	Frontal	.0025
HC (DA>SA)				
L_MFGO	Frontal	R_HIPP	Temporal	.0034
R_MFGO	Frontal	R_AMYG	Temporal	.0082
R_OLF	Frontal	L_FG	Temporal	.0072
R_SFG	Frontal	R_OLF	Frontal	.0082
L_SFGO	Frontal	R_OLF	Frontal	.0012
L_MFGO	Frontal	R_IOG	Occipital	.0063
R_MTP	Temporal	R_MOG	Occipital	.0052
HC (DA<SA)				
L_ROL	Frontal	L_PCNU	Parietal	.0042
L_OLF	Frontal	R_LING	Occipital	.0034

For the abbreviations of the regions, see Table 2.

new evidence that acupuncture has a greater effect on patients than HC with a pathological imbalance.

Finally, we made the comparisons of DA vs. SA in each group to test the effect of acupuncture with different needling depths. When DA and SA were compared, we found enhanced correlations related with the temporal regions (amygdala, insula) in MCI patients. As mentioned, these regions are implicated in memory encoding and retrieving. The enhanced correlations related with these memory-related regions following DA may relate to the therapeutic effect of acupuncture for the treatment of MCI. We also found enhanced correlations related with the temporal regions (amygdala, hippocampus) in the HC group. This is consistent with previous neuroimaging studies of acupuncture which have demonstrated its significant modulatory effect on the temporal regions [9]. Deep insertion of the needle affects several structures — the skin, muscle fascia and muscle — and acupoints may better overlap with their proximity to ascending nerve tracks than to the density of cutaneous afferences [19]. The muscular afferences affect a greater number of receptors to achieve a special clinical effect than the cutaneous afferences. Therefore, the enhanced correlations associated with the memory-related regions following DA may suggest that deep muscle acupuncture has a stronger therapeutic effect for the treatment of MCI. The term “superficial acupuncture” signifies that the needle insertion affects only the skin and may not have enough appreciable clinical effect. Therefore, it can be considered a kind of placebo acupuncture. The heterogeneous functional connectivity patterns between DA and SA may suggest the importance of the muscular afferences in acupuncture.

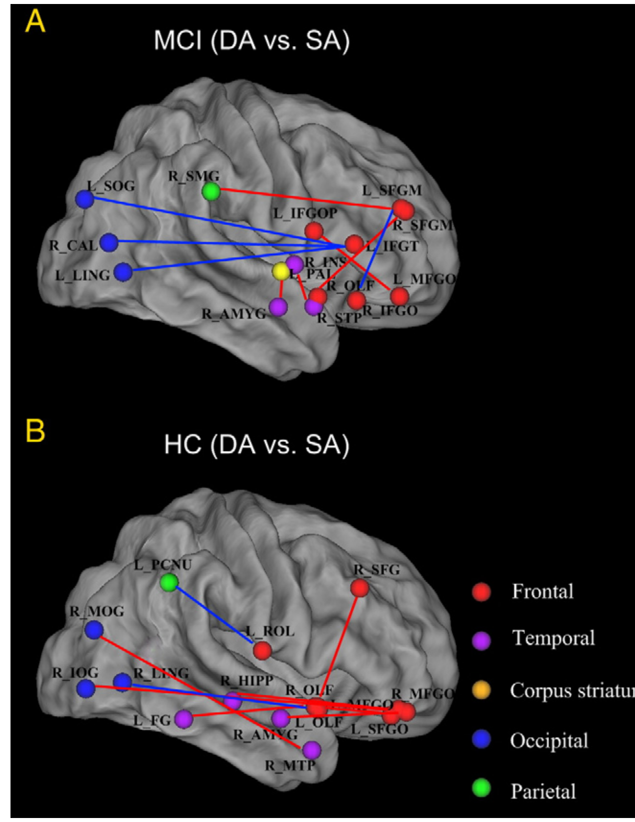

Fig. 7. Significant differences in functional correlations between DA and SA. (A) MCI group. (B) HC group. The red and blue lines indicate the significantly increased and decreased functional correlations between the corresponding regions (P<.05, multiple comparisons corrected), respectively. For the abbreviations of the regions, see Table 2.

In conclusion, our results revealed some previously unreported features of the neural mechanism of acupuncture on MCI. Consistent with previous studies, we also found the regions showing abnormal functional connectivity in MCI patients compared to HC. More importantly, we found significant changes in functional connectivity related with the abnormal regions in MCI patients following acupuncture. The significantly enhanced correlations in the memory-related brain regions (hippocampus, amygdala, insula) following acupuncture may be related to the therapeutic effects of acupuncture for the treatment of MCI. The heterogeneous functional connectivity patterns between DA and SA may suggest that deep muscle insertion of acupuncture is necessary to achieve the appreciable clinical effect.

Acknowledgments

This paper is supported by the Knowledge Innovation Program of the Chinese Academy of Sciences under grant no. KGCX2-YW-129; the National High Technology Research and Development Program of China (863 Program) under grant nos. 2008AA01Z121 and 2007AA01Z338; the Project for the National Key Basic Research and Development Program (973) under grant no. 2011CB707700; and the National Natural Science Foundation of China under grant nos. 30873462, 30970774, 60901064, 90924026, 81071137, 81071217 and 81071141.

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