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Investigation of Acupoint Specificity by Whole Brain Functional Connectivity Analysis from fMRI Data

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***Abstract*—Previous neuroimaging studies on acupuncture have primarily adopted functional connectivity analysis associated with one or a few preselected brain regions. Few have investigated how these brain regions interacted at the whole brain level. In this study, we sought to investigate the acupoint specificity by exploring the whole brain functional connectivity analysis on the post-stimulus resting brain modulated by acupuncture at acupoint PC6, with the same meridian acupoint PC7 and different meridian acupoint GB37. We divided the whole brain into 90 regions and analyzed functional connectivity for each condition. Then we identified statistically significant differences in functional correlations throughout the entire brain following acupuncture at PC6 in comparison with PC7 as well as GB37. For direct comparisons, increased correlations for PC6 compared to PC7 were primarily between the prefrontal regions and the limbic/paralimbic and subcortical regions, whereas decreased correlations were mainly between the parietal regions and the limbic/paralimbic and subcortical regions. On the other hand, increased correlations for PC6 compared to GB37 were primarily between the prefrontal regions and somatosensory regions, whereas decreased correlations were mainly related with the occipital regions. Our findings demonstrated that acupuncture at different acupoints may exert heterogeneous modulatory effects on the post-stimulus resting brain, providing new evidences for the relatively function-oriented specificity of acupuncture effects.**

I. Introduction

ACUPUNCTURE is an ancient Chinese healing technique which has been used to treat various illnesses for thousands of years [1]. Although it has gained great popularity as an alternative and complementary therapeutic intervention in the Western medicine, the neural mechanism underlying acupuncture is still unknown. In the past decades, noninvasive functional Magnetic Resonance Imaging (fMRI) techniques have provided new insights into the anatomy and physiological function underlying acupuncture [2]-[11].

Previous neuroimaging studies have primarily focused on the neural activities involving the acute effects of acupuncture [8]-[11]. According to the theory of the Traditional Chinese Medicine (TCM), acupuncture can induce long-lasting effects even after the needling manipulation being terminated [12]. Recently, several researchers have begun to pay attention to the sustained effect of the acupuncture and its influence on the resting brain [2][3][4][7][13][14][15]. Dhond et al. revealed that acupuncture can influence intrinsic connectivity of the default mode network and sensorimotor network in the post-stimulus resting brain [13]. Our group also reported that acupuncture could exert modulatory effect on the insula-anchored brain network during the post-stimulus resting period [2]. Evidence from another report suggested that a set of brain regions activated by acupuncture stimulation exhibited high temporal coherences in the post-stimulus resting state [7]. Collectively these studies demonstrated the existence of various function-guided brain networks underlying the prolonged effect of acupuncture.

Most of previous studies on acupuncture focused on the functional connectivity associated with one or a few preselected seed regions of interest (ROIs). Although ROI-based analysis can identify brain regions functionally connected to the initially selected brain regions, it is unable to completely characterize the joint interactions among multiple brain regions in the entire resting brain networks. Recently, several researchers have brought a fresh perspective to investigate the functional correlations in the resting brain networks at the whole brain level [15][16][17]. This allows us to quantitatively characterize the global organization and also provides new insight into the topological reconfiguration of the whole brain in response to external task modulations [18]. As a peripheral input to transduce signals into the brain, acupuncture may induce the reorganization of the functional connectivity across different functional brain subsystems. Therefore, it may be helpful to investigate the functional correlations from the perspective of whole brain for a better understanding of the basic neurophysiological mechanisms underlying acupuncture.

In this study, we employed acupuncture at PC6 (Neiguan, an important acupoint for preventing nausea and vomiting) as the study acupoint to test 1) whether acupuncture at PC6 can specially modulate the neural correlates underlying the control

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of nausea and vomiting, compared with acupuncture at antiemetic-irrelevant gallbladder 37 (GB37) (Guangming, located at different meridians serving as treatment for multiple vision-related disorders); and 2) whether there were some similarities and differences between PC6 and PC7 (belonging to the same meridian and median innervation). We assumed that the introduction of multiple controls can help elucidate the specificity of the neural expression underlying the antiemetic acupoint PC6. To this end, we performed whole brain functional connectivity analysis on the post-stimulus resting brain modulated by acupuncture at the PC6, PC7 and GB37. 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 [15]. Firstly, we divided the whole brain into 90 cortical and subcortical regions and obtained the functional connectivity for each condition. Subsequently, the statistically significant differences were identified by comparing the correlation coefficients of each pair in the resting brain following acupuncture at acupoint PC6 in comparison with PC7 as well as GB37. This allowed us to test whether the modulatory effects of acupuncture on the whole resting brain networks were functional-specific.

II. Methods

A. Subjects

In order to reduce intersubject variabilities, thirty-six healthy right-handed college students (18 males, aged 21.4 ± 1.3) were recruited from a homogeneous group. All subjects were acupuncture naïve according to the Edinburgh Handedness Inventory [19]. Subjects were screened and excluded for major medical illnesses, head trauma, neuropsychiatric disorders, intake of prescription medications within the last month, and any contraindications for exposure to a high magnetic field. After given a complete description of the study, each of the subjects signed the informed consent form. Subjects were allocated to receive once acupuncture at one of the three acupoints in a random sequence by using an envelope method, with the gender ratio balanced (ANOVA, $P > 0.6$) among different groups. All protocols were approved by a local subcommittee on Human Studies.

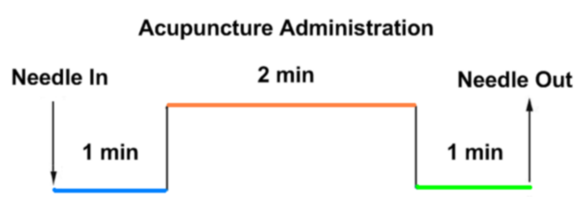

Fig. 1. Experimental paradigm. The images acquired during the time points labeled by green color were used for the whole brain functional connectivity analysis.

B. Experimental paradigm

The fMRI scanning for each acupoint incorporated 2-min needling manipulations, preceded by a 1 min rest, and followed by another 1 min rest scanning (Fig. 1). Acupuncture was performed on three acupoints PC6 (Neiguan), PC7 (Daling) and GB37 (Guangming) (Fig. 2). During the experiment, the subjects were instructed to keep their eyes closed and remain relaxed without engaging in any mental tasks. A sterile disposable 38 gauge stainless steel acupuncture needle (0.2 mm in diameter and 40 mm in length) was used to deliver acupuncture stimulation. During acupuncture, the needle was rotated manually clockwise and counterclockwise for 1 min at a rate of 60 times per min by a balanced "tonifying and reducing" technique [9]. None experienced the sharp pain among the thirty-six participants.

Fig. 2. Illustration of anatomical locations of three acupoints: PC6 (Neiguan), PC7 (Daling) and GB37 (Guangming).

C. Data Acquisition and Analysis

Magnetic resonance imaging data were acquired using a 3.0 Tesla Signa (GE) MR scanner. Head movements were prevented by a custom-built head holder. The images were parallel to the AC-PC line and covered the whole brain. Thirty-two axial slices were obtained using a T2*-weighted single-shot, gradient-recalled echo planar imaging (EPI) sequence (FOV = 240 mm x 240 mm, matrix = 64 × 64, thickness = 5 mm, TR = 2000 ms, TE= 30 ms, flip angle = 90°). After that, all images were pre-processed using 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^3. After that, the functional images were spatially smoothed with a 3D Gaussian kernel (FWHM = 6 mm). In addition, several procedures were adopted to remove possible spurious variances from the data through linear regression [16][20]. Finally, the fMRI waveform of each voxel was temporally band-pass filtered ($0.01\ Hz < f < 0.08\ Hz$).

D. 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. [21]. For each subject, we obtained the mean time series of each of the 90 regions by averaging the fMRI time series over all voxels in the region respectively for each acupoint. Then the time series were correlated region by region with Pearson's correlation coefficient in each dataset [15][16][17]. Fischer's r to z transformation was applied to improve the normality of the correlation coefficients. For direct comparisons, we performed two-sample two-tailed t-test ($P < 0.05$, FDR corrected) on all 4005 possible connections represented in the two 90×90 correlation matrices to determine the brain regions showing differences in functional correlations between PC6 and PC7, as well as between PC6 and GB37 [22].

III. Results

The results are visualized using Pajek software (www.vlado.fmf.uni-lj.si/pub/networks/pajek).

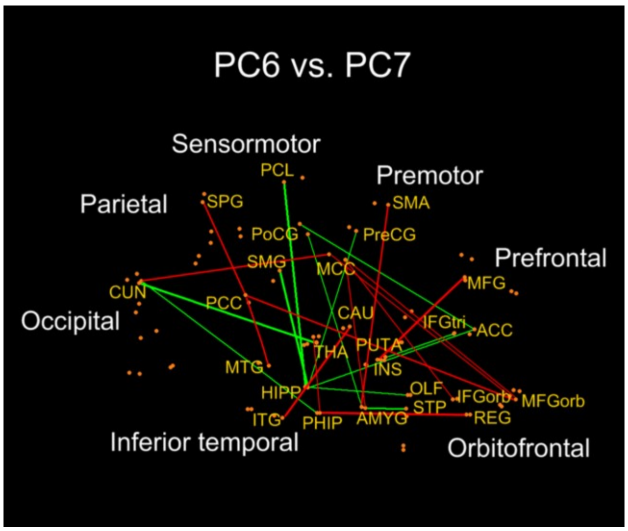

Fig. 3. Graph visualization of the significant differences in functional correlations for PC6 vs. PC7. Nodes are located according to the y and z coordinates of the regional centroids in Talairach space. The red and green lines indicate the significantly increased and decreased correlations between the corresponding regions, respectively (all lines meet $P < 0.05$, FDR corrected).

As shown in Fig. 3, increased correlations for PC6 compared with PC7 were primarily between the prefrontal regions and the limbic/paralimbic and subcortical regions such as the parahippocampal gyrus (PHIP), amygdala (AMYG), putamen (PUTA) and caudate (CAU). In addition, decreased correlations for PC6 compared with PC7 were mainly between the parietal regions and the limbic/paralimbic and subcortical regions such as the anterior cingulate gyrus (ACC), PHIP, hippocampus (HIPP), AMYG and thalamus (THA).

As shown in Fig. 4, compared with GB37, increased correlations for PC6 were primarily between the prefrontal regions and the somatosensory regions, whereas decreased correlations for PC6 compared with GB37 were mainly related with the occipital regions such as the inferior occipital gyrus (IOG), superior occipital gyrus (SOG), middle occipital gyrus (MOG) and fusiform gyrus (FG).

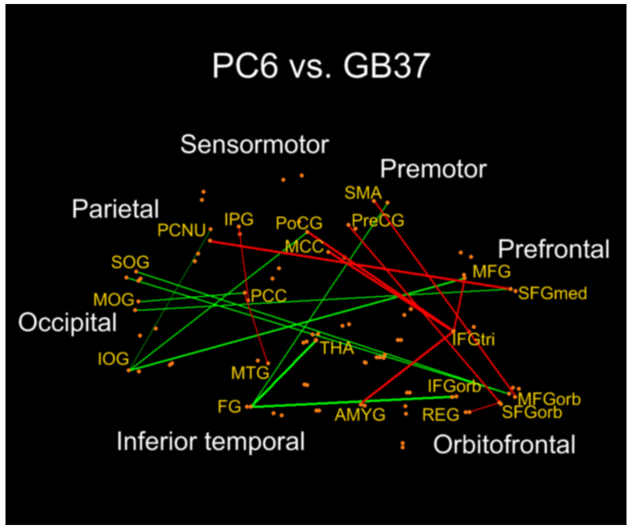

Fig. 4. Graph visualization of the significant differences in functional correlations for PC6 vs. GB37. Nodes are located according to the y and z coordinates of the regional centroids in Talairach space. The red and green lines indicate the significantly increased and decreased correlations between the corresponding regions, respectively (all lines meet $P < 0.05$, FDR corrected).

IV. Discussion and Conclusion

In this paper, we investigated acupoint specificity by whole brain functional connectivity analysis on the post-stimulus resting brain following acupuncture at three acupoints PC6, PC7 and GB37. To our knowledge, few previous studies have reported the modulation patterns exerted by acupuncture on the entire resting brain. Therefore, identifying functional correlations at the whole brain level may further shed light on how such peripheral inputs are conducted and mediated through the central nervous system.

The result presented that the increased and decreased correlations were mainly related with limbic/paralimbic and subcortical regions for PC6 compared to PC7. Previous studies also demonstrated that the limbic/paralimbic and subcortical brain regions can be modulated by acupuncture [9][10]. Given that limbic-cerebellum and subcortical areas are more engaged in affective motivation and autonomic drive of bodily responses, researchers speculate that acupuncture at these acupoints generally produces antistress and antianxiety effects [23]. Along the same line, acupuncture at PC6 and PC7 (belonging to the pericardium meridian) has been used clinically to treat mental and psychosomatic disorders. Therefore, we proposed that the connections related with these regions following acupuncture administration may be related to its clinical efficacy to produce a sedative or tranquilizing effect after a variety of stressors or depression [24].

Even with the relative similarity, the increased correlations for PC6 compared to PC7 were primarily between the prefrontal regions and the limbic/paralimbic and subcortical regions, whereas decreased correlations were mainly between the parietal regions and the limbic/paralimbic and subcortical regions. The prefrontal cortex has been reported for its central role in pain-modulation [25]. Simulation of PC 6 can relieve the pain of the patients according to previous studies [26]. The increased correlations related with prefrontal cortex for PC6 compared to PC7 may relate to such pain relief effects of PC6. In addition, the parietal lobe dysfunction in the depression has been previously demonstrated [27]. Therefore, the increased correlations between the parietal and other regions for PC7 compared to PC6 may provide an explanation for the mediate effect of PC7 to treat depressive disorders. Results indicated that acupuncture at different acupoints along the same meridian, even with the relative similarity, presented heterogeneous modulation patterns on the whole functional brain network during the post-stimulus period.

Compared to GB37, the increased correlations for PC6 were primarily between the prefrontal regions and somatosensory regions, whereas decreased correlations were mainly related with the occipital regions. The occipital regions mainly support vision-related processing. As its name "brightness" implies, GB37 was described as a very effective acupoint influencing multiple vision-related disorders, such as the cataracts, night blindness and optic atrophy [28]. The increased correlations related with occipital regions for GB37 compared to PC6 may elucidate the treatment of GB37 in vision-related disorders. The results indicated that acupuncture at acupoints along different meridian may induce distinct reorganization of the functional connectivity across different neural subsystems.

In conclusion, our findings demonstrated that acupuncture at different acupoints may exert heterogeneous modulation patterns on the post-stimulus resting brain. Results implied that therapeutic effects of acupuncture may depend on the modulation of certain brain areas related with special functional disorder. These findings may provide new evidences for the functional specific modulatory effects of acupuncture.

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