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The correlated network of acupuncture effect: a functional connectivity study

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***Abstract*—A functional connectivity, which are temporally correlated in functionally related brain regions, before and after acupuncture manipulation was measured by MRI. Amygdala, as the control system of endogenetic analgesia, was selected for "seed" point. We found that compelling similarity existed in the network of resting state before and after acupuncture manipulation. A paired student t-test was implemented to investigate under the different conditions. The main difference was found in the limbic system, brainstem and cerebellum. We conclude that the default endogenous analgesia functional network exists in human brain at a low level, and it could be increased to a higher level by acupuncture modulation.**

I. Introduction

Acupuncture is an important therapeutic modality in Traditional Chinese Medicine. In clinical practice, the effect of acupuncture is coupled with a local tingling sensation, the so-called "deqi"-phenomenon. Among the versatile clinical practice, acupuncture analgesia was one of the important aspects to be explained on the basis of physiology and anatomy [1] [2]. Results from human and animal studies [2] indicated that acupuncture acts as a neuromodulating input into the central nervous system (CNS) that can activate multiple analgesia systems, and stimulate pain modulation systems to release neurotransmitters such as endogenous opioid peptides.

As we know, the Limbic system is composed with hypothalamus, hippocampus, amygdala, nucleus accumbens and anterior cingulated. And these areas are associated with the release of various neurotransmitters, such as beta-endorphins, dopamine and 5-HT. These neurotransmitters are related with acupuncture analgesia. Some researchers [4] [5] [6] indicated the key role of limbic system in their fMRI acupuncture study. Amygdala, the critical nucleus of limbic system, has an intimated relationship with acupuncture analgesia [5] [6].

Cho et al. first investigated the CNS modulating effect of acupuncture through a block designed paradigm on fMRI. They demonstrated that both the static and dynamic activation of the visual cortex by acupoint VA1 (a vision-related acupoint) stimulation are consistent with visual light stimulation previously observed by fMRI [24]. Then, Wu et al. confirmed that acupuncture at ST.36 and LI.4 (known as hegu acupoint, having the analgesia and sedation effect in clinic) activated structures of descending antinociceptive pathway and deactivated multiple limbic areas subserving pain association though a block designed paradigm on fMRI [4]. Hui et al. further confirmed the results of Wu and provided evidence supporting a coordinated effect of acupuncture needle manipulation on a network of cortical and subcortical limbic and paralimbic structures in the human brain [5]. Amount of block fMRI acupuncture studies also demonstrated the CNS modulation effects of acupuncture with electric acupuncture or laser acupuncture. The similarity of above acupuncture studies are that they are all based on a block designed paradigm. That is, on and off periods repeated several times, in the on state the acupuncture stimulus imposed on the subjects at ST.36 or other acupoints lasting for 1 to 3 minutes and in the off state the acupuncture stimulus removed [4][5][6][24]. With blocked methods, detection of brain activation is based on probable assumptions of the temporal activation patterns suggested by the experimental paradigm. For example, when subjects perform visual or motor tasks, it is expected that the corresponding visual or motor cortex areas will be activated almost simultaneously with the performance of the task. This approach, however, is not appropriate in cases that lack clear temporal information, such as drug stimulation or eating [25]. As for acupuncture stimulus, we also do not know the accurate temporal response patterns. The effect of acupuncture can last for several minutes to several days in clinic [26]. In addition, lasting for 1 to 3minutes off state may not obtain a maximal effect of acupuncture. Moreover, in previous studies, we obtained a lot of activated areas through block designed paradigm, but which the activated areas are correlated with acupuncture effect? We can not answer these questions through previous studies.

The term functional connectivity describes the interdependence of brain regions functionally related and connected [7]. It reflects the spontaneous fluctuation of blood oxygenation level-dependent (BLOD) [8]. Recently, the analysis of low frequency (< 0.08 Hz) temporal fluctuations in MR signals in brain during resting state attracts a lot of attention. Such correlations were first observed between motor regions [14] [15] and, of course, reported within the visual system [12] [16] as well as other brain networks with known anatomical information. The functional connectivity has been widely studied for the interactions between the language regions of brain [9]. In terms of acupuncture, the functional connectivity not only

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determines the corresponding correlation network, but also makes the process more similar to clinical practice.

In present studies, we applied "seed" point correlation analysis to investigate functional connectivity of acupuncture before and after acupuncture manipulation on healthy subjects at ST36 of right leg. So a region of interest contained left amygdala was selected as the seed point (see methods) according to prior anatomic information. To explore the related acupuncture analgesia network, we hypothesized that the amygdala plays an important role in the pain modulating network existed in the human brain and acupuncture can modulate this analgesia network.

II. Method

A. Subjects

The study was performed on 14 right-handed Chinese healthy college students, seven male and seven female at the age of 22–26 years old (24.3 +/- 1.5) with informed consent. All of subjects had never experienced acupuncture.

B. Experimental protocol

The subject was settled into the scanner and instructed to close the eyes and relax throughout the imaging session. First, a scan was performed during resting state without any stimulation lasting for 15 minutes. Then, a scan was performed during persistent acupuncture needle manipulation on the ST36 followed by 15 minutes steady states scan. These two runs are interval 30 minutes.

The needle was inserted perpendicularly to the skin surface to a depth of 2-3 cm for 1 minute. The needle was rotated clockwise and counterclockwise 120 times /min for 2 minutes [4] [5]. The needle was remained in the place for 15 minutes and then removed. The procedure was performed by the same experienced and licensed acupuncturist on all subjects. At the end of the experiment, the subject was questioned for the intensive sensation of aching, pressure, soreness, heaviness, fullness, warmth, coolness, numbness, tingling, dull pain, sharp pain and others, etc. The intensive sensation rate was from 0 to 10 (0 = no sensation, 1–3 = mild, 4–6 = moderate, 7–8 = strong, 9 = severe and 10 = unbearable sensation) [5]. The rate was used to distingush de-qi frompain. The subjects were chosen for obvious de-qi or de-qi was prominent than pain.

C. Imaging

Subjects lay in a supine position in the quadrature head coil of a 3T Seimens Trio scanner (Siemens, Erlangen, Germany) for shot gradient echo-planar imaging. Foam pillow and a band across the forehead were used to restrict head movement. Functional images were collected with sagittal sections parallel to the AC-PC plane in 6 mm thickness without gap. Imaging encompassed the entire brain, including the cerebellum and brainstem. The data was acquired by a T2*- weighted gradient echo sequence (TE 30 ms, TR 2 s, matrix 64 x 64, FOV 220 mm, flip angle 90, in-plane resolution 3.4 x 3.4 mm). A set of 3D MPRAGE (magnetization-prepared rapid acquisition gradient echo) images, voxel size 1 mm^3, 176 images per set, and a set of T1-weighted high-resolution structural images were acquired prior to functional scan.

D. Data Analysis

Twelve of fourteen subjects experienced the obvious de-qi sense participated in the study and two subjects showed the remarkable sense of pain was excluded.

The first 10 time points of the resting state data set were discarded because of the instability of the initial MRI signal leaving 460 time points. For acupuncture data, the time points after manipulation were discarded (90 time points). 15 minutes resting state dataset was taken after acupuncture. As the same as the resting state, the first 10 time points were discarded. Overall, we have got the data set of two 460 time points for both conditions before and after acupuncture manipulation. All data were motion-corrected using the INRIalign toolbox in SPM [10]. (cf. www.madic.org) Then a histogram process was performed to create a mask (this removed those pixels outside the brain) [11]. Data were further examined, those ghosting and with obvious artifacts were excluded [9]. In addition, the translation and rotation were checked and the images that associated with the movement in any direction of greater than 0.8mm or head rotation of greater than 0.8 degree were excluded from analysis.

The data were processed (spatially normalization to NMI space and re-sampled at $3mm^3$) by SPM2 (www.fil.ion.ucl.ac.uk/spm/). An algorithm proposed by http://www.mrc-cbu.cam.ac.uk was used to transform the MNI coordinates to Talairach coordinates. Then global mean and linear trends were removed to eliminate the gross signal drifts, which were ostensibly due to scanner instabilities or gross physiological changes in the subjects. Finally, the data were spatially smoothed using a 4mm FWHM Gaussian kernel. After those procedures, the data were low-pass filtered using FIR filter with a cutoff frequency of 0.08 Hz to remove the oxygenation fluctuations from direct sampling of respiratory and cardiac-related oxygenation fluctuations.

E. Regions of Interest

The left side amygdala area (Talairach coordinate [-20, -8, -10]) was defined based on anatomic structure, Talairach coordination and three expert neural physician as the regions of interest (ROI). The ROI was chosen carefully in each subject to avoid including pixels containing large vessels. The timecourses of voxels within the ROI were averaged to generate a single low-frequency reference time series, named "seed" timecourse. For each subject, the seed timecourse was cross-correlation with all other low-pass filtered voxels to form functional connectivity maps. These correlation coefficient maps were then normalized to an approximately normal distribution using Fisher's transformation [14]. These approximately normal distributions were corrected to approximately standard normal distributions using the methods described in Lowe et al [9][12]. Finally, the normality of the distribution was tested using the kurtosis test ($P < 0.05$). Then, the individual two z-maps entered into a random effect one-sample t-test respectively [13] to determine brain regions showing significant functional connectivity across subjects. Finally, the t-maps were superimposed on high-resolution anatomical images (Fig. 1).

As to compare the difference of resting state connectivity and the resting state connectivity after acupuncture, a paired student t-test was implemented across subjects. The final t-maps were superimposed on high-resolution anatomical images (Fig. 2).

The above procedures were coded in MATLAB (The MathWorks, Inc.), SPM2 package and FMRIstat package.

III. RESULTS

From figure 1a, it was clear that the left amygdala showed significant resting-state connectivity in the regions of superior/middle frontal gyrus superior occipital sulcus, posterior cingulated cortex (PCC), anterior cingulated cortex (ACC), nucleus accumbens, and hypothalamus, caudate, a part of putamen, posterior thalamus, hippocampus, frontal lobe and parietal lobe.

The connectivity network of acupuncture resting state is more extensive and significant. Figure 1b indicated that the left amygdala showed significant connectivity with the regions of superior/middle frontal gyrus, parietal lobe, frontal lobe, PCC, ACC, nucleus accumbens, and hypothalamus, caudate, a part of putamen, posterior thalamus, hippocampus, dorsal thalamus, cerebellar vermis and mesencephalon periaqueductal grey matter (PAG).

From the result (Fig.2) of paired student t-test of the resting state and resting state after acupuncture, we confirmed that the compelling differences mainly exist at anterior frontal cortex and brain stem and cerebellum. That is ACC, PCC, nucleus accumbens, hypothalamus, PAG.

IV. DISCUSSION AND CONCLUSION

This article presents the first systematic study of functional connectivity to analyze the correlated network of amygdala before and after acupuncture manipulation. Functional connectivity focus on the temporal correlation of voxels. A lot of animal studies confirmed that the amygdala had been implicated in fundamental functions for the survival of the organism, such as pain. The animal study also showed the high density of opioid receptor in amygdala, and the threshold of the pain can obviously be increased by stimulation [17] [18]. Petrovic et al. suggested amygdala may contribute to pain processing both directly by regulating nociceptive modulating systems in the brainstem and indirectly by controlling behavioral and autonomic output during pain [20]. At the same time, amount of fMRI studies of acupuncture discovered the activation of amygdale [4] [5] [6]. Therefore, we proposed the amygdala play a critical role in the acupuncture analgesia circuit. We selected the amygdala as the seed voxels of acupuncture effect network is logical. Through analyzing the activity of amygdala in acupuncture analgesia we should obtain the relative network of acupuncture effect.

Our results showed that the amygdala is correlated with superior frontal gyrus, middle frontal gyrus, superior temporal gyrus, ACC, PCC, thalamus, insula, angular gyrus, caudate, putamen, a part of hypothalamus, parahippocampal gyrus and nucleus accumbens in resting state. The results are basically similar to the result of Greicius et al [21]. They provided the default mode of brain in resting state. Their article proposed that human brain exists a default mode state under resting state. This default network is associated with affective and autonomic processing including nucleus accumbens, hypothalamus, midbrain, ACC and PCC.

The results of correlative map of the resting state after acupuncture manipulation were very similar with the resting state except for higher significance. The compelling difference between these two correlated maps was the PAG, in addition, for cerebellum was involved after acupuncture manipulation.

The PAG is an important structure in midbrain and it is established as the gate region for the afferent pathway in brain [23]. Studies displayed that stimulation of acupuncture points caused potentials to occur in the PAG [22]. Meanwhile, physiological research confirmed that PAG is full of 5-HT neuron and endorphins. These materials play an important role in endogeous analgesia.

To compare the difference between resting state before and after acupuncture manipulation, we performed a paired student t-test between these two conditions. The results showed that the main difference was in anterior frontal cortex, ACC, thalamus, nucleus accumbens, hypothalamus, brainstem, PAG and cerebellum. The cerebellum has closely correlated with acupuncture manipulation. Many fMRI works reported the cerebellum activation [4] [5] [6].

We found the compelling similarity between resting state and resting state after acupuncture manipulation. (The PAG and cerebellum appeared in resting state correlated map with a lower t value. $T = 2.68$ $P < 0.025$). We believe that the default endogenous analgesia network exists in human brain at a low level. And it can be modulated to a higher level by acupuncture. Figure 1 confirms that the statistic significance for the resting condition can be obviously increased by acupuncture. We guess this modulation of acupuncture maybe the mechanism of acupuncture analgesia.

This is the first systematic study of functional connectivity before and after acupuncture manipulation. Further experiment with larger group will be continued to approve this preliminary achievement

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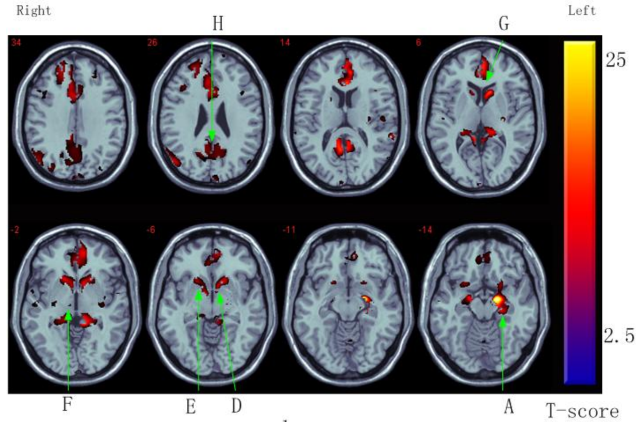
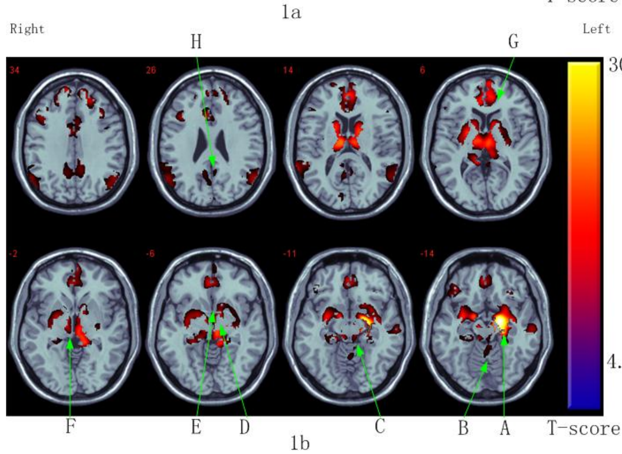


1a



1b

Fig. 1: Maps of the functional connectivity: 1a is for resting state ($t > 2.68$, df = 12, $p < 0.01$ uncorrected); 1b is for the post-effect of acupuncture resting state ($t > 4.2$, df = 12, $p < 0.001$ uncorrected). A: the approximate locations of left amygdale. We choose this cluster as the seed point. B: the cerebellar vermis. C: the mesencephalon periaqueductal grey matter (PAG). D: the nucleus accumbens. E: the caudate nucleus centered at. F: the hypothalamus. G: the anterior cingulated cortex (ACC). For more detail, see Table 1.

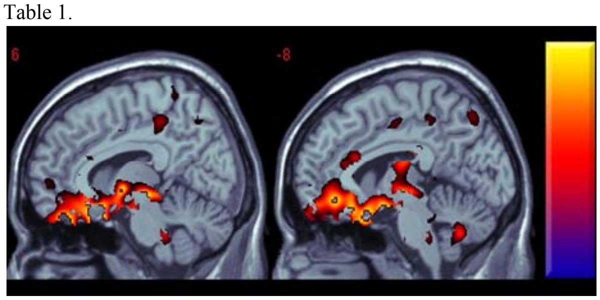


Fig. 2: Maps of the difference between resting state and post-effect of acupuncture resting state. ($t > 1.812$, df = 12, , $p < 0.05$ uncorrect)

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