ACTIVATION OF DIFFERENT CEREBRAL FUNCTIONAL REGIONS FOLLOWING ACUPUNCTURE AT BOTH H TAIXI AND TAICHONG ACUPOINTS AND TAIXI ACUPOINT ALONE: AN FMRI STUDY

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Abstract

\textbf{Background:} To explore the brain function regions characteristics of the acupoint combination, this study observed activity changes in the brain regions of healthy volunteers after acupuncture at both Taixi (KI3) and Taichong (LR3) (KI3 + LR3) and KI3 alone using resting-state functional magnetic resonance imaging (fMRI).

\textbf{Methods:} 30 healthy volunteers were randomly allocated into two groups, one group received acupuncture at KI3 and LR3, the other only acupuncture at KI3, 15 cases in each group. All volunteers underwent resting-state fMRI of the brain 15 minutes before acupuncture, and which the needle was retained in place for 30 minutes; 15 minutes after withdrawing the needle underwent a further session of resting-state fMRI. The amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) were used to analyze the changes in brain regions.

\textbf{Results:} The KI3+LR3 group compared with the KI3 group, the ALFF analysis indicated that the brain changes relatively concentrated in BA 2, 3, 7, 8, 9, 10, 18, 19, 20, 31, 32, 40, 46 and the cerebellum posterior lobe, the ReHo analysis indicated that the brain changes relatively concentrated in BA 4, 6, 7, 10, 11, 18, 19, 20, 21, 22, 31, 40 and the cerebellum posterior lobe.

\textbf{Conclusion:} Based on this study, compared with acupuncture at KI3, acupuncture at KI3 + LR3 which could specifically influence BA 7, 10, 18, 19, 20, 31, 40 and cerebellum posterior lobe, which may be related to synergy mechanism of two acupoints combination treatment.

\textbf{Keywords:} acupoint combination, acupuncture, resting state, functional magnetic resonance imaging (fMRI), amplitude of low-frequency fluctuation (ALFF), regional homogeneity (ReHo)

Introduction

Acupuncture, a form of traditional Chinese medicine, has a long history and remarkable curative effect. It is well-known that the therapeutic effect of acupuncture is based on the stimulation of acupoints; an acupoint is the fundamental element of acupuncture prescription (Chen et al., 2012). The essence of acupuncture prescription is acupoint combination. In the clinical setting, acupoint is usually used in the form of acupoint combination; combination of two acupoints is the smallest unit of acupoint combination. Currently, to approach clinical reality, clinical research or observation design of acupuncture is based on acupoint combination (Yang et al., 2014; Garrow et al., 2014; Han et al., 2014). Therefore, we think that the research of basing on acupoint combination is one of the important aspects of acupuncture mechanism study.

During the past 20 years, a large number of researchers have utilized functional MRI (fMRI), a non-invasive imaging technique mapping brain function, to investigate brain mechanisms underlying point specificity and the acupuncture therapy. In order to preliminary explore the neural mechanism of acupuncture, the signal changes of brain functional area of needling the three acupoints (ST36, SP6 and GB34) in three different meridians in human by functional magnetic resonance imaging (fMRI) were observed, the research speculated that brain response in special regions could be obtained by needling at different acupoints (Fang et al., 2006). To probe the theoretical basis of acupuncture, Hu, K. M et al. using functional magnetic resonance imaging (fMRI) to observe the cerebral functions, further to investigate the relationship of true acupuncture and sham acupuncture, they thought that acupuncture acupoint was based on certain central nervous system basis(Hu et al., 2006).
The research demonstrated that the acupuncture stimulation of the PC6 can modulate cerebellar areas, especially relevant to vestibular neuromatrix (Yoo et al., 2004). Neuroimaging signal change areas of acupuncture at LI-2 correlated to saliva production (Deng et al., 2008). Acupuncture at different acupoints (PC6, PC7 and GB37) may exert heterogeneous modulatory effects on the causal interactions of brain areas during the poststimulus resting state. These brain regions may be related to the effects of acupoint on modulating special disorder treatment. This preliminary finding may provide a new clue to understand the relatively function-oriented specificity of acupuncture effects (Fang et al., 2011).

Our research team also proposed that acupoints were related to the brain function, and thought that acupuncture researches in vivo based on brain function imaging can better reflect the clinical characteristics on the changes of brain function areas (Lai et al., 2007). Using fMRI to study the central mechanism of the acupoint combination, previous researchers have observed the different combinations of Waiguan (TE5) and acupoint combination of Baihui (GV20) and Yintang (GV29). In the study of different combinations of TE5, the activating cerebral functional areas were different from each other; therefore, we thought that different acupoint combinations can result in the activation of different brain regions (Lai et al., 2011). In the study of acupoint combination of GV20 and GV29, psychiatric disorder-related cerebral functional regions were activated/deactivated using stimulation by EA at Yintang and GV20, such as the frontal lobe, cingulate gyrus and cerebellum. We speculate that these results indicate possible mechanisms by which EA at these points brings about psychiatric and emotional adjustments (Zheng et al., 2012). In the present study, the design and methods were improved during the course of research, for instance, block design was developed to resting-state research in design of the fMRI scan, from immediate effects of acupuncture to post-acupuncture effects, from stratified observation to three-dimensional reconstruction of the brain, and from univariate analysis to collaborative research of ReHo and ALFF (Lai et al., 2011; Zheng et al., 2012).

Recently, related researches also have focused to acupoint combination. Zhao et al. compared the central difference of two different acupoint combinations by fMRI, such as Waiguan(TE5) and Qixue(GB40), Neiguan(PC6) and Taichong(LR3)( Zhao et al., 2014). Li et al. also observed the analgesic effect of Weizhong (BL40) combining with Huantiao (GB30) in patients with sciatica, and specific changes in the brain default network (Li et al., 2012). Wang et al. observed the effect of acupuncture Taichong(LR3) and Hegu(LI4) for mild cognitive impairment (MCI) and Alzheimer diseases (AD), and found that acupuncture at LR3 and LI4 can specifically activate cognitive-related regions in AD and MCI patients(Wang et al., 2012). These studies were different from acupuncture at a single acupoint, in which the participants accepted to acupuncture at two acupoints or more than two for treatment, and observed the brain regions’ changes of acupuncture acupoint combination by fMRI. However, these studies did not compare difference of between acupoint combination and single acupoint, simply observed the combined effect of acupoint combination, and only Li et al. focused on the post- acupuncture effect by a resting-state fMRI design (Li et al., 2012), the resting-state fMRI design was more in line with clinical practice.

Therefore, on basis of previous studies, in order to explore the brain function regions characteristics of the acupoint combination, and then clarify brain mechanisms that mediate therapeutic effects of acupuncture. This study focused on the post- acupuncture effect. observed and compared the influence of acupuncture acupoint combination(KI3 + LR3) and single acupoint(KI3) on brain function regions by resting-state fMRI design.

Subjects and Methods

Subjects

30 healthy volunteers (11 males and 19 females) were affiliated with universities and colleges in Guangzhou, Guangdong Province, China. All volunteers voluntarily participated experiment, and signed the informed consent before the experiment. This study was approved by the Chinese Ethics Committee of Registering Clinical Trials (ChiECRCT-2012011) and registered in the Chinese Clinical Trial Register (ChiCTR-TRC-12002427). Using randomized controlled clinical research method, random number table was generated by statistics software. 30 healthy volunteers were randomly allocated into two groups, the KI3+LR3 group acupuncture at KI3 and LR3, the KI3 group only acupuncture at KI3, and 15 cases in each group. The basic information of two groups was shown in Table 1, and there were no significant differences between each other (P>0.05) (Table 1).

Inclusion criteria: (1) Undergraduates in the city of Guangzhou aged 21–28 years, who had not undergone acupuncture more recently than 1 month prior to volunteering for our study. (2) Right-handed. (3) Regular diet, not addicted to alcohol and tobacco, tea, coffee, etc., normal sleep patterns (have a rest before 12 am), present physical and mental health. (4) Moderate weight (BMI is 18.5–23.9). (5) Acupoint site without skin damage or disease. Exclusion criteria: (1) Metal in the body, such as a pacemaker or metal dentures. (2) Acupuncture hypersensitivity during a preliminary test of acupuncture response 1 month prior to the experiment. (3) Previous recent (within 1 month) acupuncture treatment. (4) Pain

http://dx.doi.org/10.4314/ajtcam.v12i5.16

(including dysmenorrhea) or insomnia within 1 month before the experiment. (5) Fear of confined spaces or strong reactions to noise and hypothermia.

<table>
<thead>
<tr>
<th>Items</th>
<th>KI3 + LR3 (n=15)</th>
<th>KI3 (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>22.80±0.941</td>
<td>21.07±0.799</td>
<td>0.487</td>
</tr>
<tr>
<td>Height</td>
<td>166.53±7.763</td>
<td>164.93±9.765</td>
<td>0.669</td>
</tr>
<tr>
<td>Weight</td>
<td>56.20±7.655</td>
<td>55.07±11.222</td>
<td>0.304</td>
</tr>
</tbody>
</table>

Note: Data were presented as mean±SD, group difference was tested using a two-sample t-test

Methods

Experiment procedure

All subjects were asked to pass urine and stool prior to experiment. After resting for 15 minutes, fMRI scan was performed for 8 minutes, which included a 2-minute transverse scan and a 6-minute resting-state blood oxygenation level dependent sequence scan. Then, acupuncture was conducted, and the needles were maintained in place for 30 minutes. 15 minutes after withdrawing the needle, the same another 8-minute scan was performed. The volunteers’ eyes were masked (Hanjiang Xinhua Tourist Supplies Factory, Yangzhou, Jiangsu Province, China), and earplugs (Aearo, Indianapolis, USA) were worn, to avoid audio-visual stimulation during the experiment. The volunteers were unknown which acupoints were used. All procedures (including initial screening) were performed by professionals.

Acupuncture intervention

Acupuncture was performed in all subjects by the same experienced acupuncturist. The location of KI3 and LR3 were identified in accordance with Name and Location of Acupoints: Chinese National Standards GB12346-90 (2006) (Figure 1). Disposable stainless steel acupuncture needles (25 mm×0.30 mm; Hwato; Suzhou Medical Supplies Co., Suzhou, Jiangsu Province, China) were used.

Each needle was inserted using the nail-pressing method. In the LR3+ KI3 group, inserted at LR3, bilaterally, and once the subject indicated that he or she could feel each needle, the remaining two needles were inserted at KI3, bilaterally. In the KI3 group, only inserted KI3, bilaterally. All needling was performed from the subject’s left to right side. Once sensation of all needles occurred, manipulator twirled every needle at an angle of 90°–180°and frequency of 60–90 times/min, with lifting and thrusting at 0.3–0.5cm and frequency of 60–90 times/min. After manipulating the needle for 1 minute, the needle was retained for 30 minutes, during the period of retaining needle; the needles were
121 manipulated for 1 minute with an interval of 10 minutes.

**Resting-state fMRI scan**

The fMRI scanning were carried out in a 3.0 Tesla Signa HDxt MRI scanner (GE Company, Fairfield, America) at First Affiliated Hospital of Guangzhou University of Chinese Medicine. A standard 8-channel phase-array head coil and restraining foam pads were used to minimize head motion. Subjects were conscious, placed in a supine position and asked to breathe calmly. Earplugs and special earshield were used to diminish scanner noise, and eyeshades were used to avoid visual stimulation. During the fMRI scanning, subjects were instructed to move as little as possible and if they felt uncomfortable, they should tell investigators loudly and the scan would be stopped. fMRI scanning began after subjects rested for 15 min. MRI data (resting-state blood-oxygen-level dependent (BOLD) sequence) were collected 15 min before needling and 15 min after needle withdrawal. Scanning methods were identical between sham and true acupuncture.

(1) Transverse T1-weighted image (T1WI) sequence: 1 min, 51 s, Fast Spin Echo sequence; OAx T1 FLAIR, repetition time 1 750 ms/echo time 24 ms, inversion time 960 ms, field of view 24 cm × 24 cm/Z, matrix 320 × 224/number of excitations = 1, thickness 5.0 mm/interval 1.0 mm, 30 slices total, echo train length 8, and bandwidth 31.25.

(2) Resting-state fMRI BOLD data collection: gradient echo-echo-planar imaging sequence scanning was conducted for 6 min in accordance with the following parameters: repetition time 3,000 ms/minimum, echo time minimum, flip angle 90°, field of view 240 mm × 240 mm, thickness 5.0 mm/interval 1.0 mm, 30 slices each time, matrix 96 × 96/ number of excitations = 1.

**Image processing and analytical methods**

Preprocessing was performed using Data Processing Assistant for Resting-State fMRI (DPARSF V2.3; Yan & Zang, 2013, http://rfmri.org/DPARSF), which is based on Statistical Parametric Mapping (SPM8; members & collaborators of the Wellcome Trust Centre for Neuroimaging, 2009, http://www.fil.ion.ucl.ac.uk/spm) and a Resting-State fMRI Data Analysis Toolkit (REST 1.8, Song et al., 2012. http://www.restfMRI.net ) (Song et al., 2011).

The preprocessing procedure includes: (1) Convert DICOM to NIFTI; (2) Slice Timing after removing first 10 time points; (3) Realign and exclude subjects with max head motion > 1.5mm on any axis and head rotation > 1.5 degree; (4) Coregister T1 to Fun; (5) Segment and affixer Regularization according to East Asian; (6) Normalize by using EPI templates; (7) Smooth images with a Gaussian kernel with a isotropic full-width at half-maximum (FWHM) of 4 mm (data for ReHo analysis without smooth); (8) Remove linear detrend; (9) Filter (0.01 Hz-0.08 Hz).

REST1.8 software (http://www.restfmri.net/forum/REST_V1.8) was used for the ALFF and ReHo analysis. After preprocessing, the linear tendency of the data was removed by linear regression. Time and curve were convolved using Hamming bandpass filtering. The amplitude of low-frequency fluctuations was obtained (0.01–0.08 Hz), and obtained data from each subject were computed to obtain a map of the amplitude of low-frequency fluctuations.

ALFF analysis: ALFF was calculated by REST software. The time series of each voxel was converted to the frequency domain using a Fast Fourier Transform. Then computed square root of the power spectrum and averaged across a predefined frequency interval. The averaged square root was termed ALFF (Chen et al., 2012). Then, standardize the ALFF by dividing own mean ALFF within the whole brain mask.

ReHo analysis: ReHo was calculated by REST software. The Kendall’s coefficient of concordance (KCC) of each voxel was calculated by the time series of the voxel and its nearest 26 neighboring voxels (cluster size ≈ 27). Then standardize the KCC maps by dividing their own mean KCC within the whole brain mask and smooth the resulting maps with a Gaussian kernel with 4 mm FWHM (Liu et al., 2012, Song et al., 2011, Zang et al., 2007).

**Statistical analysis**

Data were analyzed using REST1.8 software. In the statistical analysis, two-sample t-test was used to explore standardized ALFF/ReHo.
values differences between the two groups with AlphaSim correction 5 and continuous voxel > 85. Finally, ALFF/ReHo values alterations differences among different groups were obtained. Rest1.8 software Viewer was employed to identify the precise anatomical position in the brain with statistical significance on the corresponding MNI coordinate. The results are presented as images.

Results

Brain areas with different ALFF values between two groups after acupuncture

15 minutes after withdrawing needle, compared with the KI3 group, the ALFF values of the LR3+KI3 group showed some changes. The ALFF values increased in BA 2, 3, 7, 18, 19, 40, whereas decreased in BA 8, 9, 10, 20, 31, 32, 46, and right cerebellum posterior lobe (Table 2 and Figure 2).

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>Number of voxels</th>
<th>Talairach (mm)</th>
<th>T (Peak intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Cerebrum, Parietal Lobe, Inferior Parietal Lobule</td>
<td>3, 40, 2</td>
<td>217</td>
<td>-45 -36 57</td>
<td>4.7481</td>
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<td>Left Cerebrum, Parietal Lobe, Precuneus</td>
<td>7, 19</td>
<td>110</td>
<td>-30 -75 39</td>
<td>4.1238</td>
</tr>
<tr>
<td>Left Cerebrum, Frontal Lobe, Inferior Frontal Gyrus</td>
<td>9, 8, 32</td>
<td>124</td>
<td>-18 15 39</td>
<td>-3.8424</td>
</tr>
<tr>
<td>Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus</td>
<td>10</td>
<td>374</td>
<td>-33 48 21</td>
<td>-4.1604</td>
</tr>
<tr>
<td>Left Cerebrum, Temporal Lobe, Inferior Temporal Gyrus</td>
<td>20</td>
<td>271</td>
<td>-51 -3 -30</td>
<td>-4.1915</td>
</tr>
<tr>
<td>Left Cerebrum, Occipital Lobe, Cuneus</td>
<td>7, 19, 18</td>
<td>1575</td>
<td>0 -84 27</td>
<td>5.6049</td>
</tr>
<tr>
<td>Right Cerebrum, Parietal Lobe, Precuneus</td>
<td>31</td>
<td>120</td>
<td>21 -57 24</td>
<td>-4.5399</td>
</tr>
<tr>
<td>Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus</td>
<td>10, 9, 46</td>
<td>572</td>
<td>48 39 12</td>
<td>-4.6552</td>
</tr>
<tr>
<td>Right Cerebellum, Cerebellum Posterior Lobe</td>
<td>1759</td>
<td>12</td>
<td>18 -33</td>
<td>-6.8436</td>
</tr>
</tbody>
</table>

Note: Positive T value represented the ALFF value increased, the corresponding brain regions were activated, if not, the corresponding brain regions were inactivated.

Brain areas with different ReHo values between two groups after acupuncture

15 minutes after withdrawing needle, compared with the KI3 group, the ReHo values of the LR3+KI3 group showed some changes. The ReHo values increased in BA 4, 6, 7, 18, 19, 31, and the posterior lobe of the left cerebellum and pyramis, whereas decreased in BA 10, 11, 20, 21, 22, 40, and left cerebellum, brainstem, midbrain(Table 3 and Figure3).
Discussion

Acupoint combination refers to according to certain principles, organizing acupoints, to make the acupoint to produce maximal synergy and clinical curative effect (Zhong et al., 2011). KI3 is widely used in the treatment of reproductive system disease, ear and throat disease, tongue disease, dizziness, limb paralysis, pain, swelling and consciousness obstacle, etc.. LR3 is used in the treatment of reproductive system disease, eye disease, dizziness, limb disease, etc.. Clinically, KI3 combining with LR3 more could enhance the curative effect of them (Wang et al., 2014; Cevik et al., 2013; Wang et al., 2012).

In the recent studies about KI3, Chen et al. found that acupuncture at KI3 could enhance the mutual connections of the dorsolateral prefrontal cortex (DLPFC) and post temporal cortex, ventromedial prefrontal cortex (vmPFC) and post temporal cortex (Chen et al., 2012). Zhong et al. reported that acupuncture at KI3 could strengthen the connection between the superior temporal gyrus and posterior central gyrus (Zhong et al., 2012). As the post temporal cortex and superior temporal gyrus are closely related to the auditory system, therefore, KI3 is widely used in the treatment of auditory diseases.

In this study, the effects of acupuncture at KI3+LR3 were compared with acupuncture at KI3 alone, brain regions with changes included BA 7, 10, 18, 19, 20, 31, 40, and posterior lobe of cerebellum in both ALFF and ReHo analysis. Furthermore, the ALFF and ReHo values of the left occipital lobe cuneus (BA18) were significantly increased, while those of the left temporal gyrus (BA20) were significantly decreased.

The functions of the above-mentioned brain regions are as follows: 1) visual information processing, combining vision and motion information; 2) cognition functions, participate in all aspects of executing cognitive function, thinking and perception, and related to memory and recall of information, recognition of object, texts and face, and perception of space relation; 3) emotion functions, including emotion...
Compared acupuncture at KI3+LR3 with acupuncture at KI3 alone, the changes in the above-mentioned brain regions speculated that clinical treatment of eye disease, dementia, emotional disorder, motion, and other aspects, which presented synergy of acupoint combination.

However, the present study also has some limitations. First, the number of observation group was limited. Second, all the subjects were healthy, and there was no in-depth study of the pathological conditions. Accordingly, the results only provided limited evidence for acupoint combination study.

In conclusion, resting-state fMRI study of acupuncture at KI3+LR3 vs. KI3 alone, acupuncture at KI3+LR3 can more activate/deactivate BA7, 10, 18, 19, 20, 31, 40, and cerebellum posterior lobe, which may be related to the synergistic mechanism of two acupoints combination treatment.

Table 3: Brain areas with ReHo alterations between two groups after acupuncture at P < 0.05

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>Number of voxels</th>
<th>Talairach (mm)</th>
<th>T (Peak intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus</td>
<td>10</td>
<td>232</td>
<td>-3 54 15</td>
<td>-3.8431</td>
</tr>
<tr>
<td>Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus</td>
<td>22</td>
<td>116</td>
<td>-54 6 6</td>
<td>-4.1201</td>
</tr>
<tr>
<td>Left Cerebrum, Temporal Lobe, Inferior Temporal Gyrus</td>
<td>20</td>
<td>85</td>
<td>-60 -33 -33</td>
<td>-3.9747</td>
</tr>
<tr>
<td>Left Cerebrum, Occipital Lobe, Cuneus</td>
<td>19, 18</td>
<td>168</td>
<td>0 -87 24</td>
<td>4.703</td>
</tr>
<tr>
<td>Left Cerebrum, Limbic Lobe, Cingulate Gyrus</td>
<td>31</td>
<td>341</td>
<td>-9 -39 39</td>
<td>4.6612</td>
</tr>
<tr>
<td>Left Cerebellum, Brainstem Midbrain</td>
<td>142</td>
<td>100</td>
<td>-3 -39 -24</td>
<td>4.6885</td>
</tr>
<tr>
<td>Left Cerebellum, Posterior Lobe, Pyramis</td>
<td>177</td>
<td>253</td>
<td>-9 -87 -30</td>
<td>4.1437</td>
</tr>
<tr>
<td>Right Cerebrum, Parietal Lobe, Superior Parietal Lobule</td>
<td>7</td>
<td>219</td>
<td>9 -69 54</td>
<td>3.6556</td>
</tr>
<tr>
<td>Right Cerebrum, Parietal Lobe, Postcentral Gyrus</td>
<td>6, 4</td>
<td>277</td>
<td>12 -45 72</td>
<td>5.1282</td>
</tr>
<tr>
<td>Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus</td>
<td>11</td>
<td>125</td>
<td>12 48 -15</td>
<td>3.8053</td>
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<td>Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus</td>
<td>22, 40, 21</td>
<td>100</td>
<td>66 -54 9</td>
<td>3.898</td>
</tr>
<tr>
<td>Right Cerebrum, Temporal Lobe, Sub-gyral</td>
<td>18</td>
<td>253</td>
<td>36 -57 -3</td>
<td>4.1902</td>
</tr>
</tbody>
</table>

Note: Positive T value represented that the ALFF value increased, the corresponding brain regions were activated, if not, the corresponding brain regions were inactivated.
Acknowledgements

Financial support provided by the National Key Basic Research and Development Project (973 Program), grant no. 2012CB518504; the National-level Undergraduate Student Innovation Venture Training Project of Local Colleges, No. 201212121048; and the Three-stage Key Subject Construction Project of Guangdong Province of China (211 Project), grant no. Yuefagaishie(2009)431.

We are very grateful to the healthy volunteers and staff from the MRI Center of the First Affiliated Hospital of Guangzhou University of Chinese Medicine in China.

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