Original Article
Blood oxygenation level dependent-functional magnetic resonance imaging (BOLD-fMRI) combined with diffusion-tensor imaging (DTI): a comparison of patients with hemiplegia after stroke treated with acupuncture and normal subjects

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Abstract: Objective: This study is to investigate the effects of acupuncture on the pyramidal tract injuries in patients with hemiplegia after stroke, with the blood oxygenation level dependent-functional magnetic resonance imaging (BOLD-fMRI) combined with diffusion-tensor imaging (DTI), in comparison with normal subjects. Methods: Totally 20 patients with hemiplegia after stroke and 20 normal subjects were treated with acupuncture at the LI4 Hegu and SJ5 Waiguan points. Right pyramidal tracts were observed, and the brain activation regions were detected, with BOLD-fMRI/DTI. Results: DTI tractography indicated intact bilateral pyramidal tracts, with normal distribution and arrangement, in normal subjects. In hemiplegia patients, injured right pyramidal tracts were noted. BOLD-fMRI detection showed that, in comparison with normal subjects, positively activated brain regions in hemiplegia patients were mainly located in the cerebellum, left basal ganglia nuclei, and midbrain reticular formation, red nucleus, and substantia nigra. Moreover, the prefrontal cortex, limbic lobe, left temporal lobe, and parietal cortex were also partially activated. On the other hand, compared with normal subjects, negatively activated brain regions in hemiplegia patients were mainly located in the anterior cingulate cortex, right precentral gyrus, and superior frontal gyrus. Furthermore, partially negative activation was also observed in the right temporal lobe, parietal lobe, and left occipital lobe. Conclusion: Pyramidal tract injuries would be detected in patients with hemiplegia after stroke. When treated with acupuncture, in comparison normal subjects, obvious activation would be observed in the extrapyramidal system and secondary motor-related regions in these patients, which might contribute to motor function rehabilitation.

Keywords: Stroke, hemiplegia, acupuncture, blood oxygenation level dependent-functional magnetic resonance imaging (BOLD-fMRI), diffusion-tensor imaging (DTI)

Introduction
Stroke is the second leading cause of death worldwide, and over 80% of the stroke cases are accompanied by paralysis with different severities, seriously affecting the patients’ quality of life [1]. Stroke lesions involve the injuries in the motor function-related regions and the pyramidal tract, which may lead to motor dysfunction. As an important part of traditional Chinese medicine, the acupuncture treatment has been shown to exert beneficial effects for stroke patients in clinic. It has been widely accepted that, acupuncture at related points could be used as a rehabilitation treatment for motor dysfunction [2, 3]. However, the underlying mechanisms have not yet been fully established, limiting the clinical development and application of acupuncture. Therefore, it is of great importance to clarify the mechanisms of acupuncture on motor function rehabilitation with modern medical methods.
In recent years, blood oxygenation level dependent functional magnetic resonance imaging (BOLD-fMRI) and diffusion tensor imaging (DTI) have been rapidly developed, which contribute to the investigation of the motor function rehabilitation in stroke [4-10]. Moreover, evidence concerning the functional brain imaging is accumulating in numerous animal experiments and clinical studies [3, 11-18]. The fMRI studies in hemiplegia patients indicate that, activation of the extrapyramidal system and the motor function-related regions might contribute to the action of acupuncture. However, due to the differences in meridian integrity and status, controlled studies of patients treated with acupuncture and normal subjects are still needed to clarify the detailed mechanisms.

In this study, totally 20 patients with hemiplegia after stroke (left upper limb) and 20 normal subjects were treated with acupuncture at the LI4 Hegu and SJ5 Waiguan points in the upper limb. The pyramidal tracts and the brain activation regions, in these patients and normal subjects, were detected and analyzed with BOLD-fMRI/DTI. The effects of acupuncture on the pyramidal tract injuries in these patients and the underlying mechanisms were investigated.

**Materials and methods**

**Patients**

Totally 20 patients with hemiplegia after stroke, 14 males and 6 females, with an average age of 58.60±8.12 years (ranging from 44 to 70 years), were included in this study, who had been admitted to our hospital from Dec 2011 to June 2014. Among these patients, there were 6 cases with the lesion in the right corona radiate, 9 cases with the lesion in the basal ganglia, and 5 cases with the lesion in the posterior limb of the internal capsule. Before investigation, the upper extremity strength was assessed with the medical research council (MRC) scale and the simplified Fugl-Meyer Assessment (FMA). Based on the MRC scale, there were 3 cases of level I, 7 cases of level II, 8 cases of level III, and 2 cases of level IV. On the other hand, according to FMA, the average score was 26.56±4.32, ranging from 13 to 45.

The inclusion criteria included: (1) patients diagnosed as stroke, with a duration of 3-6 w; (2) right-handed before disease onset; (3) with lesion located in the right corona radiate, basal ganglia, or internal capsule, as detected by imaging; (4) the infarct area ≤ 3 cm², with no obvious mass effects on surrounding brain tissues; (5) patients who were conscious and in stable condition during investigation; (6) patients with spastic paralysis in the left upper limb as the main clinical symptom; (7) patients who were willing and able to complete the MRI detection and acupuncture treatment. The exclusion criteria were as follows: (1) intracranial organic diseases, such as mass, vascular malformation, infection, trauma, and congenital abnormality; (2) with previous history of stroke; (3) with previous history of disorders that would affect the brain function, such as epilepsy, dementia, and depression; (4) with acupuncture syncope, claustrophobia, metallic implant, or severe physical weakness, due to which satisfactory images cannot be obtained.

On the other hand, another 20 gender- and age-matched healthy subjects were used as control, with 14 males and 6 females, and an average age of 54.65±5.37 years (ranging from 41 to 65 years). Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of the Yangzhou No. 1 People’s Hospital, the Second Clinical Medical College of Yangzhou University.

**Acupuncture treatment**

Acupuncture points were located at the LI4 Hegu in the Hand Yangming large intestine meridian and the SJ5 Waiguan in the Hand Shaoyang triple energizer meridian, in the left upper limb. The acupuncture treatment was performed with a 1-inch sterile silver needle (0.4 mm in diameter, 30 mm in length; Suzhou Universal Acupuncture Medical Devices Co., Ltd., Suzhou, Jiangsu, China). The needling depth at the acupuncture points was 0.6 inch, with the needling angle of 90°. The needle tail was connected to a low frequency pulse therapeutic apparatus (G6805-2A; Shanghai Huayi Medical Instrument Co., Ltd., Shanghai, China), which provided continuous-wave electrical stimulation of 0.1-0.3 mA and 9 V, at 1 Hz. Pre-scanning was performed for the first 6 s, followed by non-stimulation for 20 s and stimulation for 20 s, for totally 12 cycles.

**BOLD-fMRI/DTI scanning**

BOLD-fMRI scanning was performed with the Siemens Verio 3.0T scanner (Siemens,
Erlangen, Germany), with an 8-channel quadrature head coil. The patient was in a supine position, and the head was fixed to minimize the movement. T1-weighted images (T1WI) were obtained with the following parameters: TR, 2000 ms; TE, 30 ms; section thickness, 4 mm; no intersection gap; matrix, 64 × 64; NEX, 1; FOV, 240 mm × 240 mm. DTI scanning was performed using the spin-echo echo-planar (SE-EPI) sequences, with the following parameters: TR, 3800 ms; TE, 106 ms; section thickness, 4 mm; no intersection gap; matrix, 128 × 128; FOV, 230 mm × 230 mm. In this process, diffusion gradients were applied along 20 orientations (b = 1000 s/mm²), with a non-diffusion weighted acquisition.

**Image post-processing and statistical analysis**

The raw data from DTI were processed and analyzed with the software provided by the work station. Reconstruction was performed...

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**Figure 1.** DTI tractography of the pyramidal tract of a 51-year-old female stroke patient. The infarction was detected in the right corona radiate, and the right pyramidal tract was interrupted and destroyed (as indicated by arrows). A. Conventional 2D T2-weighted TSE sequence indicated the lesions in the right corona radiate, with the high signal demonstrating the cerebral infarction. B. Three-dimensional diffusion tensor tractography (DTT) images of the fiber tract combined with the fractional anisotropy (FA), indicating the interrupted and destroyed right pyramidal tract (arrow). C, D. Three-dimensional fiber tract DTT images with different postures, indicating the interrupted and destroyed right pyramidal tract (arrow).
BOLD-fMRI/DTI detecting stroke patients

with the seed regions of interest (ROIs) of the cerebral peduncle and the intermediate limb of the internal capsule, and a three-dimensional map of the pyramidal tract was obtained. Moreover, eFilm, dcm2nii.exe, DPARSF, SPM8, REST, and xjview were used for the collection, processing, analysis, and exhibition of the data from BOLD-fMRI scanning. Two sample $t$-test was used for comparison, with a P value threshold of $< 0.001$ and a minimum cluster size 10 voxels.

Results

**DTI tractography of the pyramidal tract**

Results from the DTI tractography of the pyramidal tract showed that, in the normal control subjects, intact bilateral pyramidal tracts were observed, with normal distribution and arrangement. On the other hand, in the patients with hemiplegia after stroke, injuries with different severities were noted in the right pyramidal tract. A representative result from a female stroke patient was shown in Figure 1. The infarction was detected in the right corona radiate, and the right pyramidal tract was interrupted and destroyed.

**BOLD-fMRI performance in patients with hemiplegia after stroke in comparison with normal subjects**

Results from the BOLD-fMRI detection showed that, compared with the normal control subjects, the positively activated regions in the patients with hemiplegia after stroke were mainly located in the both sides of cerebellum, the left basal ganglia nuclei, and the midbrain reticular formation, red nucleus, and substantia nigra. Moreover, obvious activation was also observed in the both sides of prefrontal cortex. Furthermore, partial activation was observed in the limbic lobe, left temporal lobe, and both sides of parietal cortex.

![Figure 2. Positively activated brain regions in the patient with hemiplegia after stroke. Compared with the control group, the positively activated brain regions in the patient with hemiplegia after stroke were mainly located in the both sides of cerebellum, the left basal ganglia nuclei, and the midbrain reticular formation, red nucleus, and substantia nigra. Moreover, obvious activation was also observed in the both sides of prefrontal cortex. Furthermore, partial activation was observed in the limbic lobe, left temporal lobe, and both sides of parietal cortex.](image)
Moreover, partially negative activation was also observed in the right temporal lobe, parietal lobe, and left occipital lobe (Figure 3; Tables 3 and 4).

Discussion

In the present study, the patients with hemiplegia after stroke were subjected to the acupunc-
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In comparison with normal subjects, the activated brain regions in these patients were mainly located in the bilateral cerebellum, the left basal ganglia nuclei, and the midbrain reticular formation, red nucleus, and substantia nigra. According to neuroanatomy, these regions belong to the extrapyramidal system [19]. It has been shown that the new striatum (putamen and caudate nucleus) is directly connected to the thalamus with afferent fibers, and there is a bidirectional signaling between the new striatum and the substantia nigra.

Figure 3. Negatively activated brain regions in the patient with hemiplegia after stroke. Compared with the control group, the negatively activated brain regions in the patients with hemiplegia after stroke were mainly located in the anterior cingulate cortex, right cingulate gyrus, and superior frontal gyrus. Moreover, partial activation was observed in the right temporal lobe, parietal lobe, and left occipital lobe.
The nigrostriatal fibers are dopaminergic neurons, and the striatonigral fibers are γ-aminobutyric acid (GABA)-ergic neurons. The two closed feedback loops are formed between the putamen, caudate nucleus, and substantia nigra by those dopaminergic and GABAergic neurons, which regulate the limb muscle tension. Studies have found that acupuncture at the motor function-related Yanglingquan point could raise the GABA level in the cerebrospinal fluid (CSF) [20, 21]. In this study, the activated brain regions in the cerebellum mainly received the fibers from the contralateral cerebral cortex through the pons, regulating the voluntary movement and coordination initiated by the cerebral cortex. On the other hand, the spinocerebellum mainly functions to control the muscle tone and coordination, and the vestibular cerebellum could also modulate the muscle tension through the extrapyramidal system. The hemiplegia case investigated herein was the spastic paralysis. Therefore, the clinical acupuncture treatment mainly focused on improving the spasticity and promoting the separatist movement. Compared with the normal control subjects, the acupuncture treatment significantly activated the above extrapyramidal regions. These results suggest that, in the pyramidal tract injuries, acupuncture could affect the extrapyramidal pathways and promote the reorganization of the motor function-related brain regions. The alleviated muscle tension and coordination might contribute to the motor function rehabilitation. In line with our findings, Xie et al. [22] have found that, the acupuncture treatment could improve the motor function in stroke patients, which is primarily attributed to the improvement of the movement coordination and learning capacity.

In addition to the activation of the extrapyramidal system, differential activation could also be observed in the bilateral anterior prefrontal cortex [Brodmann area (BA) 6, 8-10, 45, 46] and posterior parietal cortex (BA5, 7, 40). Premotor cortex (PMC) is located on the outer surface of the frontal lobe, which is associated with the motor function. A previous study shows that, the movement recovery in the early stroke depends on the functional reconstruction of the movement and non-movement regions in bilateral hemispheres [23].

A complex network exists among different functional regions in the cerebral cortex. When some function regions were affected due to the nerve fiber injuries, other function regions within the same loop would be influenced. During the repairing process, the secondary motor function-related regions or linking loops might partially compensate the damaged brain functions. We believe that, in the patients with damaged nerve fibers, the acupuncture treatment could enhance the activity of the motor function-related brain regions, and the function compensation of the secondary motor-related regions might promote the reconstruction of the motor pathway and contribute to the functional recovery. Our results showed that, acupuncture at the LI4 Hegu and SJ5 Waiguan points in the left upper limb could activate the contralateral brain regions. In addition, obvious activation was also observed in the ipsilateral brain regions. We suppose that the injuries in the right cerebral cortex could induce the functional compensation in the contralateral brain regions. In addition, obvious activation was also observed in the ipsilateral brain regions. We suppose that the injuries in the right cerebral cortex could induce the functional compensation in the contralateral brain regions. In line with this, the treatment could not only influence the specific brain regions, but also affect the compensatory regions. The gray matter in the extrapyramidal pathway was dramatically activated in the patients with hemiplegia after stroke, and most of the extrapyramidal system did not decussate in the medulla oblongata, which could explain why the ipsilateral brain regions were activated.

Table 3. Negatively activated brain regions in the patient with hemiplegia after stroke

<table>
<thead>
<tr>
<th>Anatomic site</th>
<th>Size (Voxels)</th>
<th>t value</th>
<th>MNI coordinate</th>
<th>Brodmann area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left anterior cingulate cortex</td>
<td>57</td>
<td>-6.45</td>
<td>-24 -69</td>
<td>6 30, 32</td>
</tr>
<tr>
<td>Right superior frontal gyrus</td>
<td>19</td>
<td>-4.57</td>
<td>24 53 -19</td>
<td>11</td>
</tr>
<tr>
<td>Right occipital gyrus</td>
<td>20</td>
<td>-4.94</td>
<td>35 -75 -7</td>
<td>18, 19</td>
</tr>
<tr>
<td>Right occipital lingual gyrus</td>
<td>15</td>
<td>-4.85</td>
<td>23 -92 -9</td>
<td>18, 19</td>
</tr>
</tbody>
</table>

In addition to the functional reconstruction of the movement and non-movement regions in bilateral hemispheres [23].

Our results showed that, in comparison with normal subjects, the negatively activated brain regions in the patients with hemiplegia after stroke mainly included the anterior cingulate cortex (BA24, 32), right precentral gyrus, and superior frontal gyrus (BA4, 6, 8). Wang et al. [24] have shown that, in rat models, the cingu-
late gyrus is connected with the primary motor center in the cortex with fibers. Cingulate motor regions play important roles in the complex voluntary movements. The right precentral gyrus (BA4) belongs to the primary motor regions. In this study, the acupuncture treatment in the left limb of the normal control subjects could activate the primary sensorimotor cortex in the right hemisphere, which is consistent with the cerebral cortex projection. In addition, the fMRI results further confirmed the activation of the motor-related regions in the cortex and the dominant contralateral cross-over effect, which were in line with the neuromodulation and functional division in neuroanatomy. Moreover, these results provided strong evidence for the positioning accuracy of fMRI in living human brains. Superior frontal gyrus (BA6, 8) is an important part of the premotor area, and the feedback loop between the BA4 area, BA6 area, and the basal ganglia nuclei influences the activity of the cone system.

In this study, our results showed that, differences in the activated brain regions were observed between the normal control subjects and the patients, indicating the differential regulatory effects of the acupuncture treatment under different physiopathological conditions. Under specific conditions, the acupuncture treatment could induce a variety of corresponding feedbacks in the central nervous system, and regulate the overall activities of the physical functions. These findings are in line with the overall regulation theory of the acupuncture and points.

This study is limited with the lack of the objective criteria for the acupuncture treatment, and the effects of the differences between individuals on the results are still unknown. On the other hand, this is a cross-sectional study, and further in-depth studies are still needed to investigate the dynamic changes in the brain structure and the functional recovery at different stages in the patients with hemiplegia after stroke.

In conclusion, our results showed that, in comparison with normal control subjects, acupuncture at the LI4 Hegu and SJ5 Waiguan points in the limbs of the patients with hemiplegia after stroke could induce significant activation in the motor function-related brain regions. Moreover, obvious activation was also observed in the gray matter (basal ganglia, red nucleus, and substantia nigra) and cerebellum in the extrapyramidal system, as well as in the secondary motor-related regions. The activation of the extrapyramidal system could regulate the tension feedback loop, and then affect the motor coordination and integration function, improving the spasticity and promoting the movement flexibility. In addition, the functional compensation of the secondary motor-related regions could promote the reconstruction of the motor pathway, which might contribute to the motor function recovery of the affected limbs.

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Disclosure of conflict of interest

None.

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Table 4. Negatively activated brain regions in the normal control subjects

<table>
<thead>
<tr>
<th>Anatomic site</th>
<th>Size (Voxels)</th>
<th>t value</th>
<th>MNI coordinate</th>
<th>Brodmann area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left parahippocampal gyrus, hippocampus</td>
<td>89</td>
<td>-5.39</td>
<td>-24 -12 -30</td>
<td>35, 36</td>
</tr>
<tr>
<td>Left superior temporal gyrus, middle temporal gyrus</td>
<td>33</td>
<td>-4.76</td>
<td>-42 12 -36</td>
<td>38</td>
</tr>
<tr>
<td>Right cingulate gyrus</td>
<td>113</td>
<td>-6.54</td>
<td>-12 33 -3</td>
<td>23, 24, 31</td>
</tr>
<tr>
<td>Right parahippocampal gyrus, hippocampus</td>
<td>112</td>
<td>-5.49</td>
<td>30 -9 -30</td>
<td>35, 36</td>
</tr>
<tr>
<td>Right cingulate gyrus</td>
<td>89</td>
<td>-4.32</td>
<td>13 32 -4</td>
<td>24</td>
</tr>
<tr>
<td>Right middle frontal gyrus</td>
<td>40</td>
<td>-4.81</td>
<td>33 42 -6</td>
<td>46</td>
</tr>
</tbody>
</table>
References


