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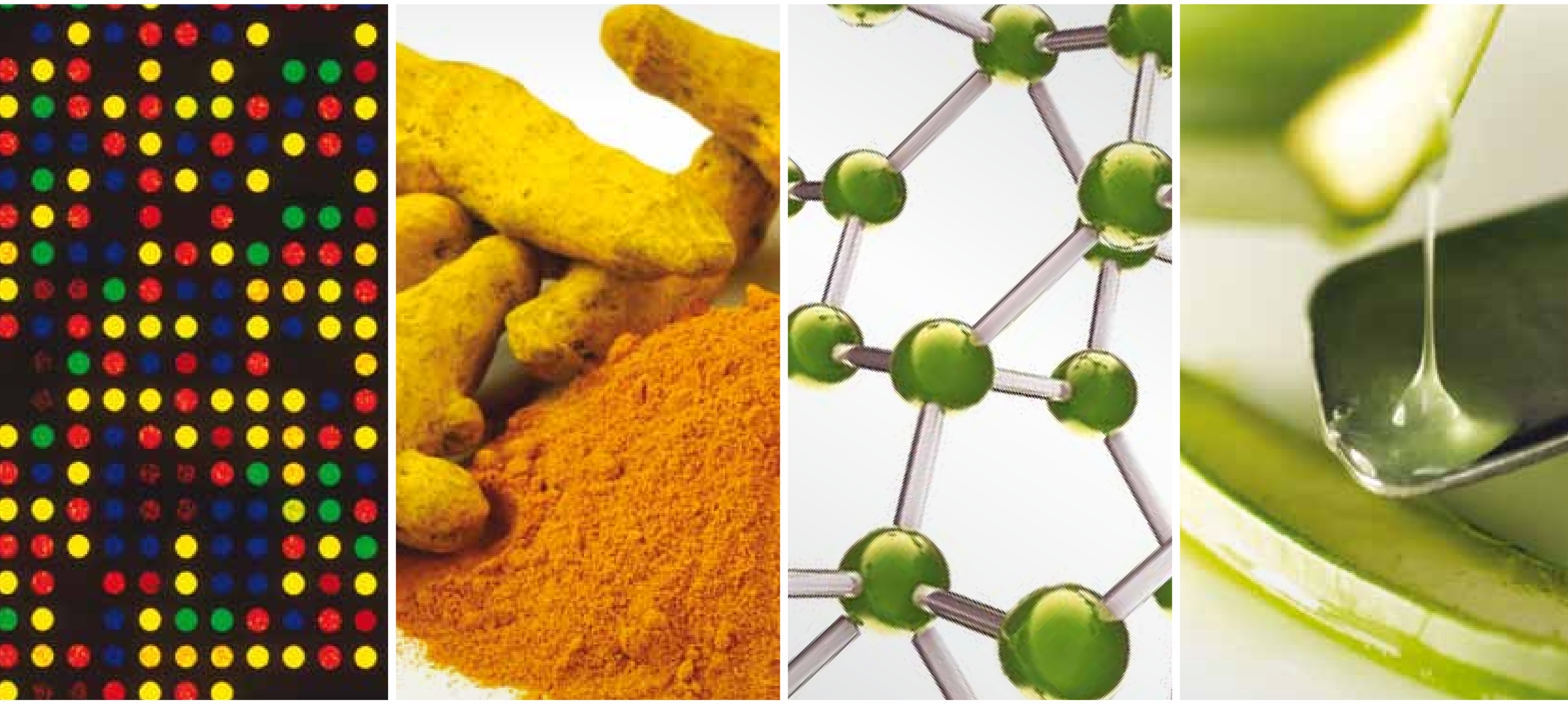
Neurobiological Mechanisms of Acupuncture 2014

Guest Editors: Lijun Bai, Richard E. Harris, Jian Kong, Lixing Lao, Vitaly Napadow, and Baixiao Zhao



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Editorial Board

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Editorial

Neurobiological Mechanisms of Acupuncture 2014

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Acupuncture is currently gaining popularity as an important modality of complementary and alternative medicine (CAM) in the western world. Acupuncture has shown efficacy in the treatment of postoperative and chemotherapy nausea and vomiting. It has also become a beneficial adjunct for pain management, stroke rehabilitation, and depression. Partly as a consequence of its public acceptance, increasing attention is being paid to explore the scientific explanation regarding the physiological mechanism of acupuncture. This special issue on neurobiological mechanisms of acupuncture compiled 9 articles, most of which represent novel primary research and explore the neurophysiologic mechanisms of acupuncture contributing to current hypotheses of acupuncture action.

For instance, stroke is responsible for increasingly high rates of mortality and disability. Acupuncture treatments for improved motor performance following stroke may lead to a remodeling of the neural network architecture of the entire motor system towards a more physiological state. Dr. Z. Xie et al. indicated that acupuncture can enhance bidirectional effective connectivity between the cerebellum and primary sensorimotor cortex, which contributed to the improvement of movement coordination and motor learning in the subacute stroke patients. This study demonstrated additional acupuncture mechanisms supporting stroke rehabilitation, which expand the scope of the study population and relay additional longitudinal observations focusing on the relationships between different brain-based variables and the degree of clinical recovery after acupuncture.

Two papers focus on potential neural mechanisms underlying acupuncture treatment for insomnia and peripheral facial nerve palsy. Acupuncture is widely used in insomnia clinically and empirically; however, the potential neural mechanism underlying the therapeutic effects of acupuncture is still unknown. Acute sleep loss, or sleep deprivation (SD), to some extent, is an alternative form of acute insomnia. Dr. L. Gao et al. investigated the activation patterns of acupuncture in SP6 under different sleep conditions, that is, normal sleep and after a night of total SD. They found that acupuncture at SP6 can induce more widespread and significant brain activations in the SD condition, compared with that of normal sleep. The salience brain network, which also processes interoceptive and autonomic information, may partly underpin the mechanism underlying acupuncture in the restoration of sleep deprivation. Dr. H. Tang et al. investigate the effects of electroacupuncture on the alleviation of peripheral facial nerve palsy (PFN) symptoms induced by herpes simplex virus type 1 (HSV-1) infection. Facial nerve function recovered more quickly in the electroacupuncture group, which was significantly lower in HSV-1 DNA quantity at day 3 and day 7, compared to the animal control group.

Electroacupuncture alleviated symptoms, facilitated affected nerve recovery, and promoted the reduction of HSV-1. The authors suggested that further study is needed to elucidate the precise mechanism of acupuncture in reducing HSV-1.

Manual acupuncture (MA) mainly contained monotype manipulations and multitype manipulations with different stimulation parameters (frequency, angle and depth, etc.). Dr. S. Hong et al. found that MA with different frequencies can induce distinct changes of the firing rate of excitatory gastric-related wide dynamic range (WDR) neurons in spinal dorsal horn (SDH) in normal rats following graded acute gastric distension (GD). Dr. L. Yu et al. further explored the different role of wide dynamic range (WDR) and subnucleus reticularis dorsalis (SRD) neurons in response to acupuncture at different acupoints. They suggested that the function of viscerosomatic convergence-facilitation at the spinal and medulla levels may be related to an acupoint sensitization phenomenon.

It is not known which aspects of the acupuncture treatment, such as the mode of stimulation or location of acupuncture points, are specific to produce different physiological effects. Dr. Y. Shan et al. aimed to evaluate the functional specificity of the Siguan acupoint, a combination of Hegu (LI4) and Taichong (LR3), and used a sham point as control. They found that real acupuncture can induce more increased activity in the somatosensory cortex, limbic-paralimbic system, and basal ganglia, compared with the sham control. They also found that brain activity induced by multiacupoint acupuncture correlates closely with that following individual acupoint stimulation, which has important implications for interpretation of the many previous single acupoint stimulation studies. Dr. C. Wu et al. investigated the changes in amplitude of low-frequency fluctuation and regional homogeneity in the brain fMRI signal induced by acupuncture at Taichong (LR3) acupoint. They found that acupuncture at LR3 can specifically activate or deactivate brain areas related to vision, movement, sensation, emotion, and analgesia, while sham acupuncture has a certain effect on psychological processes and does not affect brain areas related to function.

By gathering these papers, we hope to enrich our readers with respect to the underlying neurobiological mechanisms of acupuncture and consider important research questions for future studies, such as expanding research through larger samples, building clinical relevance into the design of basic research, and developing reliable biomarkers (i.e., neuroimaging) and clinical outcome measures of physiological responses to needling in humans and animals.

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Research Article

An fMRI Study of Neuronal Specificity in Acupuncture: The Multiacupoint Siguan and Its Sham Point

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Clarifying the intrinsic mechanisms of acupuncture's clinical effects has recently been gaining popularity. Here, we choose the Siguan acupoint (a combination of bilateral LI4 and Liv3) and its sham point to evaluate multiacupoint specificity. Thirty-one healthy volunteers were randomly divided into real acupoint (21 subjects) and sham acupoint (10 subjects) groups. Our study used a single block experimental design to avoid the influence of posteffects. Functional magnetic resonance imaging data were acquired during acupuncture stimulation. Results showed extensive increase in neuronal activities with Siguan acupuncture and significant differences between stimulation at real and sham points. Brain regions that were activated more by real acupuncture stimulation than by sham point acupuncture included somatosensory cortex (the superior parietal lobule and postcentral gyrus), limbic-paralimbic system (the calcarine gyrus, precuneus, cingulate cortex, and parahippocampal gyrus), visual-related cortex (the fusiform and occipital gyri), basal ganglia, and the cerebellum. In this way, our study suggests Siguan may elicit specific activities in human brain.

1. Introduction

Acupuncture is a traditional Chinese medicine (TCM) that has been used for thousands of years with empirical evidence of effectiveness. Recently, clarifying the intrinsic mechanisms of its clinical effects has become increasingly popular research. According to the traditional theory of acupuncture, stimulation at specific acupoints will produce effective bodily responses that can be used to treat certain diseases.

Much research has been devoted to demonstrating the mechanisms underlying acupoint specificity. Since the 1990s, development in noninvasive brain imaging techniques such as positron emission tomography and functional magnetic resonance imaging (fMRI) has accelerated the progress of connecting the effects of acupuncture with the central nervous system [1, 2]. Findings have revealed relationships between therapeutic acupuncture and brain activity in areas such as visual cortex [3, 4], language regions [5], limbic system, pain regions [1, 6–10], and somatosensory cortex [11]. While these results have shed some light on the issue of neuronal acupuncture specificity, defining an appropriate control when evaluating specificity is still controversial [12–16]. A sham point design that entails needling at the same depth and with the same pattern but at a nonacupoint located 10 millimeters away from the real one. However, Cho et al. reported that acupuncture is effective regardless of the choice of point at least for pain and analgesic response, which directly questioned the existence of point specificity [17]. Some studies also support the statement that differences between effects produced by sham and real acupuncture have remained unclear [18, 19].

The Siguan acupoint is a combination of bilateral LI4 (Hegu) and Liv3 (Taichong). In TCM, multiacupoint acupuncture is widely used to enhance the therapeutic effects as well as to avoid side effects. Siguan has been conventionally used for several symptoms, especially those of gastrointestinal and neurological disorders [20, 21]. Here, we chose to evaluate the multiacupoint specificity of Siguan using fMRI, which has rarely been reported. Based on results from studies using a single acupoint, we hypothesized that Siguan acupuncture may activate more specific brain regions than its sham point. We also expected to find both differences and commonalities between responses elicited by Siguan and its single acupoint components (Liv3/LI4). Thus, this study aimed to demonstrate whether multiacupoint acupuncture elicits specific activity in the human brain.

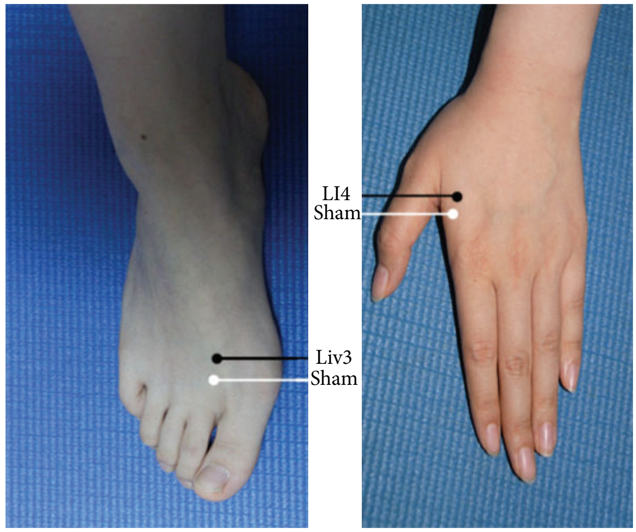

Figure 1: Anatomical location of Siguan and nearby sham acupoints: Liv3 (Taichong), LI4 (Hegu), and two sham points located 10 mm anterior to the corresponding real ones.

2. Materials and Methods

2.1. Subjects. Thirty-one healthy volunteers (14 men, 17 women; age range: 45–75 years; mean: 61.7 ± 7.9 years; all right-handed) were recruited after giving written informed consent with a basic understanding of this study. The inclusion criteria were as follows: (a) no neurological or psychiatric disorders such as stroke, depression, or epilepsy; (b) no neurological deficiencies such as visual or hearing loss; (c) no cognitive complaints; (d) no contraindications for MRI; and (e) no abnormal findings such as infarctions or focal lesions from conventional brain MRI. All the participants met the following exclusion criteria: (a) incapable of enduring pain or other physiology reaction caused by acupuncture stimulation and (b) severe head motion during scanning.

2.2. Stimuli and Scanning Procedure. MRI data acquisition was performed on a 3-Tesla scanner (Verio; Siemens, Erlangen, Germany). During scanning, hands and feet were exposed, and subjects were instructed to stay awake, hold still, keep eyes closed, and think nothing in particular. Functional images were acquired axially using an echo-planar imaging (EPI) sequence (repetition time [TR], 2000 ms, echo time [TE], 40 ms; flip angle [FA], 90°; field of view, 24 cm; image matrix, 64 × 64; slice number, 33; thickness, 3 mm; gap, 1 mm; bandwidth, 2232 Hz/pixel). Structural images, three-dimensional T1-weighted magnetization-prepared rapid gradient echo sagittal images, were also obtained (TR, 1900 ms; TE, 2.2 ms; inversion time [TI], 900 ms; FA, 90°; image matrix, 256 × 256; slice number, 176; thickness, 1 mm).

Subjects were randomly divided into real acupoint (21 subjects) and sham acupoint (10 subjects) groups. The real Siguan consists of four acupoints as mentioned above: Taichong (Liv3) on the dorsum of the left and right feet, in the depression anterior to the junction of the first and second metatarsals and Hegu (LI4) on the dorsum of the left and right hands, at the midpoint on the radial side of the second metacarpal. The sham acupoints were located approximately 10 mm to Liv3 or LI4 (Figure 1). We used stainless nonmagnetic needles that were 0.3 mm in diameter and 25 mm long. All acupuncture manipulations were performed by the same two skilled acupuncturists in synchrony. This ensured the consistency and accuracy of inserting needles into the four stimulation points at the same time during the scanning.

We adopted a 16-min scan time for the functional sequence. Here, our study used a single block experimental design to avoid the influence of unpredictable posteffects caused by acupuncture stimulation that may last several minutes to several hours. Baseline resting-state data were acquired in the initial 3 min. Then, fMRI scanning began while acupuncture stimulation was administered for 3 min. Needles were inserted (to the depth of 2 cm) into the four points simultaneously, with the needles rotated continuously (±180°, 60 times per minute) for both real and sham stimulation. Finally, needles were withdrawn and the scan continued acquiring data for 10 minutes.

2.3. Data Analysis. The preprocessing and data analysis were performed using analysis of functional neuroimages (AFNI) software (http://afni.nimh.nih.gov/). The first four images of each functional session were excluded from data processing to ensure image stabilization. The functional datasets of the two groups were preprocessed (corrected for slice acquisition time, corrected for motion, and spatially smoothed with a Gaussian filter of 6-mm full-width at half maximum). The realigned volumes were spatially standardized into the Talairach stereotaxic space by normalizing to the standard EPI template via their corresponding mean images. Then, all the normalized images were resliced into 3 × 3 × 3 mm voxels.

For the first level statistical analysis, we used the 3d Deconvolve program, part of the AFNI package, to analyze the impulse response functions on a voxelwise basis by modeling the response as a summation of tent-basis functions lasting 20 TRs (60 s) after acupuncture stimulation. The subject-specific contrast images were then used to perform the second level random-effects analysis. Then, two-sample t-tests were performed between data from real and sham acupoint groups. Multiple comparisons were analyzed using clustering and P value criteria established by AlphaSim software (http://afni.nimh.nih.gov/). Minimum cluster sizes were 17 voxels (459 mm^3), and the maximum P value for

Table 1: Brain regions activated by acupuncture stimulation at the real acupoint (Siguan) (compared with the resting-state).

Brain areas	BA	Side	Cluster size	Talairach x	Talairach y	Talairach z	t-value
Calcarine gyrus		L	419	−1.5	−67.5	20.5	5.54
Middle occipital gyrus*		R	85	34.5	−61.5	29.5	5.39
Middle occipital gyrus	19	L	21	−28.5	−82.5	20.5	4.53
Middle temporal gyrus		R	42	46.5	−70.5	5.5	4.55
	22	L	79	−49.5	−58.5	14.5	4.29
Inferior temporal gyrus		L	39	−43.5	−19.5	−15.5	4.19
Inferior frontal gyrus		L	74	−40.5	22.5	−6.5	4.77
Superior medial prefrontal gyrus	10	L	29	−4.5	58.5	2.5	4.26
Anterior cingulate cortex	10	R	17	4.5	49.5	8.5	3.35
Postcentral gyrus		L	21	−28.5	−31.5	47.5	4.06
Caudate nucleus		L	37	−19.5	28.5	11.5	3.88
Cerebellum (crus 2)		L	41	−31.5	−67.5	−33.5	4.34
		R	28	22.5	−79.5	−27.5	4.47
Cerebellum (VIII)*		R	30	7.5	−49.5	−54.5	3.66

The peak voxel for each cluster and the corresponding name of the anatomical region are given. Asterisks indicate peak voxels located in the white matter. In these cases, we labeled the brain region in which most voxels in the cluster were located.
BA: Brodmann area. $P < 0.05$ (AlphaSim correction).

voxels was 0.01. All surviving voxels had an adjusted P value < 0.05.

3. Results

3.1. Results of Acupuncture Stimulation at the Siguan Acupoint. Compared with the resting-state, acupuncture at the real acupoints activated brain regions primarily in the left calcarine gyrus, bilateral middle occipital gyrus (BA19), bilateral middle temporal gyrus (left BA 22), left inferior temporal gyrus, left inferior frontal gyrus, left superior medial prefrontal gyrus (left BA 10), right anterior cingulate cortex (right BA 10), left postcentral gyrus, left caudate nucleus, and the bilateral cerebellum (Crus 2 and VIII). This result is based on the anatomical location of the peak voxel in the activated cluster. When taking whole clusters into consideration, we also found higher activity in the right superior medial prefrontal gyrus (right BA 10), right calcarine gyrus, and the bilateral precuneus. The details of these regions are presented in Table 1 and Figure 2.

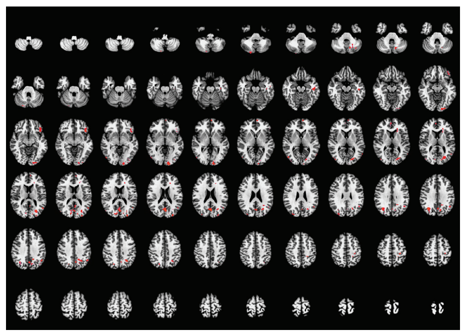

Figure 2: Brain regions activated by acupuncture stimulation at real acupoints. Left side of the images is the right side of the brain. $P < 0.05$ (AlphaSim correction).

3.2. Results of Acupuncture Stimulation at the Sham Acupoint. Compared with the resting-state, acupuncture at the sham acupoints activated brain regions primarily in the left anterior and middle cingulate cortices, right caudate nucleus, right insula, left angular gyrus, and right cerebellum (VIII). Considering the entire clusters, increased activity was also seen in right anterior and middle cingulate cortices. The details of these regions are presented in Table 2 and Figure 3.

3.3. Comparison between Real and Sham Acupuncture. Brain regions that were activated more by real acupuncture stimulation than by sham point acupuncture were mostly located in the left inferior frontal gyrus, right superior medial prefrontal gyrus (right BA 10), left mid-orbital gyrus, right medial temporal pole (right BA 38), left parahippocampal gyrus, left precuneus (left BA 23), left fusiform gyrus (left BA 20), left pallidum, and left middle occipital gyrus. When taking whole voxels into account, increased signal was also present in the right precuneus (right BA 23), right fusiform gyrus, left putamen, right mid-orbital gyrus, bilateral rectal gyrus (BA 11), and the left inferior temporal gyrus. The details of these regions are presented in Table 3 and Figure 4.

4. Discussion

In general, the fMRI result shows increased brain activity after stimulation at Siguan and significant differences between real and sham point acupuncture. Many brain regions were activated, including somatosensory cortex (such as the superior parietal lobule and postcentral gyrus), limbic-paralimbic system (such as the calcarine gyrus, precuneus, cingulate cortex, and parahippocampal gyrus), visual-related cortex

Table 2: Brain regions activated by acupuncture stimulation at the sham acupoint (compared with the resting-state).

Brain areas	Side	Cluster size	Talairach x	Talairach y	Talairach z	t-value
Middle cingulate cortex	L	68	−1.5	4.5	32.5	8.17
Anterior cingulate cortex	L	28	−1.5	25.5	26.5	6.23
Insula*	R	19	25.5	−10.5	26.5	6.84
Angular gyrus	L	17	−40.5	−52.5	23.5	4.82
Caudate nucleus	R	28	22.5	4.5	20.5	4.56
Cerebellum (VIII)*	R	31	22.5	−61.5	−48.5	5.36

The peak voxel for each cluster and the corresponding name of the anatomical region are given. Asterisks indicate peak voxels located in the white matter. In these cases, we labeled the brain region in which most voxels in the cluster were located. $P < 0.05$ (AlphaSim correction).

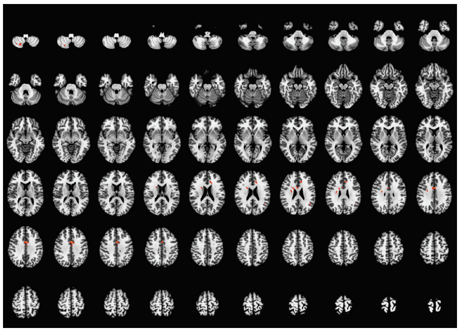

Figure 3: Brain regions activated by acupuncture stimulation at the sham point. Left side of the images is the right side of the brain. $P < 0.05$ (AlphaSim correction).

(such as the fusiform and occipital gyri), pain regions (such as basal ganglia), and the cerebellum. Some of these brain responses correlate with specific function areas. The middle occipital gyrus (BA 19) is visual cortex that has been reported to be activated by eye-related acupoints that affect vision, including Liv3 [3, 22]. The medial frontal prefrontal gyrus (BA10), superior parietal lobe, and the limbic system are regarded as visceral-related cerebral regions. In addition to these areas, other regions including the postcentral gyrus, a well-known somatosensory area [10, 13, 22–25], basal ganglia, and the cerebellum [10, 12–15] were generally activated upon stimulation, regardless of location. Yoo et al. suspected that activation of cerebellar loci could be explained by pain or possible hand motion caused by needling [14].

These findings support the idea that neuronal responses observed via fMRI are inclined to be unique and specific [19]. As Siguan is a combination of bilateral LI4 and Liv3, comparing brain activity during Siguan acupuncture to that during each single acupoint component should be informative. Indeed, studies on LI4 and Liv3 acupuncture have already identified activity in similar regions to those found here [8, 13, 22–25]. Liu et al. reported that stimulation of Liv3 selectively activated the middle occipital gyrus (BA 19), superior medial prefrontal gyrus (BA 10) [22]. Wu et al. found that Liv3 could elicit increased activity in the basal ganglia, which may have possibly been correlated with age [24]. Yan et al. reported that Liv3 acupuncture specifically activated more areas in the cerebellum, which is in accordance with its known effects on motor-related disorders [13]. Many studies have indicated that LI4 acupuncture induces activation in the insula, superior parietal lobule, middle temporal gyrus, and postcentral gyrus [13, 23]. Kong et al. found that the insula and putamen both were activated in two different types of simulation patterns at LI4 (electroacupuncture and manual acupuncture). They suggested that the insula activation occurs during the administration of pain while the putamen is essentially related to motor activity [23]. Yan et al. reported that LI4 and Liv3 acupuncture both activated bilateral middle temporal gyrus but that stimulation at LI4 could specifically elicit responses in the temporal pole [13].

The similarities suggest that brain activity induced by multiacupoint acupuncture may correlate closely with that of the individual acupoint components. In this way, single acupoint specificity can be extended further into multiacupoint specificity. Nevertheless, there are still some discrepancies between Siguan acupuncture and those of separate Liv3 and LI4 stimulation. The studies mentioned above showed that the thalamus and insula were closely associated with pain-related acupoints [12, 22, 25]. Fang et al. found increased thalamus activation at Liv3 when rotating the needle during acupuncture [12]. Claunch et al. reported activation of the anterior insula during LI4 stimulation was specific because the insula has roles in both visceral sensations and emotions [25]. However, in our present work, specific single acupoint responses in the insula and thalamus were not obviously seen after Siguan acupuncture, which indicates that brain activity after Siguan stimulation may not be a simple combination of activity resulting from Liv3 and LI4 stimulation.

Here, the sham point was also a combination of four separately located points. This allows a meaningful discussion of the activation results. The areas activated here by sham point stimulation, such as bilateral cingulate cortex, basal ganglia, insula, and cerebellum, are exactly part of the reported pain-related regions that are not thought to be related to any specific needling location. In this way, the sham multipoint did not show significant specificity. Therefore, the design and implementation of sham point need further investigation [18, 19].

One limitation of this study is that we did not focus on any reduction of brain activity that may have resulted from either real or sham stimulation. Actually, acupuncture at Liv3 and LI4 is reported to reduce activity in the limbic system

Table 3: Brain regions activated more by stimulation at the real acupoint than by stimulation at the sham acupoint.

Brain areas	BA	Side	Cluster size	Talairach			t-value
				x	y	z	
Inferior frontal gyrus		L	116	−31.5	28.5	−12.5	3.30
Superior medial prefrontal gyrus	10	R	32	4.5	61.5	−0.5	3.18
Mid orbital gyrus		L	27	−1.5	37.5	−12.5	2.68
Medial temporal pole	38	R	39	31.5	13.5	−33.5	3.14
Parahippocampal gyrus		L	26	−28.5	−22.5	−18.5	3.12
Precuneus	23	L	68	−1.5	−58.5	17.5	3.53
Fusiform gyrus	20	L	76	−31.5	−10.5	−27.5	3.08
Pallidum		L	33	−19.5	−1.5	5.5	3.05
Middle occipital gyrus		L	80	−25.5	−88.5	17.5	3.17

The peak voxel for each cluster and the corresponding name of the anatomical region are given. In these cases, we labeled the brain region in which most voxels in the cluster were located.
BA: Brodmann area. $P < 0.05$ (AlphaSim correction).

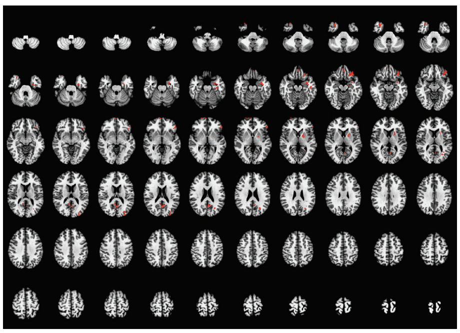

Figure 4: Brain regions activated more by acupuncture at the real acupoint than by acupuncture at the sham acupoint. Left side of the images is the right side of the brain. $P < 0.05$ (AlphaSim correction).

and other pain-related brain areas, which is directly linked to their clinical use in analgesia [8, 13, 23, 25]. Although we paid more attention to increased activation, future work can attach more importance to deactivation specificity of multiacupoint acupuncture. Another limitation is that we did not perform any single acupoint stimulation design as an experimental group. In our present result, it seems that multiacupoint acupuncture may elicit specific activity beyond that of its individual components. Therefore, we expect that further studies can be performed to demonstrate this hypothesis.

5. Conclusion

Here, we focused on confirming the specificity of multiacupoint acupuncture using Siguan and a sham acupoint design. Extensive bilateral cortical and subcortical structures showed specific activation during Siguan stimulation while sham point only activated brain regions that are not thought to be related to specific needling location. Our findings suggest Siguan may elicit more specific and extensive activities in human brain than its sham point.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Changes in Responses of Neurons in Spinal and Medullary Subnucleus Reticularis Dorsalis to Acupoint Stimulation in Rats with Visceral Hyperalgesia

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The purpose of this study was to explore the mechanism of acupoints sensitization phenomenon at the spinal and medulla levels. Experiments were performed on adult male Sprague-Dawley rats and visceral noxious stimuli was generated by colorectal distension (CRD). The activities of wide dynamic range (WDR) and subnucleus reticularis dorsalis (SRD) neurons were recorded. The changes of the reactions of WDR and SRD neurons to electroacupuncture (EA) on acupoints of "Zusanli-Shangjuxu" before and after CRD stimulation were observed. The results showed that visceral nociception could facilitate the response of neurons to acupoints stimulation. In spinal dorsal horn, EA-induced activation of WDR neurons further increased to $106.84 \pm 17.33\%$ (1.5 mA) ($P < 0.001$) and $42.27 \pm 13.10\%$ (6 mA) ($P < 0.01$) compared to the neuronal responses before CRD. In medulla oblongata, EA-induced activation of SRD neurons further increased to $63.28 \pm 15.96\%$ (1.5 mA) ($P < 0.001$) and $25.02 \pm 7.47\%$ (6 mA) ($P < 0.01$) compared to that before CRD. Taken together, these data suggest that the viscerosomatic convergence-facilitation effect of WDR and SRD neurons may underlie the mechanism of acupoints sensitization. But the sensitizing effect of visceral nociception on WDR neurons is stronger than that on SRD neurons.

1. Introduction

Due to different structural function units of related somites, there exist specific relationships between acupoints and target organs. While previous studies on the relationship of acupoints and organs mostly focus on the acupoints' regulation of visceral function in healthy state neglecting its function of reflecting visceral diseases, recently, Yu et al. [1] put forward the concept of "dynamic states of acupoints," deeming that the size and function of acupoints are not static but change dynamically. The function and size of acupoints will vary along with the state of the body. Clinical studies have shown that the pressure-pain threshold of related acupoints decreases in patients with functional intestinal disorder [2] and gastric ulcer [3]. These findings suggest that acupoint is a dynamic functional unit and can be sensitized when viscera is under pathological process.

Many features of the acupoint sensitization phenomenon resemble the features of referred pain in Western medicine. Referred pain is an important symptom of visceral irritation or inflammation [4–6]. It is characterized by visceral hypersensitivity, expanded dermatomes of referred sensation, and somatic hyperalgesia including the presence of tenderness on examination of the skin or muscle [7, 8]. Many studies have shown that referred pain often occurs in body regions somatotopically different from the sites of target organs [9, 10] and has segmental pattern related to target organs [11], suggesting the involvement of central hyperalgesic mechanisms [12]. Our working hypothesis is that central sensitization produce the increased pain sensitivity in sensitized acupoints.

Both the wide dynamic range (WDR) neurons [13, 14] in spinal dorsal horn and the subnucleus reticularis dorsalis (SRD) neurons [15] in the caudal portion of the medulla could be activated by afferent signals from acupoints and inner organs. Many morphological studies observed the neuronal projections between spinal cord and SRD area [16–20], suggesting the existence of a neuronal circuit interconnecting the spinal cord and the medulla oblongata. In our previous studies [21, 22], we observed that visceral inflammation could facilitate the responses of WDR and SRD neurons to acupoint stimulation and a linear relationship existed between the intensity of nociceptive stimulation to inner organs and the sensitivity of related acupoint. These findings suggest that the function of viscerosomatic convergence-facilitation at the spinal and medulla levels maybe related to acupoint sensitization phenomenon.

To further test the different role of WDR and SRD neurons in the dynamic change of the sensitivity of acupoints, in this study, we compared the responses of WDR and SRD neurons to acupoint stimulation in rats before and after receiving visceral nociception. The aim of this study was to obtain mechanism of acupoint sensitization phenomenon at spinal cord and medulla levels.

2. Method

2.1. Animals and Preparation. The experiments were performed on adult male Sprague-Dawley rats (body weight 250–280 g), obtained from the Laboratory Animal Center of China Academy of Military Medical Sciences (License number: SCXK- (Military) 2007–004). Rats were housed in standard laboratory conditions under artificial 12 h light/dark cycle and an ambient temperature of 22 ± 0.5°C for one week. Food and water were available ad libitum. Following an intraperitoneal injection of 100 μg atropine sulfate, the animals were anesthetized with an intraperitoneal injection of 10% urethane (1.0–1.2 g/kg, ip) and artificially ventilated through a tracheal cannula. The body temperature of animals was maintained at 37 ± 0.5°C by means of a feedback-controlled homeothermic heating blanket system (RWD CL-8).

All experimental procedures were approved in accordance with the *Guide for the Care and Use of Laboratory Animals* issued by China Academy of Chinese Medical Science.

2.2. Animal Surgeries

2.2.1. Surgery on Spinal Cord. A laminectomy was performed at spinal segments L1–L3 and the corresponding vertebrae mounted on a rigid frame. The spinal cord was exposed by removing the spinal dura mater and pia mater.

2.2.2. Surgery on Medulla Oblongata. The animals were mounted in a stereotaxic frame with the head fixed in a ventroflexed position by a metallic bar cemented to the skull. The caudal medulla was exposed by removing the overlying musculature and dura mater.

After the surgeries, the flap was sewn into skin cell and covered with 38°C paraffin oil to avoid drying.

2.3. Experimental Procedure

2.3.1. Recordings. Unitary extracellular recordings were made with glass micropipettes filled with a mixture of 2% pontamine sky blue and 0.1 M of natrium aceticum (cusp: 5 μm, impedance: 8–12 MΩ). According to the three-dimensional spatial coordinates of the nucleus, the micropipettes were inserted into the nuclei under the control of micropipette manipulator (SM-21, Japan). The coordinate of WDR neuron is 0.5–1.5 mm lateral to the midline of the back of spinal cord and 500–1500 μm beneath the surface of spinal cord. The coordinate of SRD neuron is 1.0–2.0 mm caudal to the obex and 0.5–1.5 mm lateral to the midline of medulla oblongata.

Single-unit activities were fed into a window discriminator and displayed on an oscilloscope screen. Outputs of the window discriminator and amplifier were led into a data collection system (PowerLab) and a personal computer-based data acquisition system (Chart 5.0) to compile histograms or wavemark files.

2.3.2. Identification of Neurons. Nonnoxious and noxious electrical stimuli were used to isolate unitary activities, and the skin receptive fields were mapped by means of gentle tapping and brushing stimuli. The recorded neurons were classified on the basis of their responses to different stimuli applied on their peripheral receptive fields. At the experiment on spinal cord, neurons that could be activated by both nonnoxious and noxious stimuli applied to the skin receptive fields were identified as WDR neurons. At the experiment on medulla oblongata, neurons that could be activated by noxious stimuli applied to any part of the skin but could not be activated by nonnoxious stimuli were identified as SRD neurons.

2.3.3. Stimulation. Visceral nociceptive stimulation was generated by colorectal distension. A 4–6 cm long inflatable balloon catheter was inserted into rats' colorectum with a depth of 4 cm. CRD stimulation was carried out by pressure supplied by an 80 mmHg sphygmomanometer for 60 s. In order to prevent possible sensitization triggered by overstimulation to the colorectum, the interval between two CRD stimulations was at least 10 minutes. Other technical details have been described in our previous reports.

EA stimulation was applied at the acupoints of "Zusanli-Shangjuxu" at the recorded homolateral discharging neurons with the frequency of 15 Hz for 30 seconds. EA stimulation output comes from stimulator (88–102 G, Nihon Kohden) and the intensity was 1.5 mA and 6 mA.

2.3.4. Recordings Procedures. A standard conditioned recording procedure was set as follows: the background activity was recorded for 60 s, and from 30 s to 60 s the EA stimuli were administered. After 60 s of recovery time for neuronal discharge, neuron activity was recorded for 30 s followed by

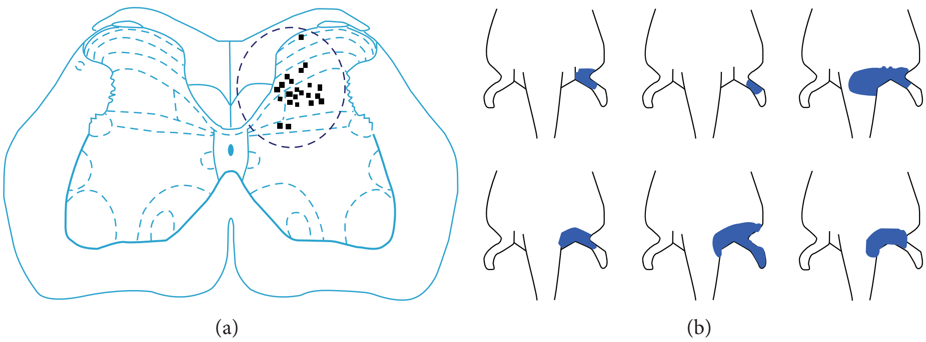

Figure 1: (a) Positions of WDR neurons marked by pontamine sky blue in the spinal dorsal horn. (b) The skin receptive fields of WDR neurons.

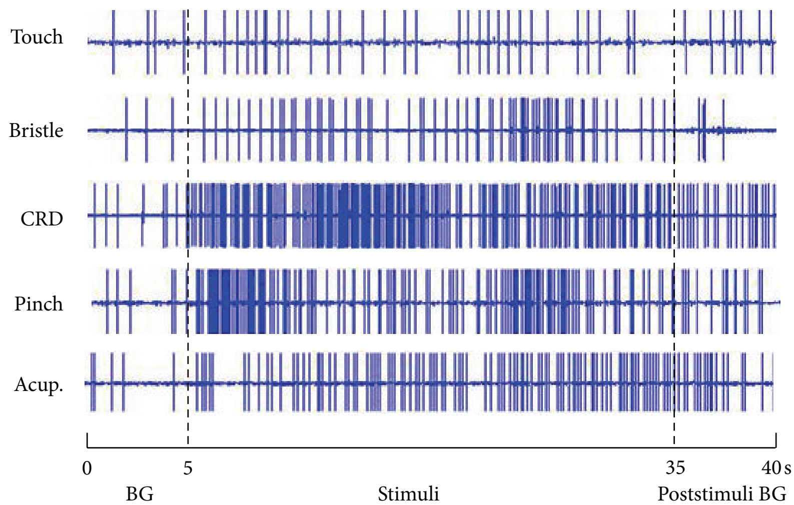

Figure 2: The response patterns of WDR neurons to different stimuli applied at skin receptive fields and viscera. The whole recording time was 40 s, of which the first 5 s was the duration for recording spontaneous background (BG) discharges; from the 5th to 35th s, the stimuli of touch, bristle, 80 mmHg CRD, pinch, and acupuncture were consecutively administered; the poststimuli BG discharges were recorded from the 35th to 40th s.

a test of the neurons' responses to CRD. After another 60 s of recovery of neuronal discharge, EA stimuli with the same intensity were applied at the acupoint before CRD, and the responses of neurons to EA after CRD stimuli were observed.

2.4. Histological Location. After single-unit recording, the recording sites were marked by electrophoretic deposition of pontamine sky blue and checked by HE coloration. Locations of the recording sites were then determined with reference to the brain atlas of the rat (Paxinos & Watson, 2007).

2.5. Data Collection and Statistical Analysis. Neuronal discharges per second and the activation/suppression ratio (identified as $\overline{X} \pm$SE%) were calculated with PowerLab, Chart 5.0, and SPSS13.0. Descriptive analyses were carried out for the average and differences of the pre- and postintervention data (identified as $\overline{X} \pm$ SE%). Paired t-test was used for cross-group comparison. $P < 0.05$ is deemed statistically significant.

3. Results

3.1. Results of Experiment on Spinal Cord

3.1.1. General Features of WDR Neurons. 30 WDR neurons were well isolated from the background. Most of the WDR neurons were located in laminas IV and V, and a few in laminas I and VI of the gray matter marked by electrophoresis of the pontamine sky blue dye at the end of experiment (see Figure 1(a)). The skin receptive fields of the majority of the WDR neurons responding to somatic stimuli were distributed along the ipsilateral caudal parts of the body, including the scrotum, hip region, tail root, hind limb, and the hind paw (see Figure 1(b)).

When a neuron recorded, we observed it's response to different stimuli applied at skin receptive fields and viscera. Figure 2 illustrated that WDR neurons not only responded to nonnoxious stimulation (such as stimuli of touch and bristle), but also responded significantly to noxious stimulation (such as stimuli of pinch and acupuncture) applied on skin receptive fields. We recorded 8 WDR neurons and observed their

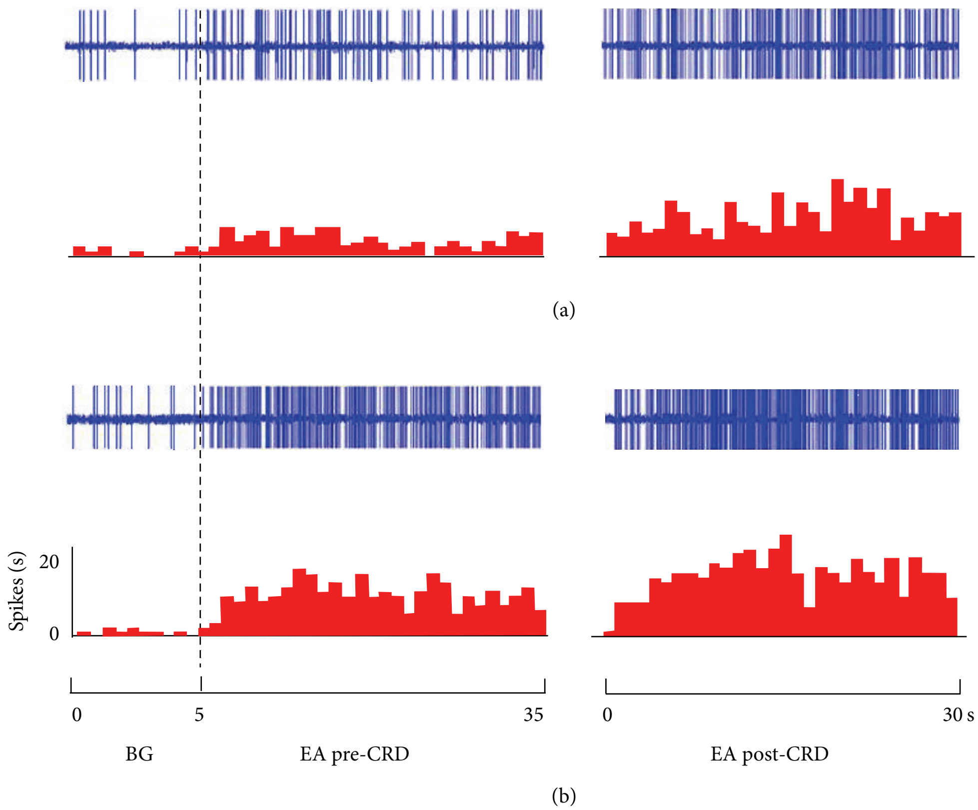

Figure 3: Responses of WDR neurons to EA at different intensities ((a) 1.5 mA, (b) 6 mA) before and after CRD. Upper rows show original unit discharges and lower rows show histograms. BG shows spontaneous activities. EA pre-CRD shows neuronal activities induced by EA before CRD. EA post-CRD shows neuronal activities induced by EA after CRD stimuli.

discharge reactions induced by 80 mmHg CRD stimulation. After CRD, the neuronal discharges increased from 2.48 ± 0.57 spikes/s of the background to 6.71 ± 1.80 spikes/s. The increasing rate was 171.97 ± 47.55% ($P < 0.001$), indicating that 80 mmHg CRD stimulation could activate the activity of WDR neurons. WDR neurons that could be activated by both EA at acupoints of "Zusanli-Shangjuxu" and CRD were chosen as research neurons for further experiment.

3.1.2. Comparison of the Effects of EA on WDR Neurons before and after CRD. Different intensities of EA were applied at acupoints of "Zusanli-Shangjuxu" before and after rats were given 80 mmHg CRD for 60 s. The responses of WDR neurons to EA before and after CRD were observed. Results showed that the numbers of discharges of WDR neurons evoked by EA were significantly increased after CRD (see Table 1 and Figure 3).

When EA intensity was set at 1.5 mA, before CRD, the activation rate of WDR neurons caused by EA was 39.42 ± 11.10%, while after CRD, the activation rate of EA with the same intensity rose to 188.44 ± 33.54%, with a further increasing rate of 106.84 ± 17.33% compared to its effect before CRD. There was a very significant difference before and after CRD ($P < 0.001$) (see Figure 4).

When EA intensity was set at 6 mA, the activation rates of WDR neurons caused by EA were 206.67 ± 38.30% and 333.79 ± 46.40% before and after CRD, respectively, with a further increasing rate of 42.27 ± 13.10% after CRD. There was a significant difference before and after CRD ($P < 0.01$) (see Figure 4).

Table 1: The numbers of discharges of WDR neurons evoked by EA before and after CRD.

EA intensity (mA)	n	Background activity (spikes/s)	EA before CRD (spikes/s)	EA after CRD (spikes/s)
1.5	11	2.62 ± 0.50	3.64 ± 0.63	7.46 ± 0.92
6	11	2.74 ± 0.31	8.35 ± 0.98	11.80 ± 1.06

3.2. Results of Experiment on Medulla Oblongata

3.2.1. General Features of SRD Neurons. 28 SRD neurons were recorded in the dorsal medulla and their positions were mapped by electrophoretic deposition of pontamine sky blue at the end of experiments (see Figure 5). The responses of neurons to touch, brush bristles, pinch, CRD, and different intensities of EA stimulation were observed. Results showed that SRD neurons had significant responses to noxious stimulations (such as pinch, CRD, and 4 mA EA) but had no responses to any kind of nonnoxious stimulations (such as touch, brush bristles, and 1.5 mA EA) (see Figure 6).

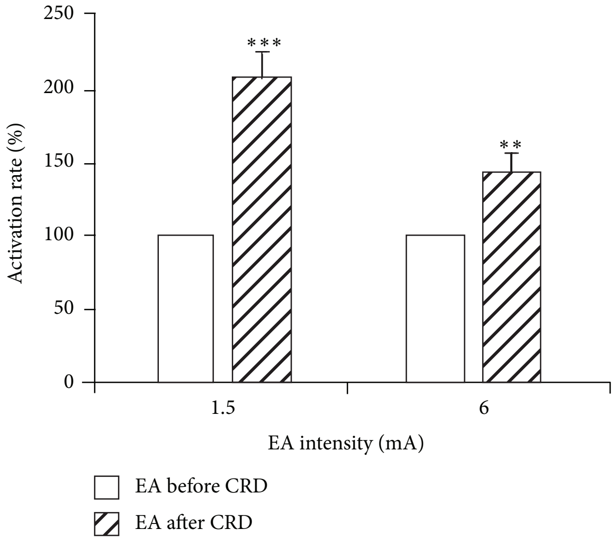

Figure 4: The activation rate of WDR neurons induced by EA at different intensities before and after CRD (left: before; right: after). After CRD, EA stimulation further increased the discharges of WDR neurons to EA ($^{**}P < 0.01$, $^{***}P < 0.001$).

Table 2: The numbers of action potentials of SRD neurons evoked by EA before and after CRD.

Intensity	N	Background activity (spikes/sec)	EA before CRD (spikes/sec)	EA after CRD (spikes/sec)
1.5 mA	10	2.99 ± 0.47	3.09 ± 0.61	4.99 ± 0.87
6 mA	10	2.84 ± 0.42	11.15 ± 0.98	13.93 ± 1.45

3.2.2. Comparison of the Effects of EA on SRD Neurons before and after CRD. We recorded 8 SRD neurons and observed their discharge reactions induced by 80 mmHg CRD stimulation. After CRD, the neuronal discharges increased from 3.10 ± 0.69 spikes/s of the background activity to 13.73 ± 2.24 spikes/s. The increasing rate was 352.32 ± 68.03% ($P < 0.001$). It indicated that nociceptive CRD stimulation could significantly activate the activity of SRD neurons.

Different intensities of EA were applied at acupoints of "Zusanli-Shangjuxu" before and after rats were given 80 mmHg CRD for 60 s. Figure 7 indicates that the responses of SRD neurons to EA before and after CRD were different. After CRD, the numbers of discharges of SRD neurons evoked by EA of the same intensity before CRD were also significantly increased (see Table 2).

Responses of 10 SRD neurons to nonnoxious EA stimulation (1.5 mA) before and after CRD were compared. Before CRD, SRD neurons had no activating reaction to EA compared with background activity ($P > 0.05$), but after CRD, EA had significant activating effect on SRD neurons. Compared to the background activity, the activating rate was 67.59 ± 21.68% ($P < 0.001$). Compared to the effect of EA before CRD, the activating rate was 63.28 ± 15.96%; there was a very significant difference in the effect of EA before and after CRD ($P < 0.001$) (Figure 8).

Responses of anther 11 SRD neurons to noxious EA stimulation (6 mA) before and after CRD were compared. Results showed that 6 mA EA stimulation had significant activating effect on SRD neurons both before and after CRD, but a stronger activation effect was observed after CRD. The neuronal discharge increased to 25.02 ± 7.47% after CRD compared with that before CRD. There was a significant difference in the effect of EA before and after CRD ($P < 0.01$) (Figure 8).

4. Discussion

Acupoints are regarded as specific spots on the body surface where Qi from meridian and viscera is infused. It is believed that acupoints have dual functions of diagnosis and treatment of visceral diseases. Pathological changes in viscera can be manifested on the body surface and cause the sensitization of acupoints with various pathological reactions mainly led by pain when pressed. The size and function of acupoints will vary along with the state of viscera. The so-called acupoint sensitization phenomenon is the specific reflection of visceral diseases through acupoints [21, 22].

Recently, some researchers have focused on the phenomenon of acupoint sensitization. Morphological studies showed that, under inflammatory state, sick organs can promote the extravasation of Evans blue on the body surface. For rats with ovarian inflammation, extravasated EB points mainly distribute around the "Guanyuan (RN4)"-"Uterus" area and the "Shenshu (BL23)"-"Mingmen (DU4)" area [23]. For rats with acute gastric mucosa inflammation, extravasated EB points distribute along with nerve segments and highly coincide with "Pishu (BL20)," "Shenshu (BL23)," and nearby acupoints [24]. But the extravasation of EB points is rarely observed on healthy rats. It indicates that acupoint is a dynamic functional unit. When the organs change from the healthy state to the pathological state, acupoints shift from the silent model to the sensitized model.

The mechanism of acupoint sensitization phenomenon is largely unknown. But both acupoints' functions of diagnosis and treatment of visceral diseases are related to the convergence of visceral and somatic afferent at spinal cord and/or supraspinal centrum. Results of the present study showed that nociceptive CRD stimulation could facilitate the response of WDR and SRD neurons to EA stimulation applied to acupoints "Zusanli-Shangjuxu." It is in accordance with the convergence-facilitation mechanism that explains referred pains. Many studies have shown that the body-viscera convergent neurons in the spinal dorsal horn and supraspinal centrum can be sensitized by stimulation from the inner organs, and the sensitized convergent neurons' responses to inputs from the body surface also become stronger. For instance, for animals with referred muscle hyperalgesia caused by ureteral calculus, the number and frequency of background discharges of spinal cord cells exceed those of normal animals [25]; after chemical stimulation in the bladder, the background discharge level of neurons in the dorsal horn of the spinal cord is elevated [26]; compared with normal rats, rats with ureteral calculus have larger amount and higher frequency of background discharges

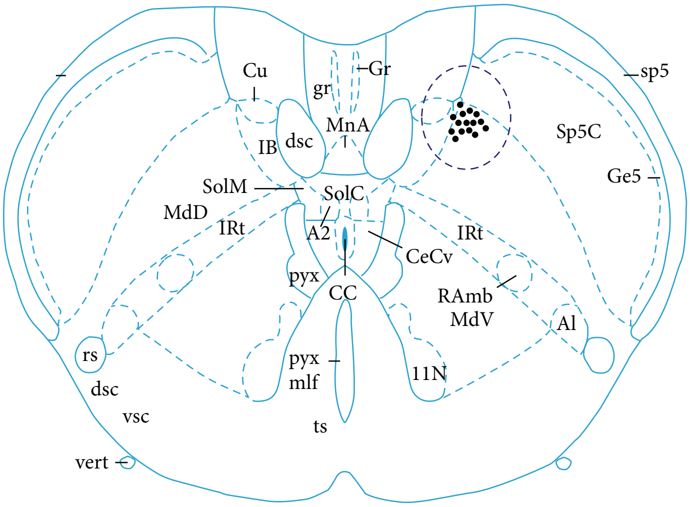

Figure 5: Positions of SRD neurons marked by pontamine sky blue in the dorsal medulla.

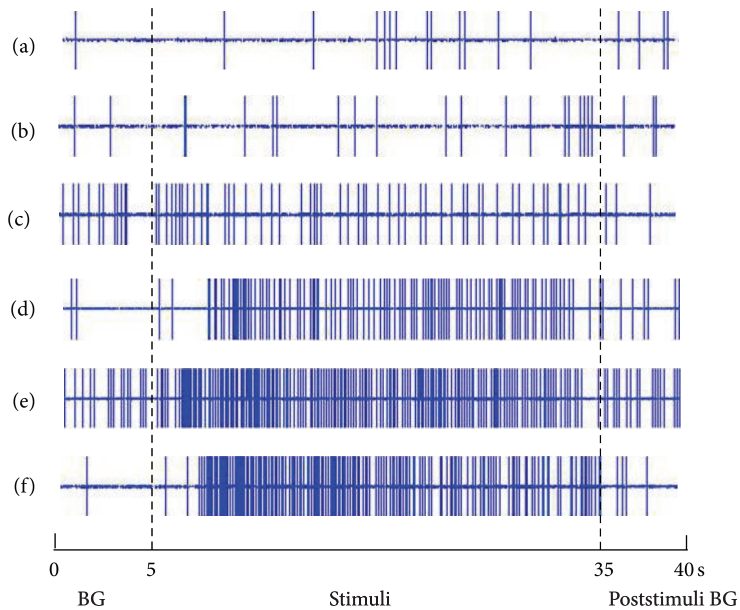

Figure 6: The responses of SRD neurons to different stimuli from skin receptive fields and viscera. The whole recording time was 40 s, of which the first 5 s was the duration for recording spontaneous background (BG) discharges; from the 5th to 35th s, the stimuli of (a) touch, (b) the bristles stimuli, (c) 1.5 mA EA, (d) pinch, (e) CRD, and (f) 4 mA EA were consecutively administered; the poststimulus BG discharges were recorded from the 35th to the 40th s.

in neurons from the dorsal horn of the spinal cord; the authors considered it as referred hyperalgesia [27]. Besides, inflammation in the esophagus [28] and colon [29] may lead to a reduction in response threshold. These findings suggest that the function of viscerosomatic convergence-facilitation at spinal cord (WDR) and medulla oblongata (SRD) may underlie the mechanism of acupoint sensitization.

In addition, large numbers of morphological studies [16–20] observed the neuronal projection between the spinal cord and the SRD area. Experiment compared the responses of WDR and SRD neurons to acupoint stimulation in rats before and after receiving visceral nociception. Results showed that spinal cord (WDR) and medulla oblongata (SRD) play different roles in acupoint sensitization. The sensitizing effect of visceral nociception on WDR neurons is stronger than that on SRD neurons. Therefore, we concluded that the neuronal projections between the spinal cord and the SRD area may form a neuronal circuit and play an important role in the interaction of somatic inputs and visceral inputs. These studies provided scientific lines of evidence for the phenomenon of referred pain in Western medicine and acupoint sensitization in traditional Chinese medicine.

The original meaning of acupoints is the locations that “cause pain or ease when pressed.” The original definition

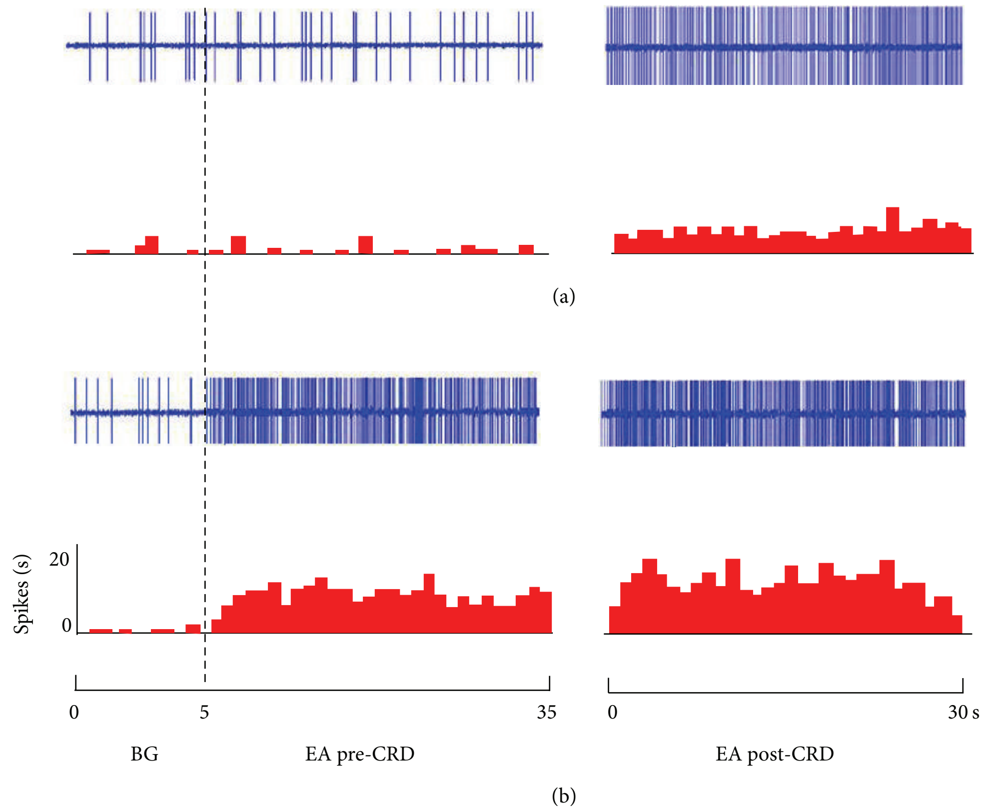

Figure 7: The response patterns of SRD neurons to EA before and after CRD. (a) A neuron not activated by 1.5 mA before CRD but activated after CRD. (b) A neuron activated by 6 mA both before and after CRD but showing a stronger response to EA after CRD.

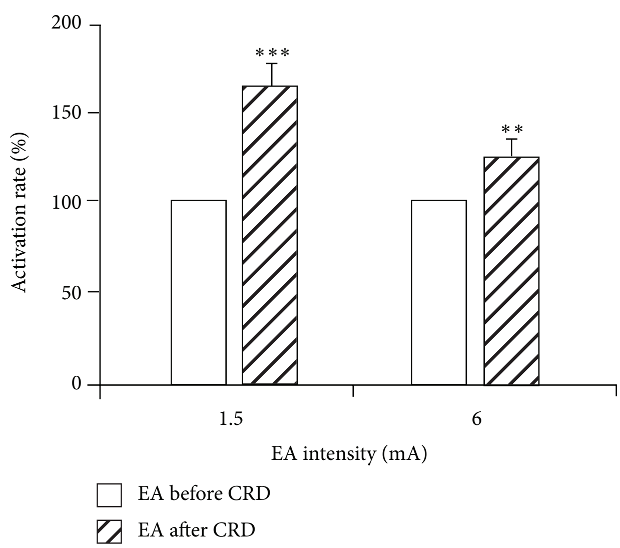

Figure 8: The activation rate of SRD neurons induced by EA at different intensities before and after CRD (left: before; right: after). After CRD, EA stimulation further increased the discharges of SRD neurons ($^{**}P < 0.01$, $^{***}P < 0.001$).

completely covers the two basic functions of acupoints: diagnosis (cause of pain) and treatment (ease). When visceral organs change from the physiological state to the pathological state, the corresponding acupoints on the body surface change from the silent model to the sensitized model. The phenomenon is the concrete manifestation of acupoints' function of diagnosis and treatment of diseases. We hypothesized that activated acupoints are not only the new points of pathological reaction on body surface, but also the best points for the treatment of visceral diseases. The analgesia effect of activated acupoints for corresponding internal organs is better than silent acupoints. But further studies are needed to test the hypothesis.

5. Conclusion

The above results indicated that visceral nociception could facilitate the response of WDR and SRD neurons to acupoints stimulation. The function of viscerosomatic convergence-facilitation at spinal cord and medulla oblongata may underlie the mechanism of acupoint sensitization. But spinal cord and medulla oblongata play different roles in acupoint sensitization. The sensitizing effect of visceral nociception on WDR neurons is stronger than that on SRD neurons.

Conflict of Interests

All authors have approved the paper and declared no competing financial interests for this paper.

Acknowledgments

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Research Article

Differential Activation Patterns of fMRI in Sleep-Deprived Brain: Restoring Effects of Acupuncture

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Previous studies suggested a remediation role of acupuncture in insomnia, and acupuncture also has been used in insomnia empirically and clinically. In this study, we employed fMRI to test the role of acupuncture in sleep deprivation (SD). Sixteen healthy volunteers (8 males) were recruited and scheduled for three fMRI scanning procedures, one following the individual's normal sleep and received acupuncture SP6 (NOR group) and the other two after 24 h of total SD with acupuncture on SP6 (SD group) or sham (Sham group). The sessions were counterbalanced approximately two weeks apart. Acupuncture stimuli elicited significantly different activation patterns of three groups. In NOR group, the right superior temporal lobe, left inferior parietal lobule, and left postcentral gyrus were activated; in SD group, the anterior cingulate cortex, bilateral insula, left basal ganglia, and thalamus were significantly activated while, in Sham group, the bilateral thalamus and left cerebellum were activated. Different activation patterns suggest a unique role of acupuncture on SP6 in remediation of SD. SP6 elicits greater and anatomically different activations than those of sham stimuli; that is, the salience network, a unique interoceptive autonomic circuit, may indicate the mechanism underlying acupuncture in restoring sleep deprivation.

1. Introduction

Acupuncture is an important element of traditional Chinese medicine (TCM) that can be traced back for at least 4,000 years. In recent years, it has gained great popularity as an alternative and complementary therapeutic intervention in the Western medicine. Neuroimaging techniques have provided new insights into the anatomy and physiological function underlying acupuncture [1–13].

Acupuncture is widely used in insomnia clinically and empirically; however, the potential neural mechanism underlying the therapeutic effects of acupuncture remains little known. As one of the most prevalent health complaints worldwide, insomnia affects approximately 10% of the population in Western industrialized countries [14] and is associated with a marked reduction in quality of life, increased fatigue, cognitive impairments, mood disturbances, and physical complaints due to its chronic sleep loss [15]. Acute sleep loss, or sleep deprivation (SD), to some extent, is an alternative form of acute insomnia. Because of its maneuverability, much research work has been carried out in short-term sleep deprivation (24 h) and found that sleep deprivation adversely affects brain function and cognitive domains [16, 17].

Many studies have suggested a remediation role of acupuncture on Sanyinjiao acupoint for sleep disturbance [18, 19]. The Sanyinjiao acupoint, also known internationally as Spleen 6 (SP6), is the junction point of the liver, spleen, and

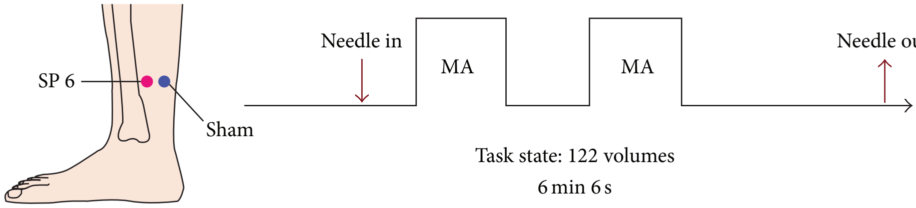

FIGURE 1: Experimental paradigm. SP6 and Sham were located on the right leg. The arrows indicated the time points of needle insertion and withdrawal. The epoch of acupuncture manipulation lasted for "2 min-MA-2 min-rest-2 min-MA" as shown by the framework.

kidney meridians based on principles of TCM, and it is proposed to strengthen the spleen, resolve and expel dampness, and restore balance to the yin and blood, liver, and kidneys [20]. If acupuncture induces homeostatic force in renormalizing the neuronal responses, then activation patterns involved may be differentially affected by acupuncture or sham stimuli under conditions of SD. In the present study, we employed functional magnetic resonance imaging (fMRI) to insight the role of acupuncture on SP6 in sleep deprivation induced cortical activation. The use of fMRI to assess neuronal activity in response to acupuncture stimuli allows us to examine not only neuronal processes regulating acupuncture but also the biphasic regulation effects of acupuncture.

2. Materials and Methods

2.1. Subjects. Sixteen healthy volunteers (8 females, mean age of 22.1 ± 0.8 years) were recruited in this study after giving informed consent. Participants were selected from respondents to a web-based questionnaire. They should meet the following criteria: (1) of right hand according to the modified Edinburgh Handedness Questionnaire [21]; (2) of 20 and 24 years of age; (3) of habitual good sleeping habits (sleeping no less than 6.5 h each night for the past one month); (4) of no extreme morning or evening chromotype (score no greater than 22 on a modified Morningness-Eveningness Scale, [22]); (5) of no long-term medications; (6) of no symptoms associated with sleep disorders; (7) of no history of any psychiatric or neurologic disorders; (8) of no history of drug abuse and current use of antidepressant or hypnotic medications; (9) of acupuncture naive. Participants had an average of 15.7 ± 1.2 years of education. This study was approved by the Medical Research Ethics Committee and Institutional Review Board of The First Affiliated Hospital of Nanchang University.

2.2. Experimental Protocol. All subjects were scheduled for three fMRI scanning procedures, one following the individual's normal sleep and received acupuncture at SP6 (NOR group) and the other two after a night of total SD with acupuncture on SP6 (SD group) or sham (Sham group). The sessions were counterbalanced and approximately two weeks apart to minimize the residual effects of SD on cognition.

Acupuncture was performed at the acupoint SP6 on the right leg (Sanyinjiao, located in the medial lower leg, 9-10 cm above the prominence of the medial malleolus (ankle bone), and closed to the medial crest of the tibia). The needles used in the acupuncture protocol were sterile, disposable, and stainless steel acupuncture needles, which would not distort MR images, measuring 0.3 mm in diameter and 40 mm in length. The needle was inserted in SP6 with a depth of approximately 1.5 cm. Stimulation was then delivered by a balanced "tonifying and reducing" technique [1] and rotated manually clockwise and counterclockwise for 1 min at a rate of 60 times per min. Acupuncture was performed with "2 min stimuli-2 min rest-2 min stimuli" program during the task-state scanning. The procedure was performed by the same experienced and licensed acupuncturist on all subjects.

For the control of acupuncture manipulation, subjects also received the sham stimulation at a nonmeridian focus near SP6 (2-3 cm inwards from SP6) on the right leg using the same timing protocol as in the acupuncture run. The sham stimulation was delivered with the needle depth, stimulation intensity, and manipulation method identical to those used in the SP6 run.

2.3. fMRI Scanning Procedure. Functional scanning was incorporated with three runs in each session. Two resting-state runs, each lasting 4 min, were separated by a 6 min-6 seconds task-state block-designed run (Figure 1). Resting-state data were not presented in the current study. During the scanning, subjects lay supine on the scanner bed, wearing earplugs to suppress scanner noise and with the head immobilized by cushioned supports. They were instructed to keep their eyes closed and their minds clear and remain awake. In addition, the feelings of *deqi* were collected at the end of the session, including the soreness, numbness, fullness, heaviness, and dull pain. Subjects were asked to rate each component of the *deqi* feeling they had experienced during the stimulation period using a visual analog scale (VAS). The VAS was scaled at 0 = no sensation, 1–3 = mild, 4–6 = moderate, 7-8 = strong, 9 = severe, and 10 = unbearable sensations. Because the sharp pain was considered an inadvertent noxious stimulation, we excluded the subjects from further analysis if they experienced sharp pain (greater than the mean by more than two standard deviations). Among all the participants, only one experienced the sharp pain and was removed from further analysis.

2.4. MRI Data Acquisition. fMRI data were collected on a SIEMENS Trio 3.0 T scanner. Each subject lied on supine with the head in neutral position fixed comfortably by a belt and foam pads during the test. The scanning sessions included (1) localizer, (2) T1 MPRAGE anatomy (176 sagittal

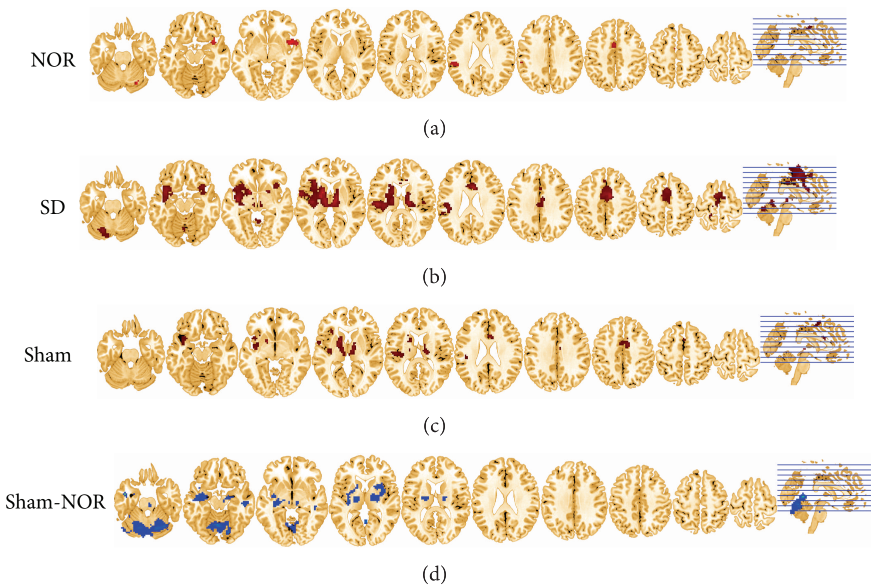

Figure 2: (a) Activations for NOR group during acupuncturing on SP6, compared with a resting baseline are shown. (b) Activations for SD group during acupuncturing on SP6, compared with a resting baseline are shown. (c) Activations for Sham group during acupuncturing on Sham, compared with a resting baseline are shown. (d) Group differences between Sham and SD. Cool color indicates that the Sham group had decreased activations compared with the SD group. All images were normalized to the standardized space defined by MNI using the structural MRI of each subject.

slices, thickness/gap = 1.0/0 mm, in-plane resolution = 256 × 256, FOV (field of view) = 240 mm × 240 mm, TR (repetition time) = 1,900 ms, TE (echo time) = 2.26 ms, and flip angle = 15°), (3) EPI-BOLD (36 axial slices, echo-planar imaging pulse sequence, thickness/gap = 5.0/1 mm, in-plane resolution = 64 × 64, TR = 3,000 ms, TE = 30 ms, flip angle = 90°, and FOV = 240 mm × 240 mm).

2.5. fMRI Data Analysis. All preprocessing and data analyses were performed by using SPM8 (Wellcome Department of Cognitive Neurology, London, UK). For each participant, the first 2 scans of each task-state run were discarded, and the remaining images were slice-time corrected and spatially realigned to the first volume of the first run to correct for motion. The structural scan was coregistered to a mean image of the realigned functional scans. The coregistered functional scans were then normalized to the Montreal Neurological Institute template brain (resampled voxel size = 3 × 3 × 3 mm^3) and spatially smoothed with a Gaussian kernel of 8 mm.

To investigate the acupuncture effect, general linear model (GLM) was used to analyze the block-designed data. Vectors of stimulus onsets were created for each of the acupuncture and rest conditions and convolved with the canonical hemodynamic response function. A 480 s temporal high-pass filter was applied to the data to remove low-frequency artifacts. Contrasts for acupuncture versus rest in three groups (i.e., NOR, SD, and Sham) were created for each subject. Thresholds for active brain regions were set at a cluster extent of >10 voxels and a voxel level of $P < 0.001$. After individual analyses, a one way within-subject ANOVA and post hoc were performed and paired t-test for group analysis was performed by using the same statistical parameters to compare regional brain activity with acupuncture versus rest for rested wakeful and sleep deprivation. Statistical analyses were performed by using SPM8. Only the coordinates from the largest cluster for each brain region are presented in the main tables for regions with multiple locations.

3. Results

4 subjects were excluded on discovery of excessive head motion or experienced the sharp pain during the task. A total of 12 participants (5 men) completed the fMRI protocol.

Rested-Wakeful Condition: Acupuncture versus Baseline. Under habitual sleep, responses to acupuncture versus baseline stimuli were found in left middle frontal area (MFA), medial frontal gyrus (MFG), precentral area (PreCG), postcentral area (PoCG), left putamen (PUT), anterior cingulate (ACC), right superior temporal gyrus (STG), insula (INS), and right inferior parietal lobe (IPL) (Figure 2(a), Table 1).

Sleep Deprivation Condition: Acupuncture versus Baseline. Under the sleep deprivation condition, greater neuronal activation was observed in the responses to acupuncture versus baseline. Activations were found in the right ACC, bilateral thalamus, bilateral INS, right MFG, bilateral STG, bilateral middle temporal gyrus (MTG), left PoCG, bilateral caudate (CAU), right uncal gyrus, left PUT, fusiform, right cerebellum anterior lobe, and so forth (Figure 2(b), Table 2).

Table 1: Activations for NOR group during acupuncturing on SP6 compared with a resting baseline are shown. ($P < 0.001$, cluster >10 voxels, uncorrected).

Brain regions	MNI coordinates (x, y, z)			BA	L/R	Voxels	T values
Middle frontal gyrus	−36	47	22	10	L	25	4.889
ACC	−3	2	46	32/24	L	22	4.9336
Superior temporal gyrus	54	8	−2	22/38	R	115	6.738
Inferior parietal lobule	−57	−34	25	40	R	50	4.928
Precentral gyrus	48	0	9	44	R	20	8.8206
Postcentral gyrus	−48	−16	22	3	L	25	5.022
Thalamus	3	−9	6	—	R	16	6.115
Putamen	−21	13	4	—	L	20	5.8152
Cerebellum	30	−70	−23	—	R	47	6.2835

Table 2: Activations for SD group during acupuncturing on SP6 compared with a resting baseline are shown ($P < 0.001$, cluster >10 voxels, uncorrected).

Brain regions	MNI coordinates (x, y, z)			BA	L/R	Voxels	T values
Inferior frontal gyrus	33	11	−14	13	R	74	5.908
Inferior/medial frontal gyrus	−34	13	−4	13/47	L	180	6.1011
Superior temporal gyrus	57	5	1	22	R	58	4.5028
Superior temporal gyrus	−40	8	−14	38	L	26	5.01
Fusiform	−15	8	−2	22/38	L	2060	10.1924
ACC	9	−1	49	24/6/8	R	720	7.608
ACC	6	14	25	24/33	R	47	7.1919
Insula	42	−16	13	13	R	69	4.6696
				13	L	366	
Postcentral gyrus	−15	−40	70	3	L	24	5.1276
Thalamus	3	−9	6	—	R	157	7.003
					L	131	—
Caudate	16	−13	20	—	L	54	5.542
Caudate	−14	−13	19		R	67	—
Cerebellum	−18	−67	−29	—	L	194	5.9275
Cerebellum	39	−55	−35		R	59	4.5446
Cerebellum	3	−52	−2		R	71	5.6171

Sleep Deprivation Condition: Sham versus Baseline. Under the sleep deprivation condition, Sham induced activations in the left superior frontal gyrus (SFG), bilateral MFG, bilateral PreCG, bilateral thalamus, bilateral INS, left pons, and left cerebellum posterior lobe (Figure 2(c), Table 3).

Sleep Deprivation Condition: Sham versus Acupuncture. To investigate the differences between Sham HAM and SP6 in sleep-deprived condition, a paired t-test was performed between SD and Sham groups. Results indicated that the group differences of activations were significantly decreased in the Sham group than that of in the SD group, including right INS/thalamus, bilateral MTG, right hippocampus, and left cerebellum (Figure 2(d); Table 4).

4. Discussion

The present study investigated the activation patterns of acupuncture in SP6 in different sleep conditions. We found that acupuncture in SP6 increased regional brain activity primarily in the ACC, INS, basal ganglia, and limbic system after sleep deprivation, while Sham induced activations in the left SFG, bilateral MFG, bilateral precentral area, bilateral thalamus, bilateral INS, left pons, and left posterior lobe of the cerebellum. Although acupuncture also elicited increases the regional brain activity in the MFC/ACC, insular, and IPL during rested wakeful, both the extent and intensity of activation were reduced and much less widespread. Our findings may suggest that sleep deprivation alters neuronal activity, which predisposes individuals to contraction and enhanced responses to acupuncture and may partly explain the biphasic regulation effects of acupuncture.

Sleep constitutes an approximate one-third of the human lifetime, and many hypotheses have been proposed about its role in physiological functions, including homeostatic restoration, thermoregulation, tissue repair, immune control, memory processing, and consolidation [23, 24]. Sleep deprivation has been shown to have a negative impact on

Table 3: Activations for Sham group during acupuncturing on SP6, compared with a resting baseline are shown ($P < 0.001$, cluster >20 voxels, uncorrected).

Brain regions	MNI coordinates (x, y, z)			BA	L/R	Voxels	T values
Medial frontal gyrus	12	3	54	6/24	R	31	6.2621
Medial frontal gyrus	−12	−18	60	6/3/4	L	285	8.7196
Thalamus	3	−9	6	—	R	146	8.1692
				—	L	210	
Pon	−18	−24	−33	—	L	20	7.5021
Insula	42	12	−9	13	R	20	4.6318
Putamen	−21	6	−12	—	L	12	5.4318
Postcentral gyrus	−48	−16	22	3	L	25	5.022
Cerebellum	−30	−66	−21	—	L	102	6.1046
Cerebellum	−33	−51	−48	—	L	72	5.6012

Table 4: Group differences between Sham and SD are shown ($P < 0.001$, cluster >20 voxels, uncorrected).

Brain regions	MNI coordinates (x, y, z)			BA	L/R	Voxels	T values
Insula/thalamus	33	21	3	13	R	165	−6.3468
Middle temporal gyrus	48	−24	−12	21	R	63	−8.3397
Middle temporal gyrus	−45	−3	−24	21/38/13	L	423	−9.6562
Hippocampus	21	−9	−12	—	R	48	−6.3265
Cerebellum	0	−57	3	—	L	963	−8.3057

the brain and health [17]. Sleep deprivation falls under the category of "fatigue" and "sleepless" in TCM and stands for "excessive lassitude," "visceral dysfunction," deficiency of "qi and blood," and yin-yang disharmony, though not yet been severe [25]. The remediation is to re-establish the equilibrium between them. Applying pressure at this acupuncture meridian can refresh the mind, sedate, nourish spleen and stomach, nourish liver, and produce other health effects [26].

In the present study, we found different neuronal activity patterns evoked by acupuncture under sleep deprivation and rested wakeful. In NOR group, a state of physical fitness, SP6 acupuncture evoked activations may indirectly reflect the neuronal responses of these regions, including MPFC, insula, putamen, lateral parietal lobes, and sensorimotor areas, which are frequently recruited in executive-control, sensory information processing, visceral regulation, social emotion, and self-awareness. Activations in these regions may be consistent with its widespread functions of SP6 in mental [27], gynecological [20, 26], and neurological diseases [27] as recorded.

In the SD group, more widespread brain regions were activated and the activation level as well as the strength was significantly higher than that of NOR and Sham group. Besides the above-mentioned activations in the NOR, ACC and insula were especially significantly activated both in extent and intensity. A prominent cognitive role of the ACC is processing errors and conflict [28, 29]. Salience network, composed of the anterior insular cortex and ACC, has received increasing attention [30–32]. This brain network is supposed to be implicated in multiple functions, ranging from attention to interoception and subjective awareness [33, 34]. The salience network integrates external sensory stimuli with internal states, and the anterior insula acts as a hub, mediating interactions between large-scale networks involved in externally and internally oriented cognitive processing [34, 35]. Most remaining nodes in the salience network are subcortical sites for emotion, homeostatic regulation, and reward [34]. Regions such as the lateral prefrontal cortex (PFC) and lateral parietal cortex are consistently recruited by cognitively demanding tasks and are critical for guidance of thought and behavior [36, 37]. In sleep deprivation, SP6 may exert an everlasting influence over the short-time period, as well as less maladaptive stimulation.

In Sham group, significant activations in the thalamus, pons, and basal ganglia were involved. This may indicate the impact of activations on the sleep deprivation itself. Under the extreme sleepy condition, more brain regions were involved in the compensation of maintaining the awareness and alert (thalamus and pons), while deactivations that occurred in the regions support advanced cognitive functions, dorsolateral prefrontal cortex, anterior cingulate cortex, and parietal lobes. It is generally believed that the Sham mainly relates to the processes of maladaptive stimulation [1, 2]. But much of the effects represent sleep deprivation, emotional and visceral processing—the left medial prefrontal. Naturally, we would suppose that low activation level of brain salience network in response to salient Sham stimuli could be explained as a failure in remediation, because such response would indicate that more stimuli are necessary to produce salient stimuli in Sham.

Interestingly, in the first individual level analysis the extent of activations in NOR was greater than SD and Sham group, and second level group statistics vice versa, that is, a relatively weak group effect in NOR and greater group effects both in SD and Sham. We speculated that the reasons for this inconsistent were likely to reflect the biphasic regulation effects of acupuncture. In the NOR group, acupuncture in SP6 reveals sparse results which may relate to its multiple functions. In sleep deprivation, an imbalance occurs; acupuncture stands for a homeostatic force to renormalize the yin and yang, biphasic regulation effects of acupuncture. Another study also reported similarities. Our results support this hypothesis that the effects of acupuncture were closing to launch homeostatic regulation [9, 38].

5. Limitations

There are several limitations in this study. First, in a relatively small sample in our study, the results for group comparison were not corrected for multiple comparisons; therefore, they should only be considered an exploratory analysis. Second, some flaws exist in our protocol, for example, a lack of sham stimuli in rested wakeful condition. The remediation of acupuncture in sleep deprivation cannot be totally inferred. Third, block design permits the observation of an immediate acupuncture effect rather than its post effects which are more valuable clinically. The mechanism still need to be further evaluated.

6. Conclusion

Different activation patterns suggest an important role of acupuncture on SP6 in remediation of SD. SP6 elicits greater and anatomically different activations than the same stimuli, that is, the salience network. A unique interoceptive autonomic circuit may, partly, indicate the mechanism underlying acupuncture in restoring sleep deprivation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Effects of Electroacupuncture on Facial Nerve Function and HSV-1 DNA Quantity in HSV-1 Induced Facial Nerve Palsy Mice

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Acupuncture is a common and effective therapeutic method to treat facial nerve palsy (FNP). However, its underlying mechanism remains unclear. This study was aimed to investigate the effects of electroacupuncture on symptoms and content of HSV-1 DNA in FNP mice. Mice were randomized into four groups, an electroacupuncture treatment group, saline group, model animal group, and blank control group. Electroacupuncture was applied at Jiache (ST6) and Hegu (LI4) in electroacupuncture group once daily for 14 days, while electroacupuncture was not applied in model animal group. In electroacupuncture group, mice recovered more rapidly and HSV-1 DNA content also decreased more rapidly, compared with model animal group. We conclude that electroacupuncture is effective to alleviate symptoms and promote the reduction of HSV-1 in FNP.

1. Introduction

Peripheral facial nerve palsy (FNP) is an acute peripheral facial nerve disorder, usually affecting unilateral facial muscle, orifices, and related tissues. The clinical symptoms vary according to the location of the lesion of the facial nerve along its course to the muscles but include drooping of the brow, incomplete eyelid closure, drooping of the corner of the mouth, impaired closure of the mouth, dry eyes, hyperacusis, impaired taste, or pain around the ear [1]. An FNP patient can appear expressionless when he or she is smiling [2]. The annual incidence of FNP is estimated to be 20–25 cases per 100,000 people [3–5]. In the United States, the annual incidence was 25/100,000 people [2, 6] compared with 258/100,000 in China [7]. FNP may influence individuals of all age groups [4], with the peak incidence lying between 20 and 40 years of age. Females and males are equally affected [4] but a slight female preponderance has been observed [8]. The etiology of FNP is controversial, but viral infection, vascular ischemia, heredity, and autoimmune inflammation have been proposed as possible underlying causes [9, 10]. Evidence suggests that viral infection with herpes simplex virus type 1 (HSV-1) may predominantly occur if the immune system is compromised [4, 11–13]. Additionally, HSV-1 DNA was detected in clinical specimens, endoneurial fluid, and saliva from FNP patients [14, 15]. The herpes simplex virus (HSV) mediated viral inflammatory immune mechanism is therefore widely accepted as the major cause of FNP [14, 16].

Treatment methods of FNP are still controversial. Clinical trials investigating the efficacy of specific antiviral treatment did not show a significant benefit compared with alternative therapy [17–19]. Acupuncture is an essential part of traditional Chinese medicine (TCM), which has a history of thousands of years. Electroacupuncture is a technique combining acupuncture with electric currents. A number of studies provide evidence for a beneficial effect of acupuncture on FNP [20–22]. However, the mechanism underlying the effects of acupuncture on FNP induced by HSV-1 infection is not fully understood.

Because of difficulties in obtaining clinical specimens from patients, basic research using animal models is necessary to investigate the effects of electroacupuncture in FNP

[23]. In this study, we established a mouse model of FNP induced by HSV-1 infection [24–27]. The purpose of this study was to investigate the effects of electroacupuncture on the alleviation of symptoms and content of HSV-1 in FNP mice.

2. Materials and Methods

2.1. Animals. We used 4-week-old Balb/c mice (18.5–20.5 g) purchased from Chengdu Dashuo Biological Technology Company for this experiment. All mice were maintained in Laboratory Animal Center of Chengdu University of TCM and cared for in compliance with the Guideline for Animal Experimentation at Ehime University School of Medicine.

2.2. Virus Inoculation and Groups. Mice were randomly divided into three groups, an FNP model group ($n = 156$), saline group ($n = 30$), and blank control group ($n = 30$). The KOS strain of HSV-1 was prepared in Vero cell sand plaque-titrated at 6.7×10^7 plaque-forming units (PFU) per milliliter. Mice in the FNP model group were generally anaesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the posterior auricular branch of right facial nerve was incised by 2 mm and inoculated with 25 μL virus solution (1.7×10^6 PFU) on a 2 mm × 3 mm gelatin sponge, which was placed in the notch. Then, the incision was closed. In the saline group, normal saline solution was applied instead. Nothing was used on the mice in the blank control group. Animals were returned to their cages upon completion of the procedure.

2.3. Evaluation of FNP Model. The model was evaluated by scores of blink reflex, vibrissae movement, and position of apex nasi daily after the virus inoculation [27]. The blink reflex was evoked twice by blowing air onto the eye through an 18-gauge needle with a 5 mL syringe. The degree of blink reflex was graded on a 0 to 2 scale (0, no difference between two sides; 1, the blink reflex was delayed compared with the unaffected side; 2, the blink reflex disappeared completely). Vibrissae movement was observed for 30 s and scored on a 0 to 2 scale (0, no difference between both sides; 1, the vibrissae movement was weaker than that on the healthy side; 2, the vibrissae movement disappeared completely). The position of the apex nasi was scored on a 0 to 1 scale (0, the position is in the middle; 1: the position is to the unaffected side). The total score was defined as the sum of all scores. When the total score was 3 or 4 points, we recognized that the FNP model was established.

Only mice that developed a transient and homolateral FNP after the primary infection were used for the following experiments. Ninety mice (58%) developed FNP exclusively on the same side as the inoculation, and 60 FNP model mice were randomly divided into two groups, an electroacupuncture group ($n = 30$) and a model animal group ($n = 30$).

2.4. Experiment Procedures. There were four groups with 30 mice in each: Group A, blank control group; Group B, saline group; Group C, model animal group; and Group D, electroacupuncture group.

2.4.1. Electroacupuncture Treatment Group. Based on previous study [22], two frequently used acupoints, Jiache (ST6) and Hegu (LI4), were selected. The location of the two acupoints was found according to *Experimental Acupuncture* [28] with both Jiache (ST6) and Hegu (LI4) on the paralyzed side. Both acupoints were punctured 3–5 mm in depth. Electroacupuncture was given by a G6805 after fixing the mice (Qingdao Xin Sheng Industrial Co., Ltd., Qingdao, China). The filiform needles were sterile Hwato acupuncture needles for single use, 25–40 mm in length and 0.30 mm in diameter (Suzhou Medical Supplies Factory Co., Ltd., Suzhou, China). The stimulation frequency was 3-4 Hz and intensity was 1-2 V in continuous wave (CW) mode to make the needle slightly vibrate and keep the mice quiet. The needles were retained for 20 min, once a day, and the treatment lasted for 14 days. Electroacupuncture practitioner in this study had 10 years of acupuncture and TCM training and 6 years of experience in academic and clinical acupuncture.

2.4.2. Control Groups. There were 3 control groups, the blank control group, saline group, and model animal group. All mice were fixed once a day for 20 min without any intervention.

2.5. Evaluation of FNP Symptoms. After electroacupuncture at days 3, 7, and 14 of treatment period, symptoms were evaluated by scores of blink reflex, vibrissae movement, and position of apex nasi.

2.6. Quantification of HSV-1 DNA. HSV-1 DNA in the intratemporal facial nerve, geniculate ganglia, brainstem, and cerebral cortex tissue was quantified after electroacupuncture at days 3, 7, and 14 of treatment period.

2.6.1. Extraction of HSV-1 DNA. Ten mice in each group were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and killed by decollation quickly at days 3, 7, and 14 of treatment period. The intratemporal portions of the right facial nerves, geniculate ganglia, brainstem, and cerebral cortex were dissected and preserved at −80°C. The facial nerves, geniculate ganglia, brainstem, and cerebral cortex were cut with scissors, placed in a sample tube containing 200 μL buffer solution, and shaken until they were suspended thoroughly. After removing the supernatant and adding 20 μL proteinase K (20 mg/mL), the tissue was digested at 56°C for 30 min, followed by a brief centrifugation and removal of supernatant. After that, 220 μL buffer solution GB was added and the sample was shaken for 15 sec and incubated at 70°C for 10 min, followed by a brief centrifugation and removal of supernatant. After adding 220 μL absolute ethyl alcohol, the sample was oscillated for 15 sec and mixed thoroughly by vigorous shaking for 15 sec. Following a brief centrifugation, this solution was poured into a CB 3 absorbing column, which was put in a 2 mL collection tube and centrifuged at 9660 g for 30 sec. Waste liquid in the collection tube

Table 1: Scores in evaluation of FNP during treatment period and at the end of treatment period ($\bar{x} \pm S$).

Group	N	At day 3 of treatment period			At day 7 of treatment period			At day 14 of treatment period		
		Blink reflex	Vibrissae movement	Position of apex nasi	Blink reflex	Vibrissae movement	Position of apex nasi	Blink reflex	Vibrissae movement	Position of apex nasi
A	10	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}
B	10	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}
C	10	15 ± 3.1	17 ± 2.8	9 ± 1.8	12 ± 2.5	11 ± 1.7	7 ± 1.9	7 ± 1.8	8 ± 2.4	5 ± 1.6
D	10	$7 \pm 2.4^{*}$	$8 \pm 1.9^{*}$	$3 \pm 2.2^{*}$	$4 \pm 1.6^{**}$	$5 \pm 1.9^{**}$	$3 \pm 1.4^{**}$	$1 \pm 2.1^{*}$	$2 \pm 1.0^{*}$	$0 \pm 0.0^{*}$

Group A: blank control group; Group B: saline group; Group C: model animal group; Group D: electroacupuncture group. *Compared with Group C, $P < 0.05$. **Compared with Group C, $P < 0.01$.

was removed, and the absorbing column was put back into the same collection tube. After that, 500 μL deproteinized solution and 700 μL and 500 μL eluent GW were added consecutively and the sample was centrifuged at 9660 g for 30 sec after each addition. Waste liquid in the collection tube was removed, and the absorbing column was put back into the same collection tube and centrifuged at 9660 g for 2 min to remove residual liquid. The CB 3 absorbing column was put in a clean centrifugal tube of 1.5 mL and incubated at room temperature for 2.5 min. Buffer TE of 50 μL was added to the adsorption film in the center of the absorbing column. The sample was incubated at 60°C for 2.5 min and centrifuged at 9660 g for 2 min.

2.6.2. Reverse Transcription PCR of HSV-1 DNA. Synthetic primers encoding parts of the HSV-1 (5′-CCACCGAGC-GGCAGGTGATC-3′ as upstream primer and 5′-GCC-GACCGCCTGCTCGTGCT-3′ as downstream primer) and the β-actin gene (5′-CGTTGACATCCGTAAAGACCTC-3′ as upstream primer and 5′-TAGGAGCCAGGGCAGTAA-TCT-3′ as downstream primer) were used for PCR amplification. After an instantaneous centrifugation of primers, deionized water was added to make a 100 μM solution. Deionized water of 380 μL was added in another EP tube, and 10 μL upstream primers and 10 μL downstream primers were added (400 μL and 2.5 pmol/μL). Deionized water was added to dilute cDNA to a proper concentration. The extracted DNA and primers were denatured initially. Forty cycles of amplification (15 sec at 95°C for denaturation; 15 sec at 95°C for renaturation; 45 sec at 72°C for extension) were performed and the sample was extended at 72°C for 5 min and incubated at 4°C. To test the amplification efficiency and specificity of primers, standard curves and melting curves were made. PCR solution of 24 μL was made with 12.5 μL SYBR Premix Ex Taq (2×), 0.5 μL PCR forward primer (10 μM), 0.5 μL PCR reverse primer (10 μM), 3 μL template cDNA, and 8.5 μL dH_2O and incubated in a real-time PCR tube. Gradient dilution of 2 μL for cDNA was added. Forty cycles of amplification (15 sec at 95°C for denaturation; 15 sec at 95°C for renaturation; 45 sec at 72°C for extension) were performed and the sample was extended at 72°C for 5 min. Fluorescence data for melting curve was acquired every 0.5°C during a temperature transition from 58°C to 85°C. Fluorescence quantitative standard curve was made. Real-time PCR were run in triplicate. Briefly, mRNA was corrected with β-actin via CT (the cycle at threshold level). The relative mRNA expression of HSV-1/β-actin was quantified according to the formula of $2^{-\Delta\Delta CT}$. Analysis of CT was performed using Sequence Detection software version 1.2.3 (Applied Biosystems group).

2.7. Statistical Analysis. All analyses were carried out using the Statistical Package for the Social Sciences, version 17.0 (SPSS, Chicago, IL, USA). Descriptive statistics including the mean ± standard deviation (SD), median, minimum (min), and maximum (max) were used to present continuous variables. One-way analysis of variance (ANOVA) was used to determine statistically significant differences between groups of variables with data that were normally distributed. The least-significant difference (LSD) test was used for homogeneity of variance, whereas Tamhane's T2 test was used for heterogeneity of variance. Hierarchical data was tested with the nonparametric test to determine differences between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Results of Melting Curve. The HSV-1 melting curve had a single peak, melting at 85 ± 1°C. This step was replicated several times. The amplified product was the intended product.

3.2. Symptom Relief of Acute Viral FNP Mice. Mice in the blank control group and saline group showed no symptoms of FNP, and the score for each item in these two groups was 0. The scores of blink reflex, vibrissae movement, and position of apex nasi in the model animal group and electroacupuncture group decreased over time. However, the scores in the electroacupuncture group decreased more rapidly. The scores of blink reflex, vibrissae movement, and position of apex nasi in the electroacupuncture group were significantly lower than those in model animal group ($P < 0.05$ at day 3, $P < 0.01$ at day 7, and $P < 0.05$ at day 14 of treatment period). The differences in FNP symptoms among all groups are shown in Table 1.

3.3. Quantity of HSV-1 DNA. No HSV-1 DNA was detected in either saline group or blank control group mice. HSV-1 DNA was detected in the model animal group and electroacupuncture group. However, the quantity of HSV-1 was significantly lower in the electroacupuncture group compared with the model animal group ($P < 0.05$ at day 3, $P < 0.01$ at days 7 and 14 of treatment period), as shown in Table 2. At day 7 of treatment period, HSV-1 DNA was decreasing in the electroacupuncture group, while HSV-1 DNA was still higher compared with 4 days prior in the model animal group. HSV-1 DNA in the model animal group was decreasing at day 14 of treatment period.

4. Discussion

The blink reflex score, vibrissae movement score, position of apex nasi score, and total score were significantly higher in the electroacupuncture and model animal group than those in saline and blank control group after modeling, which indicated that the FNP mice model was successfully induced by inoculation of HSV-1. With the development of FNP, some facial nerve function recovered and HSV-1 DNA content also decreased in the model animal group. These results reflect the disease progression of FNP. Facial nerve function recovered more quickly in the electroacupuncture group. The symptom relief after acupuncture is consistent with clinical trials evaluating the therapeutic effects of acupuncture for FNP [20–22]. In addition, HSV-1 DNA quantity in the electroacupuncture group was significantly lower than that in model animal group at day 3. At day 7, HSV-1 DNA quantity in the electroacupuncture group was decreasing, while HSV-1 DNA quantity in the model animal group was still higher compared with 4 days prior. These results indicate that electroacupuncture has therapeutic effects on FNP and promotes the reduction in HSV-1.

4.1. The Mechanism Underlying Acupuncture to Alleviate FNP Symptoms. It has been suggested that the cell-mediated autoimmune mechanism against myelin basic protein may be the pathogenesis of FNP. FNP is an autoimmune demyelinating cranial neuritis, and, in most instances, it is a mononeuritic variant of Guillain-Barré syndrome, a neurologic disease with cell-mediated autoimmune reaction against peripheral nerve myelin antigens. In FNP, viral infection or the reactivation of a latent virus may provoke an autoimmune reaction against peripheral nerve myelin components, leading to inflammation and demyelination of the facial nerve [29]. During its progression, FNP is accompanied by significant elevations in some proinflammatory mediators, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) [30]. TNF-α, IL-6, and IL-1β are linked with a wide range of inflammatory, infectious, autoimmune, and malignant conditions [31]. The abnormal high level of serum TNF-α also causes demyelination of the facial nerve via inflammation [32, 33]. Acupuncture significantly reduces plasma levels of TNF-α in patients with chronic headache [34] and mRNA levels of IL-6 and IL-1β in rats of lipopolysaccharide-induced fever [35]. A recently published study found a new pathway for anti-inflammatory pathway of electroacupuncture. Electroacupuncture at the sciatic nerve controls systemic inflammation by inducing vagal activation of aromatic L-amino acid decarboxylase, leading to the production of dopamine in the adrenal medulla and then the reduction of TNF-α [36]. By inhibiting the production of proinflammatory mediators, acupuncture might help alleviate facial nerve inflammation and demyelination and thus promote the repair of the facial nerve to improve symptoms. Overall, acupuncture might have potential benefit for inflammation in inflammatory and infectious diseases by regulating the production of proinflammatory mediators. However, there is no direct observation to support that acupuncture could reduce the contents of TNF-α, IL-6, and IL-1β in HSV-1 induced FNP *in vivo*.

4.2. The Mechanism Underlying Acupuncture to Help Control HSV-1 Infection

4.2.1. Acupuncture Might Help Reduce the Efficiency of HSV-1 Replication and Spread. HSV-1 can activate autoimmune pathways, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway. Evidence suggests that HSV-1 can induce a persistent translocation of NF-κB [37]. NF-κB complexes are bound to inhibitors of NF-κB (IκBs) in unstimulated cells, thereby maintaining NF-κB in an inactive state. The phosphorylation of IκB by the IκB kinase (IKK) complex is involved in the activation of NF-κB, leading to IκB degradation, which releases NF-κB and then allows it to translocate into the nucleus [38, 39]. The persistent translocation of NF-κB subsequently increases the efficiency of virus replication [37]. The translocation of NF-κB to the nuclei of infected cells is a necessary component to prevent apoptosis of host cells during HSV-1 infection, which helps HSV-1 evade immunological surveillance and spread effectively in the host [40]. Acupuncture can inhibit abnormal NF-κB expression and activation by inhibiting the NF-κB signal transduction pathway in host cells [41, 42]. This might help the immunological surveillance and reduce the efficiency of HSV-1 replication and spread. However, there is no direct observation to support that acupuncture could inhibit NF-κB expression and activation in HSV-1 induced FNP *in vivo*, which needs to be further studied.

4.2.2. Acupuncture Might Regulate Immune Response to Eliminate HSV-1. After HSV-1 infection, humoral immunity and cell-mediated immunity will be activated to eliminate HSV-1. In cell-mediated immunity, antigen-specific cytotoxic T lymphocytes, a type of T lymphocytes that kill cancer cells and infected cells, are activated to induce apoptosis in virus-infected cells displaying epitopes of foreign antigens on their surface. Studies regarding the effects of acupuncture on the immune system show a stimulating effect on cell-mediated immunity. Acupuncture is able to help in T lymphocyte proliferation [43]. After acupuncture, T helper lymphocytes, a type of T lymphocytes that help in the activity of other immune cells, and cytotoxic T lymphocytes were significantly increased [44, 45]. The ability of acupuncture to modulate the immune response might help the immune system to eliminate HSV-1. Therefore, acupuncture might have potential to control infection in infectious diseases by activating some immune pathways and modulating the immune response.

Table 2: Detection of HSV-1 DNA during treatment period and at the end of treatment period ($\overline{x} \pm S$).

G	N	At day 3 of treatment period				At day 7 of treatment period				At day 14 of treatment period			
		Facial nerve ($2^{-\Delta\Delta CT}$)	Geniculate ganglion ($2^{-\Delta\Delta CT}$)	Brainstem ($2^{-\Delta\Delta CT}$)	Cerebral cortex ($2^{-\Delta\Delta CT}$)	Facial nerve ($2^{-\Delta\Delta CT}$)	Geniculate ganglion ($2^{-\Delta\Delta CT}$)	Brainstem ($2^{-\Delta\Delta CT}$)	Cerebral cortex ($2^{-\Delta\Delta CT}$)	Facial nerve ($2^{-\Delta\Delta CT}$)	Geniculate ganglion ($2^{-\Delta\Delta CT}$)	Brainstem ($2^{-\Delta\Delta CT}$)	Cerebral cortex ($2^{-\Delta\Delta CT}$)
A	10	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}
B	10	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}	0^{*}	0^{**}	0^{**}	0^{**}	0^{**}	0^{**}
C	10	13.67 ± 2.16	11.82 ± 1.81	7.73 ± 1.08	6.52 ± 2.27	15.42 ± 2.71	14.18 ± 1.97	12.57 ± 3.11	6.52 ± 2.27	9.89 ± 1.47	7.63 ± 1.22	6.77 ± 1.28	6.14 ± 1.63
D	10	$9.84 \pm 1.29^{*}$	$9.04 \pm 2.29^{*}$	$3.04 \pm 2.29^{*}$	$2.17 \pm 1.14^{*}$	$2.18 \pm 2.13^{**}$	$1.78 \pm 1.14^{**}$	$1.04 \pm 1.48^{**}$	$2.17 \pm 1.25^{**}$	$0.71 \pm 0.16^{**}$	$0.18 \pm 0.21^{**}$	$0.03 \pm 0.09^{**}$	$0.05 \pm 0.13^{**}$

The quantity of HSV-1 DNA was expressed with $2^{-\Delta\Delta CT}$ of difference ratio of HSV-1 mRNA. Group A: blank control group; Group B: saline group; Group C: model animal group; Group D: electroacupuncture group. *Compared with Group C, $P < 0.05$. **Compared with Group C, $P < 0.01$.

4.3. The Compatibility of Distal-Proximal Acupoints in Acupuncture Treatment for FNP. Acupoints compatibility is a key point in acupuncture prescriptions, which influences therapeutic effects. There are many classical acupoints compatibility methods, such as yuan-source acupoints and luo-collateral acupoints combination, back-shu acupoints and front-mu acupoints combination, and distal-proximal acupoints combination. Distal-proximal acupoints combination is often used in the treatment of FNP. For example, Hegu (LI4) in upper extremity and some local acupoints in face are needled in combination. Synergistic effects exist among acupoints, so acupoint compatibility can strengthen the effectiveness of acupuncture.

The mechanism underlying Hegu (LI4) treating diseases in the face and mouth remains unclear. A morphological study suggested that the Gasserian ganglion receives the neural projection to Hegu (LI4) [46]. Furthermore, the nerve in Hegu (LI4) has an indirect project to the nucleus of the solitary tract and the facial nerve has a direct connection with the nucleus of the solitary tract [47]. This might be the morphological foundation of Hegu (LI4) treating diseases in the face and mouth. As to Jiache (ST6), this acupoint is located near the subbuccal and marginal mandibular branches of the facial nerve, which provides the morphological foundation to alleviate symptoms of FNP [48]. The combination of Hegu (LI4) and Jiache (ST6) stimulates more peripheral nerves compared with needling a single acupoint of them.

5. Limitations

There are some limitations of this study. Although the modeling method in this study is more consistent with the natural pathogenesis of FNP, the model rate is relatively lower than that of compressing the facial nerve. Additionally, this study shows the potential of acupuncture in triggering a patient's immunoreaction to further reduce HSV-1 quantity. However, this study is insufficient to fully elucidate the mechanism of acupuncture in reducing HSV-1 quantity. Future studies might be directed toward elucidating this mechanism, such as the relationship between acupuncture and NF-κB in FNP *in vivo*.

6. Conclusions

We concluded that, in the treatment for FNP, electroacupuncture alleviates symptoms, facilitates affected nerve recovery, and promotes the reduction in HSV-1. Acupuncture might have therapeutic advantages in controlling inflammation and infection in FNP.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Hongzhi Tang and Shuwei Feng contributed equally to the work.

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Research Article

Correlation between the Effects of Acupuncture at *Taichong* (LR3) and Functional Brain Areas: A Resting-State Functional Magnetic Resonance Imaging Study Using True versus Sham Acupuncture

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Functional magnetic resonance imaging (fMRI) has been shown to detect the specificity of acupuncture points, as proved by numerous studies. In this study, resting-state fMRI was used to observe brain areas activated by acupuncture at the *Taichong* (LR3) acupoint. A total of 15 healthy subjects received brain resting-state fMRI before acupuncture and after sham and true acupuncture, respectively, at LR3. Image data processing was performed using Data Processing Assistant for Resting-State fMRI and REST software. The combination of amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) was used to analyze the changes in brain function during sham and true acupuncture. Acupuncture at LR3 can specifically activate or deactivate brain areas related to vision, movement, sensation, emotion, and analgesia. The specific alterations in the anterior cingulate gyrus, thalamus, and cerebellar posterior lobe have a crucial effect and provide a valuable reference. Sham acupuncture has a certain effect on psychological processes and does not affect brain areas related to function.

1. Introduction

The traditional Chinese medical therapy, acupuncture, is used clinically as an alternative or supplementary treatment [1, 2]. A few studies have investigated the mechanism of action of acupuncture, with functional magnetic resonance imaging (fMRI) being an effective method to study the results of acupuncture [3–5]. The *Taichong* (LR3) acupoint has been used for the study of specificity of meridians and acupoints. Many studies have investigated the effects of acupuncture at LR3 on brain function using fMRI [6–9]. These studies were based on a block design using a general linear model. Because acupuncture has persistent effects [10], conclusions based on this model may show a false-positive phenomenon. In addition, the acupuncture mode described above is not fit for the clinic. To better simulate clinical acupuncture treatment, this study performed acupuncture at LR3 using resting-state fMRI and compared the preacupuncture and postacupuncture image data to verify the changes in brain functional connectivity in different brain areas in an attempt to explain the mechanism of action of acupoints.

Previous studies [6, 8, 9] concerning fMRI at LR3 have compared the difference in brain function between acupuncture at LR3 and surrounding areas (nonacupoint) and confirmed the specificity of meridians and points on brain function. However, some shortcomings were noted in these studies. The nonacupoint was near LR3, making it difficult to avoid blood vessels and nerve segments with similar regulatory effects. This activation of nerves or blood vessels could possibly affect study results in the specificity of meridians and points. In addition, the use of nonacupoints at different sites of acupuncture to verify the specificity of meridians and points has some limitations. A previous study [11] has demonstrated that different acupuncture methods at the same acupoint could induce various responses in the central nervous system. The acupuncture method used is important to demonstrate the specificity of acupuncture. This study used sham acupuncture as a control (blunt, nonpenetrating needles) and compared alterations in brain function after true acupuncture at LR3. The preacupuncture and postacupuncture brain areas affected were observed. This study compensated for the shortcomings of previous studies investigating LR3 by determining whether sham acupuncture exerted an identical effect to true acupuncture.

Previous studies mainly focused on cerebral functional imaging under acupuncture but seldom on effects after acupuncture [6–9]. This study focused on mechanisms of resting-state brain function as cerebral functional imaging studies have suggested that acupuncture can exert posteffects [10, 12]. Furthermore, the study of the posteffects of acupuncture excluded interference from sensing the needle body on cerebral functional imaging and is also more clinically relevant [13].

The combination of amplitude of low-frequency fluctuation (ALFF) and regional homogeneity (ReHo) was used in the present study. ReHo mainly reflects the synchronism of a time series in regional brain areas, but not signal intensity, and indirectly reflects the synchronism of spontaneous activity of local neurons in brain areas [14]. ALFF represents the intensity of blood-oxygen level-dependent signal in each voxel, directly reflecting the spontaneous activity of neurons [15]. Thus, this study investigated the specificity of LR3 by analyzing the common alterations in brain areas using ReHo and ALFF methods. On the basis of the theory described above, this study compared preacupuncture and postacupuncture at LR3 cerebral functional imaging and alteration-related brain areas. This study also compared cerebral functional imaging after true acupuncture and sham acupuncture at LR3 to test whether acupuncture at LR3 specifically affected brain areas by producing activation or deactivation. The effects of directly activated brain areas and their association with the mechanism of action of LR3 were then determined.

2. General Data

A total of 15 healthy subjects were from universities and colleges in Guangzhou city, China. Inclusion criteria: (1) being between 21 and 28 years of age; right handedness; (2) having regular diet; minimal liquor, tobacco, tea, and coffee; normal sleeping patterns (before 12 a.m.); moderate size body mass index of 18.5–23.9 (Chinese); no history of nervous system disease; (3) having no pain (including dysmenorrhea) or insomnia within 1 month before the test; (4) having no metallic substances in the body, such as stents; (5) being fit for noise and hypothermia; no fear of confined spaces; (6) having not undergone acupuncture within 1 month before the test.

The present study contained nine males and six females, aged from 21 to 23 years (mean = 21.80 ± 0.56 years). Subjects weighed 46–72 kg (mean = 55.40 ± 8.35 kg) and were 160–180 cm (168.6 ± 6.81 cm) tall. All subjects gave full informed consent.

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).

3. Methods

3.1. Trials and Processing Methods. The subjects first underwent sham acupuncture at LR3. Thirty minutes later, subjects were subjected to the second acupuncture (true acupuncture at LR3). These volunteers were not told the order of true or sham acupuncture. Subjects were asked to pass urine and stool prior to treatment. Volunteers' eyes were masked with eyeshades, and earplugs were simultaneously worn so their audiovisual system could not be stimulated.

3.2. Acupuncture Methods. Acupuncture was performed by the same experienced physician. Tubes (DONGBANG AcuPrime, Exeter, UK) and Huatuo needles 0.30∗(25 mm–45 mm) (Suzhou Medical Supplies Co., Suzhou, China) were used in this study.

(1) Sham acupuncture at LR3: bilateral LR3 was localized according to Name and location of Acupoints: Chinese National Standards GB/T12346 [16]. Skin was sterilized with alcohol. In accordance with a previously published method of sham acupuncture [17], the auxiliary part of the tube was applied to the skin, with a sham needle placed in the tube over the acupoint. The sham needle was then tapped to make its tip touch the skin without puncturing it and was maintained in place for 30 min.

(2) True acupuncture at LR3: bilateral LR3 were localized according to Name and location of Acupoints: Chinese National Standards GB/T12346 [16]. Skin was sterilized with alcohol. The tube needling technique was used: the needle handle was tapped softly using a forefinger. Puncturing depth was controlled. After removal of the tube, the needle was vertically punctured at 15 mm ± 2 mm. After developing needle sensation, twirling at an angle of 90–180° and frequency of 60–90 times/min and lifting and thrusting at a range of 0.3–0.5 cm and frequency of 60–90 times/min were conducted. After manipulating the needle for 1 min, the needle was held in place for 30 min. During the 30 min, physician repeated this manipulation for 1 min every 10 min.

3.3. fMRI Examination. Subjects were conscious, placed in a supine position, and asked to breathe calmly. The head was fixed with a foam mat, thus greatly reducing active and passive movement. Earplugs were used to reduce hearing and eyeshades were also used. After subjects rested for 15 min, the scan began.

Table 1: Brain areas with ALFF alteration.

Comparisons	Number of voxels	Brain areas	Right/Left	Brodmann area	Talairach (mm)			T
					X	Y	Z	
TA versus PA	101	Frontal lobe, subgyral	R	47	27	21	−3	−6.2209
	139	Superior occipital gyrus	L	19	−33	−87	30	−3.8911
TA versus SA	181	Sublobar, extranuclear	R		27	−36	15	−5.6675

Experiments were performed on a GE 3.0T MRI scanner with an 8-channel head coil. The MRI data (resting-state BOLD sequence) were collected at 15 min before needling and 15 min after withdrawing the needle. The 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: 1750 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: 3000 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, and matrix: 96 × 96/number of excitations = 1.

3.4. Image Processing and Analytical Methods. Preprocessing was carried out using Data Processing Assistant for Resting-State fMRI (DPARSF; Chao-Gan and Yu-Feng, 2010, http://www.restfMRI.net), which is based on Statistical Parametric Mapping (SPM8; http://www.fil.ion.ucl.ac.uk/spm/) and Resting-State fMRI Data Analysis Toolkit (REST, Song et al., 2011. http://www.restfMRI.net) [18, 19]. This includes DICOM format conversion, removal of 10 time points before image scanning, time correction, correction of head movement, space standardization, and space smoothing. After preprocessing, 15 subjects were included in the statistical analysis.

ReHo analysis: using REST1.8 software, linear tendency of the data after preprocessing (space standardization was completed, and space smoothing was not finished) was removed by linear regression. Time and curve were convolved using Hamming bandpass filtering. ALFF was extracted (0.01–0.08 Hz). Kendall's coefficient of concordance of each subject was computed, so each subject had a Kendall coefficient of concordance map, that is, ReHo map. This map was divided by the mean of the whole brain, and standardized ReHo was obtained and used in statistical analysis [18, 20, 21].

ALFF analysis: using REST1.8 software, linear tendency of the data after preprocessing (space smoothing was completed) was removed by linear regression. Time and curve were convolved using Hamming bandpass filtering. ALFF was obtained (0.01–0.08 Hz). ALFF of each subject was computed, so ALFF maps were obtained. ALFF value was divided by the mean of the whole brain, and standardized ALFF was obtained [15].

3.5. Statistical Analysis. The data were analyzed with REST 1.8 software. Intragroup standardized ReHo and ALFF values were detected with paired t-test (AlphaSim correction $P < 0.05$; continuous voxel >85). Finally, the preacupuncture and postacupuncture differences between the alterations of ALFF and ReHo were obtained in subjects of the same group. 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 were presented as images.

4. Results

4.1. ALFF Analysis. ALFF results showed that brain areas with alterations after true acupuncture at LR3 apparently decreased in the right frontal lobe (BA47) and left superior occipital gyrus (BA19; T value was negative; Table 1, Figure 1) versus preacupuncture. Brain areas decreased in the sublobar and extranuclear regions after true acupuncture compared with sham acupuncture at LR3 (Table 1, Figure 2). However, there were no functional areas with specific alterations after sham acupuncture at LR3 (Figure 3).

4.2. ReHo Analysis. ReHo results demonstrated that, compared with preacupuncture, the right middle temporal gyrus (BA19) decreased (T value was negative) and the left inferior temporal gyrus (BA20), left middle temporal gyrus (BA21), and left anterior cingulate gyrus (BA32; Table 2, Figure 4) increased in brain areas after true acupuncture at LR3. After true acupuncture at LR3 ReHo showed that, in the right superior temporal gyrus (BA38), left cerebrum, sublobar and extranuclear regions, and right thalamus were decreased (T value was negative), while the right cerebellar posterior lobe was increased at LR3 compared with the sham acupuncture (Table 2, Figure 5).

Comparing sham acupuncture at LR3 with preacupuncture, we found that decreased ReHo was visible in the right superior frontal gyrus (BA9) and right posterior cingulate

Table 2: Brain areas with ReHo alterations.

Comparisons	Number of voxels	Brain areas	Right/Left	Brodmann area	Talairach (mm)			T
					X	Y	Z	
TA versus PA	134	Middle temporal gyrus	R	19	36	−57	18	−4.4085
	100	Temporal lobe, subgyral	L	20	−45	−21	−27	4.3696
	276	Middle temporal gyrus	L	21	−63	−51	0	6.1186
	118	Limbic lobe, anterior cingulate	L	32	−9	24	24	5.6813
TA versus SA	94	Superior temporal gyrus	R	38	36	9	−21	−4.0397
	115	Cerebellum posterior lobe, declive	R		33	−57	−15	5.21
	331	Sublobar, thalamus	R		24	−27	0	−6.5849
	129	Sublobar, extranuclear	L		−30	−24	−6	−4.8703
SA versus PA	101	Sublobar, lentiform nucleus	R		30	−18	0	4.5274
	267	Superior frontal gyrus	R	9	24	51	36	−6.5548
	184	Limbic lobe, posterior cingulate	R	30	18	−57	12	−5.514
	132	Middle temporal gyrus	L	21	−63	−54	0	5.3984
	109	Limbic lobe, cingulate gyrus	L	24	0	−6	39	5.4237

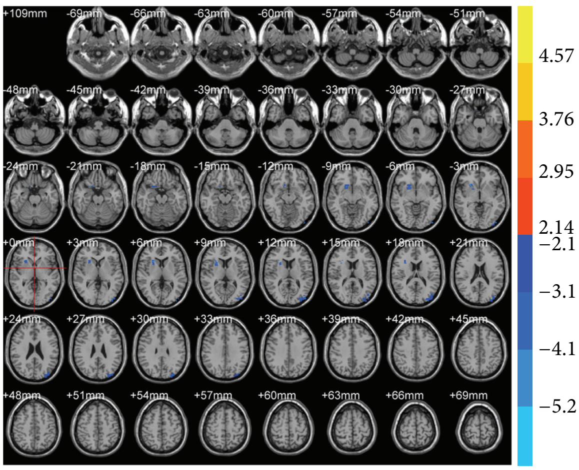

Figure 1: Brain areas with ALFF alteration after true acupuncture at LR3 versus preacupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

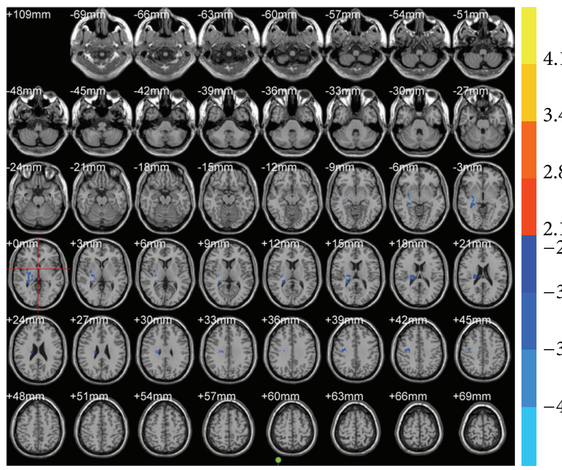

Figure 2: Brain areas with ALFF alteration after true acupuncture at LR3 versus sham acupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

(BA30). Enhanced ReHo was detectable in the left middle temporal gyrus (BA21), left cingulate gyrus (BA24), and right lenticular nucleus (Table 2, Figure 6). All these brain areas have been shown to be involved with association.

Both ALFF and ReHo results had signal deactivated in BA19 and sublobar and extranuclear regions.

5. Discussion

After true acupuncture at LR3, brain areas with ALFF alterations included BA19 and BA47. Brain areas with ReHo alterations included BA19, BA20, BA21, and BA32. Deactivation in BA19 occurred with both ALFF and ReHo. BA19, a visual association area, can perceive and integrate visual information. Deactivation represents relative decrease in regional blood-oxygen signal intensity. A previous study verified that a decrease in blood-oxygen signal intensity may have been a mark of neuronal inhibition [22]. Another study also confirmed that the decrease in blood-oxygen signal intensity was associated with local field potentials and multiunit activity [23], primarily suggesting that acupuncture at LR3 specifically suppressed vision-related neurons. Siedentopf et al. [24] suggested that acupuncture at acupoints on the foot activated the visual cortex and treated vision-related disease. Simultaneously, an fMRI study confirmed that acupuncture at LR3 specifically activated BA19 [8]. Thus, we speculate that BA19 is a specific brain area for true acupuncture at LR3. Acupuncture at LR3 could bidirectionally regulate (excitation and inhibition) visual neurons, which could be a mechanism for treating vision-related diseases. Moreover, BA20 and BA21 activated by ReHo are associated with visual processing. This observation verified the bidirectional regulation on vision after puncturing LR3 and further confirmed that vision-related brain areas are specific for acupuncture at LR3. A previous study suggested that the anterior cingulate gyrus (BA32) participated in many complicated motor functions

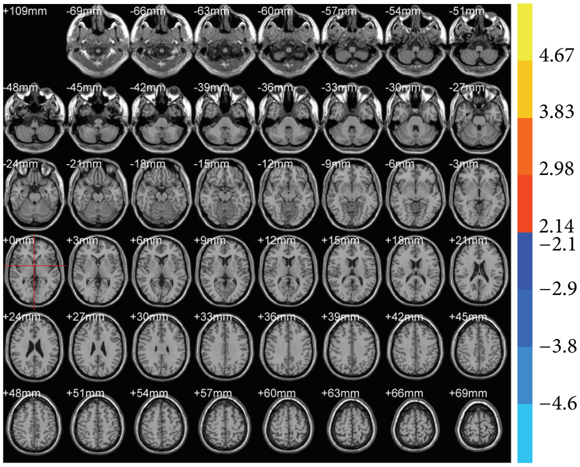

Figure 3: Brain areas with ALFF alteration after sham acupuncture at LR3 versus preacupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

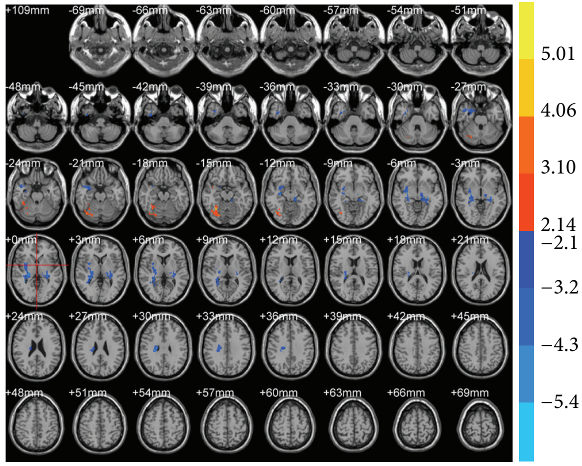

Figure 5: Brain areas with ReHo alterations after true acupuncture at LR3 versus sham acupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

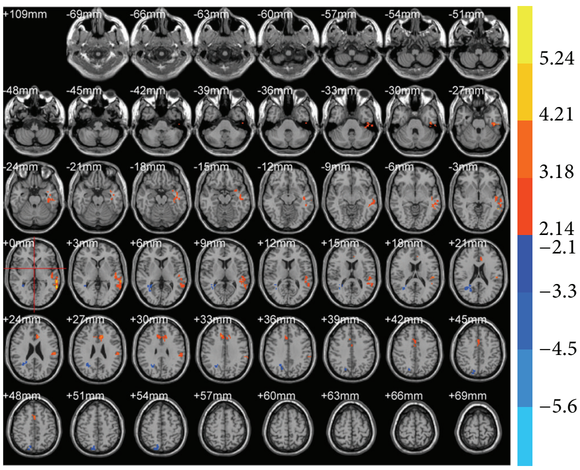

Figure 4: Brain areas with ReHo alterations after true acupuncture at LR3 versus preacupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

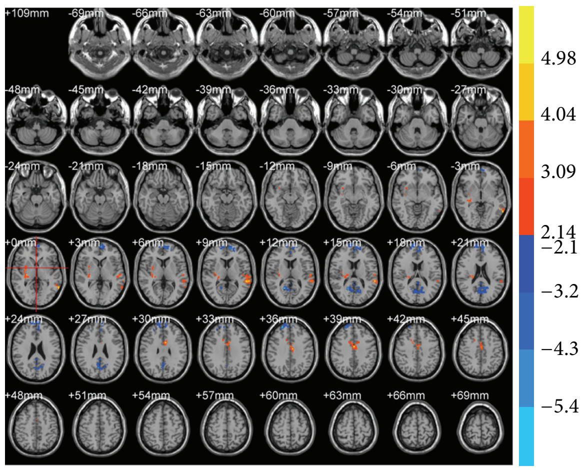

Figure 6: Brain areas with ReHo alterations after sham acupuncture at LR3 versus preacupuncture. Blue represents deactivation; red represents activation; blank represents no activation.

and pain reactions in the body [25]. Prior studies have also indicated that acupuncture at LR3 specifically activated the anterior cingulate gyrus [6, 8], indicating that the anterior cingulate gyrus was an additional specific brain area activated by acupuncture at LR3.

Brain areas after true acupuncture at LR3 versus sham acupuncture: ALFF alterations were visible in sublobar and extranuclear regions of the brain. ReHo alterations were observed in BA38, sublobar and extranuclear regions, thalamus, and cerebellar posterior lobe. ALFF and ReHo results demonstrated that deactivated brain areas included sublobar and extranuclear regions, but the functions of these brain areas were not prominent, and did not show close correlation with the effects of LR3 acupoint. This observation requires further investigation. The thalamus is a relay station buried under the cerebral cortex and is involved in sensory perception. A previous study showed that the thalamus was associated with the regulation of acute and chronic pain [26]. The thalamus is a component in the pain management network. This network was deactivated during development of needle sensation [11, 27], demonstrating that the thalamus could negatively regulate brain networks activated by pain. Thus, pain-activated brain areas were transformed into an inhibitory state, exerting an analgesic effect of acupuncture. Studies [6–9] have also found changes in the thalamus, indicating another area specifically altered after acupuncture at LR3. This observation provides further evidence for the analgesic efficacy of acupuncture at LR3. The main function of the cerebellar posterior lobe is to maintain balance, to regulate muscular tension, to coordinate voluntary movement, and to manage fine motor control. In clinical practice, acupuncture at LR3 could treat vertigo and lower limb paralysis, which could be associated with regulatory effects of LR3 on the vestibular nerve and spinal movement through the cerebellum. Moreover, the cerebellar posterior lobe is a component of the neocerebellum and has an extensive connection to the cerebral cortex. The neocerebellum is involved in affection and cognition [28], which could be a mechanism of LR3 acupuncture in the treatment of emotion-related disease. Numerous studies confirmed [29–31] that acupuncture activated the cerebellum, suggesting that this activation plays an important role in the mechanisms underlying acupuncture treatment.

ALFF results demonstrated that no brain areas were altered after sham acupuncture and reflected the specificity of meridians and acupoints from an indirect source. ReHo results revealed that, after sham acupuncture, alterations were observed in the superior frontal gyrus (BA9), posterior cingulate gyrus of the limbic lobe (BA30), cingulate gyrus of the limbic lobe (BA24), the left middle temporal gyrus (BA21), and the lenticular nucleus. Of these areas, the frontal lobe (BA9) mainly participates in prefrontal association; the middle temporal gyrus (BA21) participates in temporal association; the posterior cingulate gyrus (BA30) is mainly involved in limbic lobe association; and the cingulate gyrus (BA24) is mainly involved in emotion and cognition. These brain areas have been shown to be involved with association. It is presumed that sham acupuncture-induced sensation was different from that of a needle pricking skin. This difference would lead to thinking and association in most subjects, so association-related alterations were detected in these brain areas. The combination of ALFF and ReHo methods suggested that sham acupuncture at LR3 could not affect functional areas.

In this study, fMRI was performed 15 min after acupuncture. Activation and deactivation of brain areas further verified that acupuncture definitely caused posteffects. Moreover, true acupuncture at LR3 versus sham acupuncture specifically activated/deactivated relevant brain areas. This study primarily confirmed that regulatory effects and clinical efficacy of acupuncture were not the effects of psychological factors. The present study has some limitations. In this study, subjects were healthy. It is initially presumed that activated/deactivated brain areas are associated with the treatment of clinically relevant diseases. Whether it still has regulatory effects on brain areas in patients requires further investigation. Some brain areas activated/deactivated by LR3 acupuncture did not have clear functions. Moreover, these brain areas did not strongly associate with the effects of LR3, and we therefore did not deeply study these regions. Whether these areas exert specific effects of LR3 acupuncture also deserves further study.

In summary, using resting-state fMRI, true acupuncture at LR3 specifically activated/deactivated some brain areas related to vision, movement, sensation, emotion, and analgesia. These findings may show a mechanism underlying the regulatory effects of acupuncture at LR3. The results from the comparisons of posttrue acupuncture and pretrue acupuncture, as well as posttrue acupuncture and postsham acupuncture, confirmed that meridians and points (LR3) exert effects on specific brain areas, which were associated with the mechanism of action of LR3.

Ethical Approval

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).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Chunzhi Tang obtained funding. Yong Huang and Chunzhi Tang participated in the study concept and design and manuscript authorization. Jiping Zhang and Shaoqun Zhang recruited volunteers. Shanshan Qu and Junqi Chen analyzed the data. Chunxiao Wu wrote the manuscript. Huailiang Ouyang performed the acupuncture. Zhipeng Li and Jiarong Chen ensured the integrity of the data.

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Research Article

Acupuncture Enhances Effective Connectivity between Cerebellum and Primary Sensorimotor Cortex in Patients with Stable Recovery Stroke

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Recent neuroimaging studies have demonstrated that stimulation of acupuncture at motor-implicated acupoints modulates activities of brain areas relevant to the processing of motor functions. This study aims to investigate acupuncture-induced changes in effective connectivity among motor areas in hemiparetic stroke patients by using the multivariate Granger causal analysis. A total of 9 stable recovery stroke patients and 8 healthy controls were recruited and underwent three runs of fMRI scan: passive finger movements and resting state before and after manual acupuncture stimuli. Stroke patients showed significantly attenuated effective connectivity between cortical and subcortical areas during passive motor task, which indicates inefficient information transmissions between cortical and subcortical motor-related regions. Acupuncture at motor-implicated acupoints showed specific modulations of motor-related network in stroke patients relative to healthy control subjects. This specific modulation enhanced bidirectionally effective connectivity between the cerebellum and primary sensorimotor cortex in stroke patients, which may compensate for the attenuated effective connectivity between cortical and subcortical areas during passive motor task and, consequently, contribute to improvement of movement coordination and motor learning in subacute stroke patients. Our results suggested that further efficacy studies of acupuncture in motor recovery can focus on the improvement of movement coordination and motor learning during motor rehabilitation.

1. Introduction

Although acupuncture has been widely used in rehabilitation of hemiplegic stroke patients in many parts of the world, the potential neural mechanism underlying the beneficial effect of acupuncture remains largely unknown. Recent neuroimaging studies have demonstrated that stimulation of acupuncture at motor-implicated acupoints modulates activities of brain areas relevant to the processing of motor signals [1–4]. These findings have shed some lights on the functional substrates of the purported therapeutical effect of acupuncture in stroke rehabilitation. However, the interactions within motor-related networks as well as its influence contributing to motor recovery induced by acupuncture have remained elusive.

Models of functional connectivity and effective connectivity can be used to describe the interactions between brain areas within brain network [5]. Recent neuroimaging studies have demonstrated that rehabilitative therapies can induce changes in effective connectivity of motor-related areas in stroke patients [6, 7], and rTMS or pharmacological treatments can also ameliorate stroke-induced deficits by enhancing effective connectivity within the motor network [8–10]. Acupuncture, which is a potentially effective therapy in stroke rehabilitation, has been reported to modulate resting state functional connectivity in the default mode

Table 1: Clinical and demographic data.

Patient number	1	2	3	4	5	6	7	8	9
Age (years)	56	64	57	68	57	37	58	71	52
Gender	F	M	M	M	F	M	M	M	M
Localization of infarct	BG	IC	IC	CR	IC	IC	IC	IC	BG
Motricity index	0	60	14	72	23	60	34	76	76
	11	64	14	72	23	60	34	76	—
Rankin scale	4	1	2	2	4	2	3	2	2
	4	1	2	1	4	2	3	1	—
Barthel index	35	95	60	90	60	85	65	90	85
	40	95	65	85	60	85	75	90	—
NIHSS	14	3	9	5	8	7	7	3	5
	8	1	9	2	8	7	7	2	—
MMSE	22	30	27	29	22	30	30	24	30
	23	30	30	28	24	30	30	27	—
Brunnstrom	I	IV	II	II	I	V	II	V	II
	I	IV	II	III	I	V	II	V	—
Ashworth	0	1	1	0	0	2	2	0	0
	0	1	0	1	0	2	2	0	—

Abbreviations: BG: basal ganglia; IC: internal capsule; CR: corona radiate; NIHSS: National Institute of Health Stroke Scale; MMSE: Mini-Mental State Examination.

and sensorimotor brain networks [11, 12]. However, recent studies have shown that functional connectivity and effective connectivity between different regions are both important and essential in detailing working mechanisms of the brain's functional architecture. The resultant model is primarily concerned with the directions of neural interactions and how one neural system exerts influence over another. Such information can be used to explore the specific role of a cortical region in a distributed system [5]. Changes in the pattern of normal cortical connectivity within and across hemispheres in stroke patients with motor deficits in the subacute phase have been discovered [13]. Moreover, James et al. found that improvements in motor performance were associated with enhanced interhemispheric communication [6]. These findings provide compelling rationales to investigate the acupuncture-induced changes in effective connectivity among motor areas in hemiparetic stroke patients.

Previous neuroimaging studies mainly focused on the functional specificity of motor-related acupoint on healthy subjects. According to the theory of traditional Chinese medicine, acupuncture is believed to exert various therapeutic effects by restoring the homeostatic balance [14]. Thus, acupuncture may have more specific effects on patient with a pathological imbalance compared to healthy subjects. In the present study, we investigated acupuncture stimulation at acupoint GB 34 in stroke patients and used healthy subjects as control condition. It has been reported that acupuncture can produce sustained effects even after the acupuncture manipulation being terminated [15, 16]. In this study, a nonrepeated event-related (NRER) design [12, 17] was employed to investigate effective connectivity changes after acupuncture administration. Based on the principle of temporal predictability, the Granger causality analysis can be used to explore effective connectivity between ROIs without any *a priori* specification of a network model [18–20]. In the present study, a multivariate Granger causality model was employed to obtain causal relations among multiple brain areas. This approach was based on a multivariate vector autoregressive (MVAR) model and allowed us to detect the simultaneous directional influences between multiple ROIs without any *a priori* specification of a network model. This approach has been successfully applied in many previous brain network studies [18–20]. We hypothesized that stroke patients may exhibit different models in effective connectivity within motor network involving both passive finger movements task and resting state, and acupuncture can induce relatively specific effects on the modulation of interactions within the motor network compared with healthy controls. In order to further understand the acupuncture mechanism, we conducted an fMRI study to identify acupuncture-induced changes in the interactions between motor-related areas that potentially facilitate motor recovery after stroke.

2. Materials and Methods

2.1. Subjects. From March 2012 to February 2013, a total of 9 patients who had ischemic strokes in the anterior circulation (7 males and 2 females, mean age 57.8 ± 9.9 years, mean days from first-onset stroke 53.6 ± 41.6 days, ranged from 18 to 122 days) were recruited from Beijing Dongzhimen Hospital. Inclusion criteria were as follows: (1) >2 and <12 weeks from the onset of ischemic stroke; (2) unilateral right-sided striatocapsular lesions; (3) Mini-Mental State Examination (MMSE) score $\geq$ 21 [21]; (4) moderate to severe motor deficits of the contralesional upper extremity, Motricity Index (MI) < 80; (5) right-handed individuals according to the Edinburgh Handedness [22]; (6) age range of 35–75 y. Exclusion criteria were as follows: (1) any clinically significant or unstable medical disorder, (2) bihemispheric or brain stem infarcts; (3) severe aphasia precluding communication, (4) any neuropsychiatric comorbidity other than stroke, and (5) standard contraindications for MRI such as non-MRI compatible implanted metallic devices. All patients recruited were scored on the following function measures on the same day as MRI: (i) Motricity Index (MI) for affected upper and (ii) lower limbs; (iii) NIHSS; (iv) Brunnstrom; (v) Modified Ashworth Scale; (vi) Barthel Index; (vii) Modified Rankin Scale.

An additional 8 healthy subjects were recruited from Dongzhimen Hospital as age-matched and sexually matched control subjects (6 males and 2 females; mean age 51.6 ± 4.8 years, all right-handed individuals). There was no significant difference in age between the patients and the healthy subjects ($P = 0.132$). All control subjects had no history of drug abuse, alcohol abuse, stroke, or other neurological or psychiatric diseases. This study was approved by the local Institutional Review Board and conducted in accordance with the Declaration of Helsinki, and full written informed consent was obtained from all subjects. All subjects were acupuncture naïve and patient characteristics are listed in Table 1.

2.2. Experimental Design. Patients and healthy subjects had the same MRI procedure. Eight patients underwent the experiment twice at an interval of two weeks. One patient only underwent the experiment once. Every control subject underwent the experiment once. Every experiment consisted of three functional runs including, successively, resting state scanning before and after acupuncture stimuli, and passive finger movement. Resting-state run lasted 8 min (Figure 1(a)). During the resting-state run, subjects were asked to lie motionless with their eyes closed, not to think of anything in particular, and not to fall asleep. Cushions were used to reduce head motions. The acupuncture run employed the NRER-fMRI design paradigm (Figure 1(b)), incorporating 1 min needle manipulation, preceded by 10 seconds rest and followed by 8 min rest scanning. The motor task run employed a conventional block design in which five blocks of 20-second finger movement were alternated by five blocks of 20-second baseline, with 10 seconds rest in the beginning (Figure 1(c)). The motor task consists of a repetitive movement in which the left thumb was passively opposed to the left index finger at the frequency rate of 1 Hz. After the three runs of scanning, all subjects were asked if they fell asleep during any of the runs.

Acupuncture was performed at an acupoint GB 34 on the left leg (Yanglinquan, located in a depression anterior and inferior to the head of the fibula). This acupoint is one of the most frequently used acupoints and proved to have various efficacy in the treatments of hemiplegic stroke in some previous researches [23, 24]. A sterile disposable 38-gauge sterling silver acupuncture needle (0.3 mm in diameter and 40 mm in length) was inserted vertically to a depth of 2-3 cm to deliver acupuncture stimulation. During acupuncture stimulation, the needle was rotated bidirectionally to an amplitude of approximately 180° for 1 min at a rate of 120 times per min by a balanced "tonifying and reducing" technique. All subjects were not informed of the presumed acupuncture effects. The procedure was performed by the same experienced and licensed acupuncturist on all subjects.

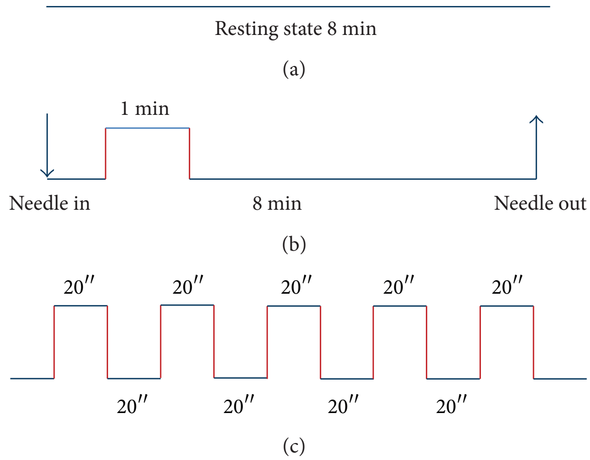

Figure 1: fMRI scan procedures. (a) Resting-state run lasted 8 min. (b) The acupuncture run employed the NRER-fMRI design paradigm, incorporating 1 min needle manipulation, preceded by 10 seconds rest and followed by 8 min rest scanning. (c) The motor task run employed a conventional block design in which five blocks of 20-second finger movement were alternated by five blocks of 20-second baseline, with 10 seconds rest in the beginning.

Following the acupuncture run, subjects were presented with a 10-point visual analog scale (VAS) in which 0 = no sensation and 1–3 = mild, 4–6 = moderate, 7-8 = strong, 9 = severe, and 10 = unbearable sensation. Subjects were asked to rate the intensity of aching, pressure, soreness, heaviness, fullness, warmth, coolness, numbness, tingling, or dull or sharp pain they felt during the acupuncture run. Subjects were excluded from further analysis if they experienced sharp pain (greater than the mean by more than 2 standard deviations). None of the subjects experienced sharp pain among the 17 subjects.

2.3. Data Acquisition and Analysis. Imaging was performed on a 3.0 Tesla Siemens MRI Scanner in Radiology Department, Dongzhimen Hospital. A custom-built head holder and firm cushions were used to minimize the head motion. Functional scans were collected with sagittal sections parallel to the AC-PC plane. Thirty-two axial slices with coverage of the whole brain were obtained by using a $T2^*$-weighted single-shot, gradient-recalled echo planar imaging (EPI) sequence. Acquisition parameters used in the functional scans were TE = 30 ms, TR = 2 s, flip angle = 90°; 3.5 mm slice thickness with 0.7 mm gap; 64 × 64 acquisition matrix with a field of view (FOV) of 225 mm × 225 mm. After acupuncture run, high-resolution structural images were acquired on each subject using a T1-weighted three-dimensional (3D) MRI sequence with a voxel size of 1 mm^3 for anatomical localization. Acquisition parameters used in the structural scans were TR = 1.9 s, TE = 2.52 ms, matrix = 256 × 256, FOV = 250 mm × 250 mm, flip angle = 9°, slice thickness = 1 mm.

All images were preprocessed and analyzed using Statistical Parametric Mapping 5 (SPM5, http://www.fil.ion.ucl.ac.uk/spm). Images were first corrected for head movement using least square minimization. None of the subjects had excessive head movements (>1.5 mm) on any axis and head rotation more than one degree. Then the image data was further normalized to the MNI template and resampled at 2 mm × 2 mm × 2 mm. Finally, images were smoothed with a 6 mm full-width-at-half maximum (FWHM) Gaussian kernel to decrease spatial noise. Then these data were filtered by using a bandpass filter (0.01~0.08 Hz) to reduce the effect of low-frequency drift and high-frequency noise.

2.4. Definition of Regions of Interest. The motor task from each subject was entered into a general linear model (GLM) "fixed-effect" framework. BOLD signal change for motor task epochs compared to rest epochs was estimated at each voxel and individual t-maps were obtained. Then individual t-maps were entered into the "random effect" group analysis framework, and statistical maps were obtained ($P < 0.05$, FDR corrected) by one-sample t-test. Brain regions activated during motor task in different groups were determined by the statistical maps. As brain regions activated during finger movement in healthy controls have been studied in lots of previous researches [25–28], we selected brain regions activated during motor task in healthy group as regions of interest ($P < 0.05$, FDR corrected) for further effective connectivity. These motor-related brain regions included the bilateral declive, bilateral culmen, bilateral inferior frontal cortex, bilateral inferior parietal lobule, lateral nucleus of thalamus, bilateral superior temporal cortex, bilateral middle temporal cortex, left precentral cortex, left postcentral cortex, right precentral cortex, right postcentral cortex, precuneus, bilateral insula, bilateral posterior thalamus, bilateral anterior cingulate cortex, bilateral caudate nucleus, bilateral middle cingulate cortex, and substantia nigra. Taking into account the intersubject anatomical variance, ROIs were defined on individual anatomical map, and the obtained individual ROIs were registered to standard MNI space to get a group probabilistic anatomical map. Finally, ROIs were defined by using the standard Talairach-Daemon-based atlas. The time series within each ROI were selected, averaged across voxels, and normalized across subjects to obtain a single vector per ROI, separately for different conditions (resting state before and after acupuncture and motor task) in different groups. For bilaterally activated areas, time series were averaged.

2.5. Effective Connectivity Analysis Using mGCA. To detect the causal interactions among those selected ROIs during the three conditions, a multivariate autoregressive model (MVAR) of time series within each ROI was established. Directed transfer function (DTF) based on the principle of the Granger causality was computed in the multivariate autoregressive model [29]. To highlight the direct connections and reduce mediated influences, we calculated the partial coherence to evaluate the direct association between every two ROIs. An approach of surrogate data was employed to test the significance of the path weights. A null distribution of 2500 sets of surrogate data was generated and DTF was calculated from these datasets [30]. Finally, a one-tailed significance test was carried out to compare the DTF value from the original time series with null distribution ($P < 0.01$, corrected).

Comparison between the resting state and the postacupuncture resting state was performed on the path weight between any two ROIs. In this way, we obtained changes in causal influence within the motor-related network between rest state and postacupuncture rest state. To better understand, the dynamic characters of the network, the "in + out" degree of each node within the network, were calculated. "in + out" degree of a node was defined as the number of all edges connected directly with it, and notes with a standard deviation more than mean degree were considered as the hubs of the network. The hubs of the network were believed to exert important influence on the network dynamics.

3. Results

3.1. Psychophysical Results. In this study, all subjects reported de qi sensations in different intensity such as soreness, heaviness, and fullness during acupuncture stimulation. The sensation of fullness was reported most frequently among both patient and control groups. According to the subjects' reports, 85% of the patients and 50% of the healthy subjects experienced the sensation of fullness, while the second most prevalent sensation experienced was numbness in patient group and aching in healthy group. The sensation intensity (mean ± standard deviation) was 4.5 ± 1.9 in healthy group and 4.1 ± 1.9 in patients groups. Although the prevalence of various sensations was significantly different ($P < 0.05$) between the two groups, there was no significant difference in the intensity of the sensations experienced between two groups ($P = 0.67$).

3.2. mGCA Mapping Results. A visual description of causal connectivity between every two ROIs within the motor-related network was carried out with nodes representing the brain regions, edges thickness indicating the strength of influence, and arrows referring to directions of the influence (Figure 2).

During motor task, the right postcentral gyrus and middle temporal gyrus served as the hubs of the network in healthy controls, receiving the most information inflows from cortical and subcortical brain regions, such as left precentral gyrus, precuneus, caudate nucleus, inferior parietal lobule, and lateral nucleus of thalamus. In stroke patients, the postcentral gyrus and precentral gyrus in left hemisphere turned into hubs of the network, receiving causal inflows from inferior parietal lobule and from each other (Figure 2(a)). This altered pattern of hubs showed a distinct shifting from right (contralateral) hemisphere to left (unaffected) hemisphere. In addition, the significant effective connectivities within the network in stroke patients were substantially reduced in comparison to those in healthy controls. Moreover, in stroke patients, there was a lack of significant connectivity in the subcortical regions such as the caudate nucleus and cerebellum.

Effective connectivity of the resting network was demonstrated, respectively, in different groups in Figure 2(b). Effective connectivity of the postacupuncture resting network was demonstrated, respectively, in different groups (Figure 2(c)). In healthy controls, left precentral gyrus, left postcentral gyrus, and inferior parietal lobule served as the hubs of the network, but in stroke patients, the hubs of the network included left precentral gyrus, left postcentral gyrus, insula, culmen, and the lateral nucleus of thalamus. It is interesting to note that although the acupuncture stimulation was performed on the left side, the left precentral and postcentral gyri, instead of the right precentral and postcentral gyri, served as hubs in the network, in both patients and healthy controls.

Changes in effective connectivity between resting and postacupuncture resting state were demonstrated, respectively, in different groups in Tables 2 and 3. In healthy controls, following acupuncture stimulation, the pre- and postcentral gyri received enhanced causal inflows from various brain regions including declive, substantia nigra, lateral nucleus of thalamus, and inferior parietal lobule. In stroke patients, following acupuncture stimulation, the pre- and postcentral gyri received enhanced causal inflows from culmen. Meanwhile, culmen also received enhanced inflows from right postcentral gyrus.

Table 2: Changes in effective connectivity during postacupuncture resting state in controls[a].

Projecting regions	Receiving regions	P
	Increased connectivity	
Declive	Postcentral GR	<0.05
MCC	Postcentral GR	<0.05
P_Thalamus	Postcentral GR	<0.01
IPL	Precentral GL	<0.05
MCC	Precentral GL	<0.01
Postcentral GR	Precentral GR	<0.05
SubN	Postcentral GL	<0.05
Insula	P_Thalamus	<0.05
	Decreased connectivity	
Precuneus	ACC	<0.01
ACC	Precuneus	<0.01
STG	Insula	<0.05
Insula	Precuneus	<0.01
P_Thalamus	Precuneus	<0.01

[a]only $P < 0.05$ was listed in the table.

Table 3: Changes in effective connectivity during postacupuncture resting state in patients[b].

Projecting regions	Receiving regions	P
	Increased connectivity	
Insula	MTG	<0.05
MTG	Insula	<0.05
Culmen	Postcentral GL	<0.01
Precentral GR	Postcentral GL	<0.01
Postcentral GR	Culmen	<0.01
Culmen	Precentral GR	<0.05
	Decreased connectivity	
ACC	Precuneus	<0.01
Insula	ACC	<0.01

[b]only $P < 0.05$ was listed in the table.

4. Discussion

In the current study, we investigated the effective connectivity changes involved in motor task in stroke patients to show motor-related connectivity deficits in stroke patients. Then, we assessed that acupuncture can induce relatively functional specificity modulation within the motor network in stroke patients, compared with the healthy controls.

4.1. Motor-Related Connectivity in Stroke Patients. In this study, we found not only remarkably reduced effective connectivity within the motor-related network but also a lack of connectivity between cortical and subcortical brain regions in stroke patients during motor task. Since effective connectivity represents the causal influence one brain region exerts on another [31], the lack of effective connectivity indicates the inefficient information transmission between motor-related regions. This observations is consistent with previous studies that the efficiency of information integration between motor-related regions was significantly decreased in stroke patients [32, 33]. Interconnected with cerebral cortex by multiple circuits, the target subcortical areas, such as the basal ganglia and cerebellum, have been traditionally regarded as important subcortical motor structures in mediating muscle tone changes and ensuring movement precision [34–36]. Therefore, the disruption of effective connectivity between subcortical and cerebral cortex may underly motor deficit in stroke patients.

4.2. Acupuncture-Induced Changes in Brain Connectivity. In healthy controls, significantly enhanced effective connectivity from various subcortical brain regions to sensorimotor cortex in both hemispheres was shown in postacupuncture resting state. By contrast, only one subcortical area (the culmen) showed enhanced effective connectivity with sensorimotor cortices in stroke patients. Moreover, the information transfers between the sensorimotor cortex and culmen were bidirectional. The target brain areas were more concentrated in stroke patients. Previous studies have reported that acupuncture can modulate the activity of sensorimotor areas [1, 2] and cerebellar structures [37, 38] at motor-implicated acupoint. However, few studies have demonstrated concentrated and bidirectional enhancements in causal inflows between the cerebellum and primary sensorimotor cortex in stroke patients. Interestingly, such increases in connectivity between the subcortical areas and sensorimotor cortex may compensate for the lack of connectivity between cortical and subcortical cortex in stroke patients when executing motor task.

Culmen is located in the anterior vermis, which is considered as part of spinocerebellum that receives sensory

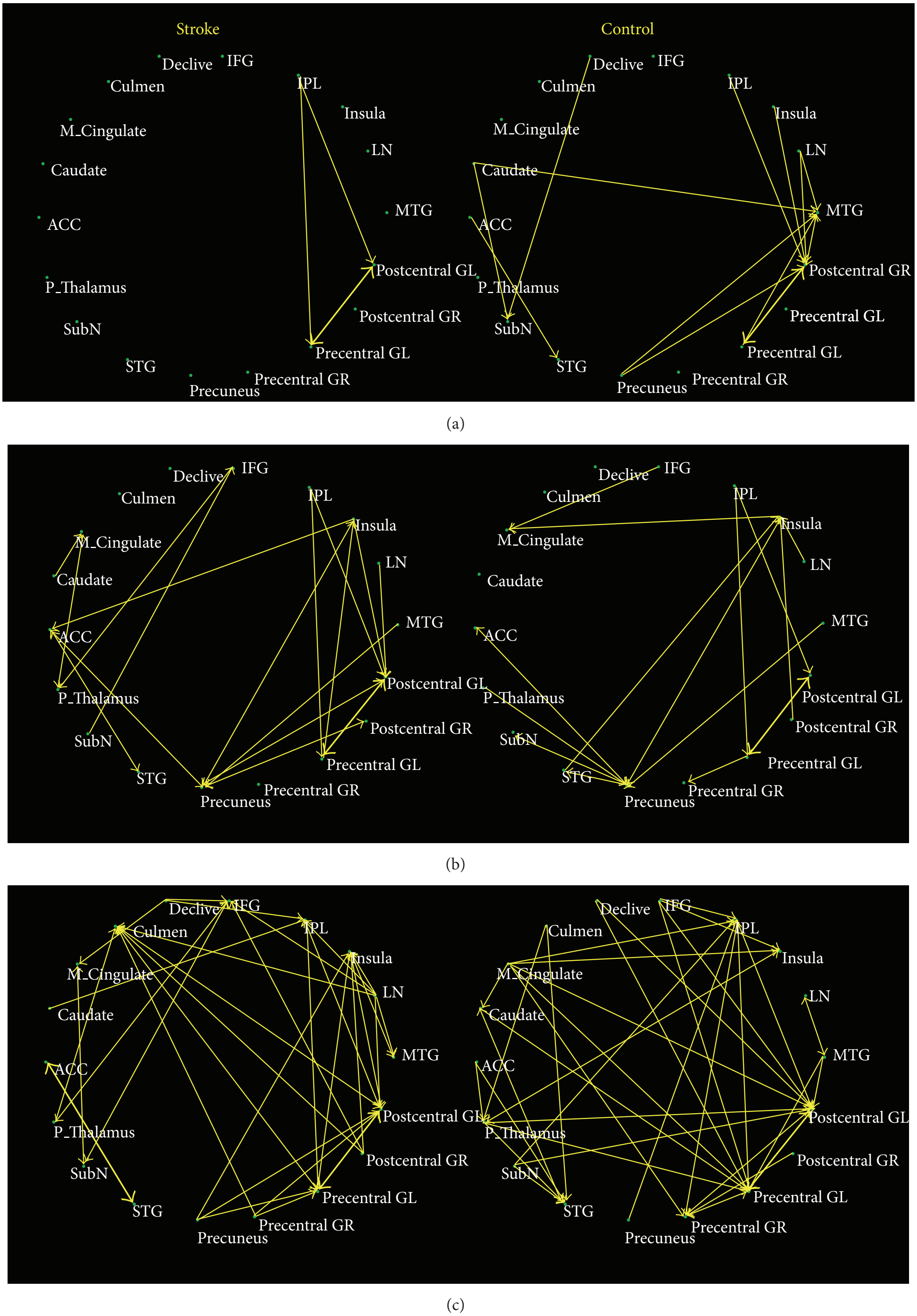

FIGURE 2: The visual description of causal connectivity between every two ROIs within the motor-related network with nodes representing the brain regions, edges thickness indicating the strength of influence, and arrows referring to directions of the influence. (a) Causal connectivity during motor task in stroke patients and controls. (b) Causal connectivity during resting state in stroke patients and controls. (c) Causal connectivity in postacupuncture state in stroke patients and controls.

information from both the primary sensorimotor cortex and the periphery and sends modulation information back to the sensorimotor cortex and brain stem via deep cerebellar nuclei [39, 40]. In this way, cerebellum modulates the descending motor systems [39, 40]. Enhancement in effective connectivity between culmen and primary sensorimotor cortex could be related to more bidirectional information transfer in the cerebrocerebellar loops, which may lead to a stronger motor coordination effect of cerebellum on motor system. Cerebellum has also been considered as a key structure in feedback processing and storage of motor skill during motor learning, and motor learning mechanism is involved in both spontaneous recovery and rehabilitative trainings including constraint-induced movement therapy (CIMT) and impairment-oriented training (IOT) [41]. In fact, involvement of cerebellum in the process of motor recovery after stroke has been demonstrated in previous researches [42–45]. Increased functional connectivity between ipsilesional primary motor region and cerebellum persisted during the 6 months from onset in stroke patients [43]. Johansen-Berg et al. found that increases in activity of specific regions in the cerebellum and sensorimotor cortex correlated with improvement in motor function after motor rehabilitation and suggested that recovery after motor rehabilitation may be facilitated by changes of activity in cerebellum and sensorimotor cortices [46]. Moreover, an experimental study on rats has demonstrated that enhancement in the output of the dentatothalamocortical pathway improved motor recovery after strokes [47]. Taken together, the bidirectional increases in effective connectivity between cerebellum and sensorimotor cortex following acupuncture may contribute to the motor recovery after stroke by improving coordination of movement and motor learning. Accordingly, future efficacy studies of acupuncture in motor recovery can focus on the improvement of movement coordination and motor learning during rehabilitative trainings.

Previous connectivity study on tactile stimulation has demonstrated stronger functional connectivity of the primary and secondary somatosensory areas in contralateral hemisphere than in ipsilateral hemisphere following tactile stimulation [48]. Unlike tactile stimulation, there was a distinct shifting of the hubs from right hemisphere to left (ipsilateral) hemisphere in both stroke patients and healthy controls following acupuncture on the left side of the body, suggesting stronger influence of the ipsilateral sensorimotor cortex on the network dynamic. This finding coincides with the results of previous laser acupuncture studies suggesting that acupuncture effect is not only based on processing of afferent sensory information [49–51]. Furthermore, the ipsilateral central neural effect of acupuncture provides rationales for the classical needling method "opposing needling" according to which acupuncture can be performed on the opposite of the affected limbs.

Decreases in effective connectivity from ACC to precuneus during postacupuncture resting state in patients were also found in both healthy controls and stroke patients. This finding is in accordance with the results of previous researches which showed interrupted correlation between the precuneus and anterior cingulate cortex (ACC) during the poststimulation sate [52]. ACC is one of the important key nodes of the salience network, while precuneus plays a pivotal role in default mode network [53, 54]. Salience network is considered to identify the most relevant among internal and extra stimuli to guide behavior [53]. Therefore, the decrease of effective connectivity between the two networks may be due to acupuncture stimulation administration in both healthy controls and stroke patients.

According to the theory of Traditional Chinese Medicine, de qi is believed to be essential to the therapeutic effectiveness of acupuncture. One of the major limitations of the study was that it is hard to record for how long de qi sensation lasted after acupuncture stimulation. Another limitation was that the sample size in this study is not very large. In the future study, the use of time measurement in de qi sensation and a larger sample size might provide a clearer picture of the therapeutic effectiveness of acupuncture.

5. Conclusions

Acupuncture induced a concentrated and bidirectional enhancement in effective connectivity between cerebellum and primary sensorimotor cortex in stroke patients, which may contribute to improving coordination of movement and motor learning. Our results suggest that future efficacy studies of acupuncture in motor recovery can focus on the improvement of movement coordination and motor learning by combining with other rehabilitative trainings.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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