The modulation of brain functional connectivity with manual acupuncture in healthy subjects: An electroencephalograph case study*

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Manual acupuncture is widely used for pain relief and stress control. Previous studies on acupuncture have shown its modulatory effects on the functional connectivity associated with one or a few preselected brain regions. To investigate how manual acupuncture modulates the organization of functional networks at a whole-brain level, we acupuncture at ST36 of a right leg to obtain electroencephalograph (EEG) signals. By coherence estimation, we determine the synchronizations between all pairwise combinations of EEG channels in three acupuncture states. The resulting synchronization matrices are converted into functional networks by applying a threshold, and the clustering coefficients and path lengths are computed as a function of threshold. The results show that acupuncture can increase functional connections and synchronizations between different brain areas. For a wide range of thresholds, the clustering coefficient during acupuncture and post-acupuncture period is higher than that during the pre-acupuncture control period, whereas the characteristic path length is shorter. We provide further support for the presence of "small-world" network characteristics in functional networks by using acupuncture. These preliminary results highlight the beneficial modulations of functional connectivity by manual acupuncture, which could contribute to the understanding of the effects of acupuncture on the entire brain, as well as the neurophysiological mechanisms underlying acupuncture. Moreover, the proposed method may be a useful approach to the further investigation of the complexity of patterns of interrelations between EEG channels.

Keywords: manual acupuncture, electroencephalograph, synchronization, functional connectivity

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1. Introduction

Acupuncture, a therapeutic technique originating from ancient China, has gained great popularity as an important modality of alternative and complementary medicine methodology. A number of clinical trials have shown that acupuncture has therapeutic effects in the treatment of various illnesses, such as chronic pain syndromes, neuropsychiatric disorders, and so on.[1] Of various types of acupunctures, manual acupuncture is one of the most popular clinical treatments in traditional Chinese medical acupuncture, which has many advantages, such as appropriate stimulus intensity, flexible operation skill, no side-effect, and so on.[2] In spite of its public acceptance, the underlying mechanism of the interactions between acupuncture and brain activity is still largely unknown, and various controversies remain.

Recently, modern neuroimaging techniques such as electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI), have provided new insights into the anatomy and physiological function underlying acupuncture.[3–9] Previous neuroimaging studies have shown that acupuncture stimulation can activate extensive brain regions.[3–8] Using fMRI, Dhond *et al.*[3] revealed that acupuncture can influence the intrinsic connectivity of the default mode network and sensorimotor network in the poststimulus resting brain. Bai *et al.*[4] demonstrated that a set of brain regions are activated by acupuncture stimulation, which clearly supports the existence of a large organized functional connectivity network related to effect of acupuncture in the resting brain. Bail *et al.*[5] also reported that acupuncture could exert a modulatory effect on the insula-anchored brain network during the post-stimulus resting period. Ren *et al.*[6] showed that the point specificity of acupuncture is supported by different modulatory brain networks with functional connectivity analysis. Qin *et al.*[7] indicated that acupuncture could change the amygdala-associated brain network into a functional state that underlies pain perception and pain modulation. Collectively, all of these studies demonstrate the existence of various function-guided brain networks underlying the therapeutic effect of acupuncture. Therefore, a further understanding of how acupuncture stimulation interacts with resting brain networks and their functional

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connectivity may be helpful and shed light on the appreciation of the physiological function and integrated mechanisms involved in acupuncture.[6]

However, most previous investigations on acupuncture only focus on the functional connectivity associated with one or a few preselected seed regions of interest. They are unable to completely characterize the interactions among multiple brain regions in the whole resting brain networks.[10] Meanwhile, a critical issue is ignored in the above investigations, i.e., the brain regions involved in the network are more important. Further, it has been proposed that to investigate the functional correlations from the perspective of the whole brain could provide a better understanding of the basic neurophysiological mechanisms underlying acupuncture,[10] which is more valuable and helpful.

Recently, several researchers have proposed a new perspective to investigate the functional connectivity by characterizing EEG signals at the whole brain level using the graph theory.[11–14] It allows us to quantitatively characterize the global organization and investigate the topological reconfiguration of the whole brain in response to acupuncture stimulation. To the best of our knowledge, few studies have used this approach to investigate the functional connectivity in the entire brain modulated by acupuncture. In addition, one recent report showed the time-variability and long-lasting effects of acupuncture.[15] Other studies have also demonstrated that neural responses induced by acupuncture have saliently time-varying characteristics.[16] The EEG is appropriate for this specialty. As a neurophysiological technique, EEG has high temporal resolution and low spatial resolution compared with other techniques, such as fMRI.[17] It can not only simultaneously record multi-channel neural electrical signals in different brain regions at the macroscopic level, but also reflect brain functions and states of the whole body. Therefore, we adopt the graph theory to extract brain functional networks by determining the coherence of the scalp-recorded EEG, which is a large scale measure of functional interrelations between pairs of neocortical regions.[18]

An alternative approach to the characterization of both local and long distance functional connectivity in complex networks is the application of measures derived from the graph theory.[19] A graph is an abstract representation of brain functional network, which can be characterized by its clustering coefficient and characteristic path length. The clustering coefficient is a measure of the local interconnectedness of the graph, whereas the path length is an indicator of its overall connectedness.[11] This approach offers a unique perspective on the balance of local and distributed interactions occurring in the brain. It has been widely used for different neuroscience studies in animals and humans, such as in studies of anatomical connectivity, fMRI BOLD, EEG, and MEG signals.[11,13,20–22] Besides, recent studies of brain functional networks[23] have shown that they exhibit "small-world" properties with many local connections and a few random long distance connections. Networks with "small-world" architecture are characterized by a combination of strong local clustering and a short characteristic path length. It has been proposed that a small world-like network architecture may be optimal for synchronizing neural activities between different brain regions during specific functions.[19,21,23]

Therefore, in our study we use the graph theory to extract brain functional networks as revealed by manual acupuncture-evoked EEG signals in healthy humans. By characterizing the topological features of functional networks, we quantitatively investigate how manual acupuncture modulates brain functional connectivity.

2. Experiment design and EEG recording

2.1. Subjects

Experiments are performed in 15 right-handed healthy volunteers (age: 23–27 years old; eight males and seven females), who are naive and have no or little experience with acupuncture. All the volunteers have no personal history of neurologic or psychiatric disorders, and are not abusing alcohol or illicit drugs. Every subject is free from neurological active medication, or any other factor that may affect EEG activities. Our study is performed with the approval of the local ethics committee. All the subjects have been provided with informed consent with the adequate understanding of the purpose and procedure of the study and their informed written consent is obtained according to the declaration of Helsinki.

2.2. Acupuncture stimulation and experimental design

In our experiment, we acupuncture at the acupoint ST36 (Zusanli) on the right leg, shown in Fig. 1(a). ST36 is an important meridian on Stomach Meridians, which has many therapeutically beneficial effects, such as keeping calm and sedation, treating sleep disorders, controlling stress, handling dizziness, insomnia and gastrointestinal diseases, and so on. This acupoint works effectively and has been widely used in traditional Chinese medical acupuncture experiments.[24] Acupuncture is administered manually by a single licensed acupuncturist who has been in clinical practice for over 25 years. The stainless steel needles used for ST36 are 0.2 mm in diameter and 40 mm in length.

During the whole procedure, all operations are conducted in a quiet room where the subjects lie on a soft bed and are awake. The subjects are instructed to keep their eyes closed and to relax during the whole acupuncture procedure. Before acupuncture, all subjects have 5 min to relax. Then, the stainless steel needles are inserted into ST36 as deep as 10 mm–20 mm. The whole acupuncture process lasts about 10 min for every subject as shown in Fig. 1(b). First, the

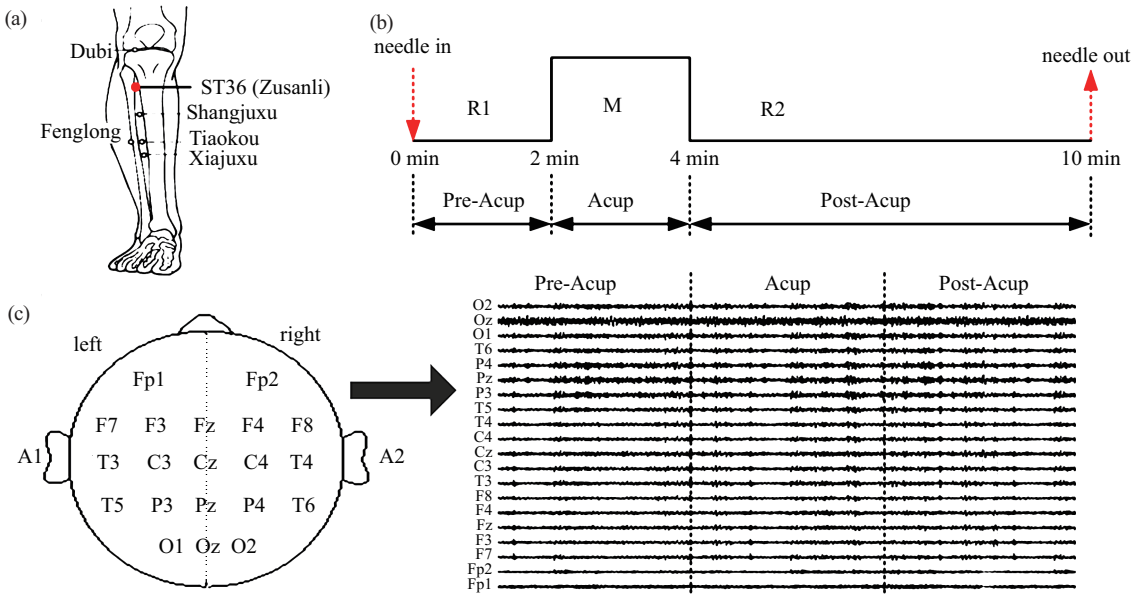


Fig. 1. (color online) Acupuncture experiment. (a) Acupoint ST36 on the right side; (b) acupuncture process; (c) electrodes positioned on the brain (left panel) and 20-channel EEG signals recorded for one subject in three acupuncture states (right panel).

needle is kept in a resting state for 2 min (R1), and then the subjects are acupunctured by using the twirling method to cause the needling sensation for 2 min (M). The twisting is within a range of 90°–180° and at a rate of about 1 Hz. After acupuncture, subjects are kept in resting state for 6 min (R2).

2.3. EEG recording and preprocessing

The recording of EEG signals is completed by a Neuroscan system using 20 Ag–AgCl electrodes, which are placed according to the 10–20 international system as shown in Fig. 1(c). Reference electrodes are placed between A1 and A2, using an earlobe as a reference ground. The cut-off frequencies of the EEG amplifiers are set to be 0.5 Hz and 100 Hz. We record 20-channel EEG signals at a 256-Hz sampling rate from 20 scalp electrodes, which are channels Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, and O2, respectively.

For 15 subjects, EEG signals are recorded continuously in the whole acupuncture process (Fig. 1(c)). We extract three EEG epochs from the recorded EEG data of each subject to represent three states of the experiments, which are the pre-acupuncture control period (Pre-Acup), during acupuncture stimulation (Acup), and the post-acupuncture stimulation period (Post-Acup), respectively. Then, we intercept the intermediate 80-s data from each EEG epoch, which are free of signs of muscle artifact, eye movements, and other artifacts. Thus, 3×80-s EEG epochs are obtained for each subject. The EEG datasets are preprocessed by a 0.5 Hz∼48 Hz band-pass digital filter, to further eliminate artifacts induced by residual electromyography (EMG) activity and noise owing to the electrical mains.

3. Method

3.1. Linear measures of synchronization—coherence estimation

The coherence estimation is a general linear measure of the synchronization in the frequency domain by normalizing the cross spectrum of two time series. For two simultaneously measured discrete time series x_n and y_n, $n = 1,\ldots,N$, the cross-correlation function can be expressed as

$$c_{xy}(\tau) = \frac{1}{N-\tau}\sum_{i=1}^{N-\tau}\left(\frac{x_i-\bar{x}}{\sigma_x}\right)\left(\frac{y_{i+\tau}-\bar{x}}{\sigma_y}\right), \qquad (1)$$

where $\bar{x}$ and σ_x are the mean and variance, τ is a time lag. The sample cross spectrum for these two series is defined as the Fourier transform of the cross correlation c_{xy} by Fourier convolution theorem, as

$$C_{xy}(\omega) = F_x(\omega) \times F_y^*(\omega), \qquad (2)$$

where F_x and F_y is the Fourier transforms of x_n and y_n, ω is the discrete frequency $(-N/2 < \omega < N/2)$ and the symbol $*$ denotes the complex conjugation.

To compute the synchronization measurement, signals x_n and y_n are subdivided into K segments of equal length M. For each segment, we use a Hamming window for smooth processing to reduce the spectrum leakage effect. The average cross spectrum $\langle C_{xy}(\omega)\rangle$ over M segments is a complex number whose normalized amplitude is

$$\Gamma_{xy}(\omega) = \frac{\left|\langle C_{xy}(\omega)\rangle\right|}{\sqrt{\langle C_{xx}(\omega)\rangle} \times \sqrt{\langle C_{yy}(\omega)\rangle}}. \qquad (3)$$

The normalized amplitude $\Gamma_{xy}(\omega)$ is also called the coherence function which gives a measure of the linear synchronization

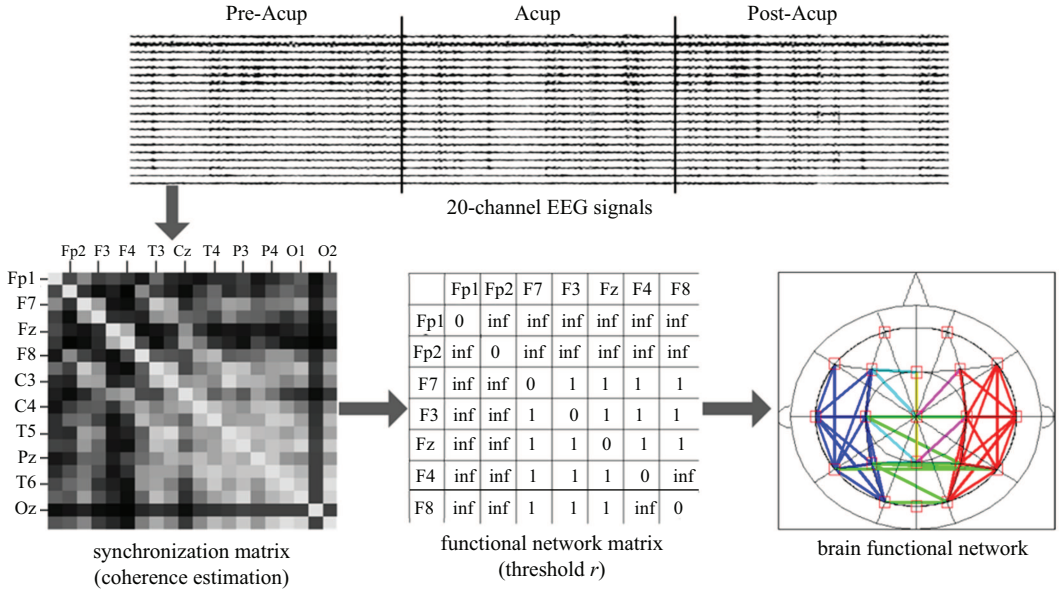
between x_n and y_n as a function of frequency ω. This measure is very useful when synchronization is limited to some particular frequency bands, which is usual in the case of EEG signals.[25]

3.2. Brain functional network

We model the brain functional network as an undirected network $G = (N, E)$, where N is a set of nodes and E is a set of edges. Each node n from the node set N corresponds to one of the EEG channels. Since electrodes measure signals in different brain areas, the nodes can represent these regions functionally. We put an edge between two nodes if the signals on the corresponding sensors are "dependent enough". The simplest way to determine whether signals at two channels are co-dependent is to measure synchronization between these two signals. In our study, we adopt coherence estimation to measure the synchronization. The end result of computing synchronizations for all pairwise combinations of channels is a square 20×20 synchronization matrix $\boldsymbol{Q}$ (20 is the number of recorded EEG channels), where each entry $q_{i,j}$ contains the synchronization measure for channels i and j.

The next step is to convert the synchronization matrix $\boldsymbol{Q}$ into a 20×20 brain functional network matrix $\boldsymbol{X}$. We can complete this by considering a threshold r. If the entry $q_{i,j} > r$, the corresponding $x_{i,j}$ in matrix $\boldsymbol{X}$ is equal to 1; if $q_{i,j} < r$ the corresponding $x_{i,j}$ is infinitely small expressed as "inf" in this study. As the synchronization measure for the same EEG signal is always equal to 1, for simplicity, the corresponding $x_{i,j}$ $(i = j)$ is set to be 0 in our study.[11,20] According to the matrix $\boldsymbol{X}$, we can construct the brain functional network by using the graph theory. Specifically, if the entry $x_{i,j} = 1$, an edge is said to exist between nodes i and j; if the entry $x_{i,j}$ is infinitely small, there is no edge between nodes i and j; if the entry $x_{i,j} = 0$, it indicates nodes i and j are the same nodes.

Figure 2 shows how the underlying functional network is exposed in our study. Once the synchronization matrix is converted to a network, the next step is to characterize the network in terms of its clustering coefficient C and characteristic path length L.



	Fp1	Fp2	F7	F3	Fz	F4	F8
Fp1	0	inf	inf	inf	inf	inf	inf
Fp2	inf	0	inf	inf	inf	inf	inf
F7	inf	inf	0	1	1	1	1
F3	inf	inf	1	0	1	1	1
Fz	inf	inf	1	1	0	1	1
F4	inf	inf	1	1	1	0	inf
F8	inf	inf	1	1	1	inf	0

Fig. 2. (color online) Methodology used to extract brain functional networks from the EEG signals.

3.3. Graph theoretic analysis—network parameters

Average path length L is a measure of the efficiency of information or mass transport on a network. Considering an unweighed network G with the set of vertices V, we denote d_{ij} as the shortest distance between v_i and v_j $(v_i, v_j \in V)$, then the average path length L is[26]

$$L = \frac{2}{N(N-1)} \sum_{i>j}^{N} d_{ij}, \tag{4}$$

where N is the number of nodes in network G.

The clustering coefficient C is a measure of the degree to which nodes in a graph tend to cluster together. If a vertex v_i has k_i neighbors in a network G, then the clustering coefficient C of vertex v_i is[26]

$$C_i = \frac{2e_i}{k_i(k_i-1)}, \tag{5}$$

where e_i is the number of connections in the neighborhood of v_i and $k_i(k_i-1)/2$ is the number of connections if the neighborhood is fully connected. The clustering coefficient for the whole network is given by Watts and Strogatz[22] as

$$C = \frac{1}{N} \sum_{i=1}^{N} C_i. \tag{6}$$

The degree of a node in a network is the number of connections or edges that the node has to other nodes. The average degree of the whole network is defined as[26]

$$K = \frac{\sum_{i=1}^{N} k(i)}{N}, \tag{7}$$

where $k(i)$ is the degree of vertex v_i.

These three parameters are the most robust measures of network topology.

3.4. Statistical analysis

The statistical comparisons are made by using t-tests and linear regression of the plots of C and L as a function of threshold. $P < 0.05$ is considered due to its significance.

4. Results

4.1. Synchronizations of EEG signals at three acupuncture states

The mean synchronization matrices $\boldsymbol{Q}$ over 15 subjects in different acupuncture states are shown in Fig. 3. It can be found that the synchronization matrices in different acupuncture states show complex but rather similar patterns, with most regions of high (light) and very few regions of low (dark) levels of synchronization. For instance, the light region in the bottom right corner corresponds to higher levels of synchronization between parietal, central, and temporal channels, while the synchronization of prefrontal channels (Fp1 and Fp2) and Oz are relatively low compared with other channels.

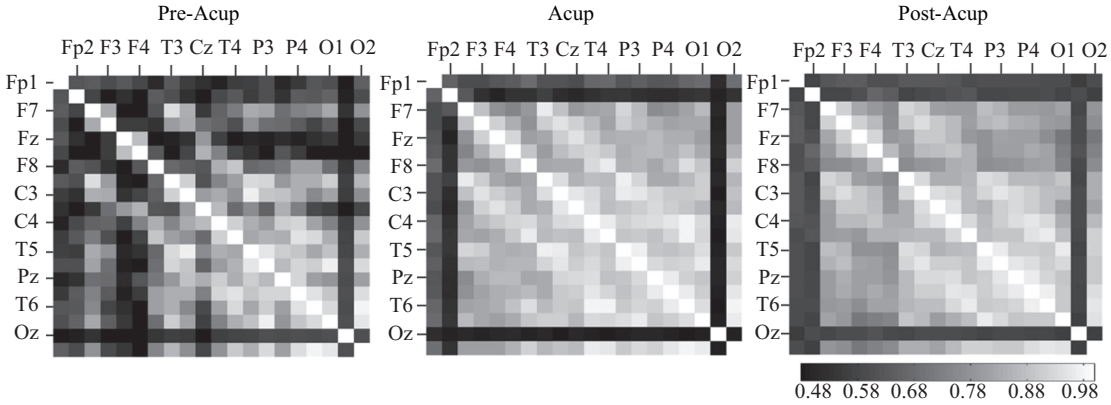


Fig. 3. (color online) Mean synchronization matrices over 15 subjects in three acupuncture states.

Overall, the synchronization of EEG during acupuncture stimulation is statistically higher than the pre-acupuncture control period ($P = 0.0026$), as shown in Fig. 4. During the post-acupuncture period, the synchronization decreases slightly compared with that during acupuncture stimulation, but is still higher than that during the pre-acupuncture control period ($P = 0.0974$). Therefore, the synchronization will be enhanced by acupuncture stimulation.

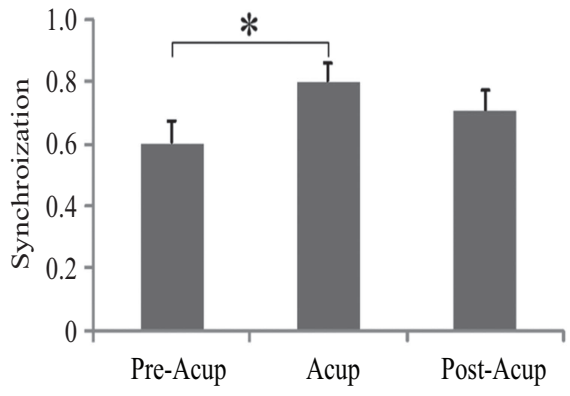


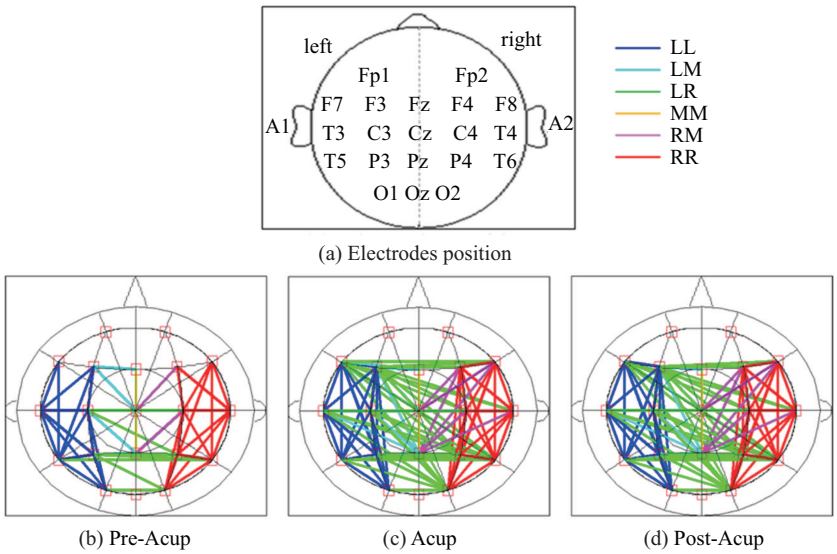
Fig. 4. Statistical analysis of the synchronization averaged over 15 subjects in different acupuncture states. The symbol $*$ refers to the case that there is a significant difference between two acupuncture states ($P < 0.05$).

4.2. Effects of acupuncture on brain functional connectivity

4.2.1. Brain functional networks in three acupuncture states

Figure 5 shows the functional networks corresponding to the mean synchronization matrices in Fig. 3 using threshold $r = 0.68$. The graphs in different acupuncture states show similar complex networks. For three acupuncture states, there are few connections between prefrontal cortex with other areas, while the connections between temporal, posterior temporal, parietal, and occipital lobes are relatively high (Figs. 5(b)–5(d)).

Compared with the functional network during the pre-acupuncture control period, the functional networks during acupuncture and post-acupuncture periods have much more connections between the central, temporal, occipital, and frontal regions for the threshold $r = 0.68$ (Figs. 5(c) and 5(d)). The statistical results of the increased connections in different brain areas over 15 subjects are summed up in Fig. 6. It can be found that the increased connections in the left hemisphere (LL, LM in Fig. 6) are higher than those in the right hemisphere (RR, RM in Fig. 6). Moreover, the increased



(a) Electrodes position

(b) Pre-Acup (c) Acup (d) Post-Acup

Fig. 5. (color online) Brain functional networks converted by corresponding mean synchronization matrices in Fig. 3 using a threshold of $r = 0.68$. Panel (a) shows the positions of the electrodes, which are indicated by small empty squares in panels (b)–(d). Panels (b)–(d) are functional networks in different acupuncture states. The connections between different brain regions are expressed by different line types, where LL denotes the connections within left brain, LM the connections between left brain and midbrain, LR the connections between left and right brain, RR the connections within the right brain, RM the connections between the right brain and midbrain, and MM the connections with the midbrain.

connections are mostly long distance ones between the left and the right hemisphere (LR in Fig. 6).

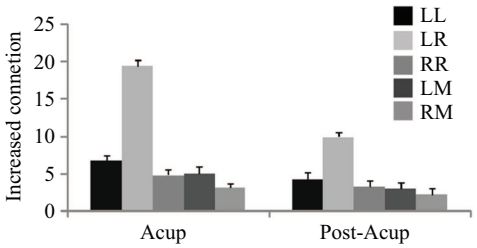


Fig. 6. Statistical analysis of the increased connections over 15 subjects during acupuncture and post-acupuncture periods with respect to those during the pre-acupuncture control period.

4.2.2. Characterizing clustering coefficients *C* and path length *L* each as a function of threshold

It has been shown that the threshold r can influence the connections of functional network greatly. Thereby, it is necessary to explore the properties of brain functional network dependent on threshold. In this study, we characterize the network in terms of its clustering coefficient C and characteristic path length L.

The graphs shown in Figs. 5(b)–5(d) represent different acupuncture state averages and primarily serve as the illustration of the main patterns. For the actual analysis, the synchronization matrix is converted into a network for each subject separately and the averages are made, respectively, over the individual values of C and L each as a function of threshold r. Because there is no unique way of choosing threshold r, we explore a whole range of values of r: $0.50 < r < 0.86$ in steps of 0.02 and repeat the full analysis for each value of r. The reason for selecting this range is that if threshold $r < 0.50$, the brain functional networks in different acupuncture states tend to fully connected states and the characteristic parameters of functional network are almost constant; what is more, the connections of network will decrease as threshold increases, so once $r > 0.86$, the connection of the functional networks during pre-acupuncture control period tends to 0. As both cases are meaningless, the whole range of threshold r in our study is $0.50 < r < 0.86$.

The values of average clustering coefficient C as a function of threshold r in three acupuncture states are shown in Fig. 7. For a lower value of the threshold r, the corresponding networks are almost fully connected with edges between almost all vertices generating a higher corresponding C close to 1. As the threshold increases, more and more edges will be lost, which provides the corresponding value of synchronization measured to be less than r. Thus, the clustering coefficient starts to decrease. Over the whole range of threshold values investigated ($r \in [0.50, 0.86]$), the clustering coefficient C during acupuncture stimulation is higher than that during the pre-acupuncture control period. For most ranges of threshold ($r \in [0.52, 0.86]$), the clustering coefficient C during acupuncture stimulation is statistically higher than that during the pre-acupuncture control period (Fig. 7(a)). This conclusion also holds true during the post-acupuncture period (Fig. 7(b)). However, there is no consistent statistical difference in state between during acupuncture stimulation and during the post-acupuncture period. Furthermore, the standard deviation of the clustering coefficient C increases as the threshold increases.

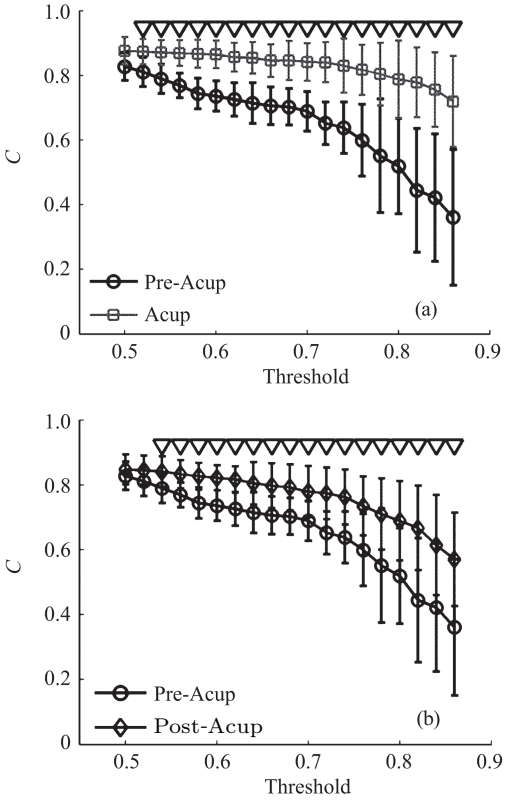


Fig. 7. Values of average clustering coefficient C over 15 subjects each as a function of threshold in three acupuncture states. The gray hollow triangles on the top of panels (a) and (b) indicate where the difference between the two states is significant ($P < 0.05$).

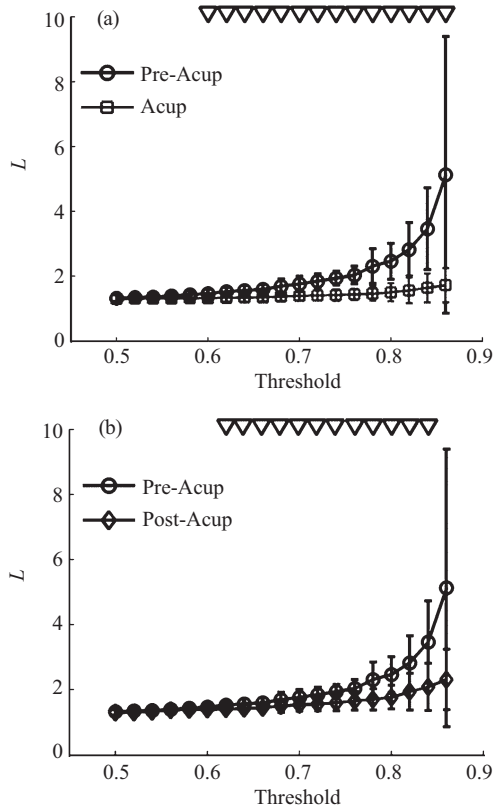


Fig. 8. Values of average path length L over 15 subjects each as a function of threshold in three acupuncture states. Gray empty triangles on the top of panels (a) and (b) indicate where the difference between the two states is significant ($P < 0.05$).

Figure 8 shows the curves of the average path length L each as a function of threshold r for three acupuncture states. For small values of r ($r \in [0.50, 0.70]$), the path length increases almost linearly with the threshold increasing. As the threshold increases, more and more edges will drop out. So the average path length between randomly chosen vertices increases. If the threshold is further increased, the average path length will continue to increase at a more rapid rate. For most of values of r ($r \in [0.60, 0.86]$), the average path length during acupuncture stimulation is significantly smaller than that in pre-acupuncture control period (Fig. 8(a)), which also holds true for the post-acupuncture period (Fig. 8(b)). In addition, the standard deviation of the average path length L increases as the threshold increases. Compared with that during pre-acupuncture control period, the standard deviation of the path length L is almost relatively low during acupuncture stimulation and post-acupuncture periods. However, there is almost no consistent statistical difference in state between during acupuncture stimulation and during post-acupuncture period. Overall, as the threshold increases, the tendency that the average path length L during acupuncture stimulation and post-acupuncture periods is lower than that during pre-acupuncture control period remains intact.

4.2.3. "Small-world" characteristic of the brain functional network by acupuncture

To determine whether the function networks by acupuncture exhibit small-world characteristic, we express the clustering coefficient C and path length L as ratios of theoretical C_{rand} and L_{rand} of the corresponding random networks for three acupuncture states. Here, to ensure that the statistical comparison is general, the brain functional network and its corresponding random network should have equal (or at least similar) degree sequences, which, as is well known, can affect all kinds of network measures. In our study, we generate the random network following the procedure described by Milo *et al.*[27] which preserves the degree distribution exactly. Otherwise, to control and correct the potential influence of subtle (non-statistical) differences in mean coherence between the different acupuncture states, additional results are obtained using the values of constant K, i.e., 6, 8, 10, 12, and 14. Here we focus on the ratios of C and L derived from the recorded EEG data to matching values derived from the reference random networks: C/C_{rand} and L/L_{rand} as shown in Table 1.

The small-world network organization is evident when C/C_{rand} is significantly greater than 1 whilst L/L_{rand} is near the value of "1".[13,14] Simultaneous values of C/C_{rand} and L/L_{rand} significantly greater than 1 are the indication of ordered networks. From the results in Table 1, it can be found that the

clustering coefficients of brain functional network in different acupuncture states are significantly higher than that of the corresponding random network, whereas the characteristic path lengths are nearly equal to that of random network. The clustering coefficient of the brain functional network in the present study is more than 1.5 times compared with that of the corresponding random network. Thus, the brain functional network shows a typical small-world pattern by acupuncture stimulation, which has a higher clustering coefficient and shorter average path length. That is to say, acupuncture stimulation does not disrupt the "small-world" pattern of the functional connectivity in brain.

Table 1. Average clustering coefficient C and average path length L expressed as ratios of the theoretical $C_{\rm rand}$ and $L_{\rm rand}$ of the corresponding random networks in different acupuncture states. Bold figures indicate the difference between the corresponding state and the pre-acupuncture control period is significant ($P < 0.05$).

Degree	Pre-Acup		Acup		Post-Acup	
	$C/C_{\rm rand}$	$L/L_{\rm rand}$	$C/C_{\rm rand}$	$L/L_{\rm rand}$	$C/C_{\rm rand}$	$L/L_{\rm rand}$
$K = 6$	2.1520	1.1031	**1.9414**	1.0954	**2.0918**	**1.1039**
$K = 8$	1.9543	1.0366	**1.8842**	**1.0875**	**1.9597**	**1.1006**
$K = 10$	1.8968	1.0198	**1.8686**	**1.0502**	1.8617	**1.0673**
$K = 12$	1.7875	1.0185	1.6447	1.0227	1.6758	1.0499
$K = 14$	1.6694	1.0175	1.5258	1.0166	1.6421	1.0299

5. Discussion and conclusion

The main purpose of this study is to investigate how manual acupuncture modulates the functional connectivity of the whole brain. To achieve this goal, we acupunctured at acupoint ST36 of the right leg to obtain EEG signals. By coherence estimation, we determined the synchronizations between all pairwise combinations of EEG channels. Then, the functional connectivity matrices of the synchronization were converted into networks and analyzed in terms of clustering coefficients C and characteristic path length L for a range of thresholds. This approach is quite general, which could be further applied to other connectivity matrices based on reconstructed sources in future studies.

By computing the synchronization matrix, it is found that acupuncture stimulation can increase the synchronization between different brain areas, which is in accordance with the previous study by Feng *et al.*[10] The synchronization of the activities in different brain areas can reflect the ability to process the information in the brain. Further, pathological synchronization has been shown to be a main mechanism responsible for a number of neurological disorders.[25,28] So the result in our study can be regarded as an underlying mechanism supporting the modulation of brain activities with acupuncture.

By investigating the functional networks with acupuncture, it is observed that the increased connections are mainly long distance ones between the left hemisphere and the right hemisphere (Fig. 6). Therefore, we can hypothesize that acupuncture at ST36 can improve communication between the brain's remote areas. In addition, the increased connections on the left cerebral hemisphere are greater than those on the right side. That is to say, acupuncture at the right leg has greater effects on the information communication of the left cerebral hemisphere than those of the right cerebral hemisphere, which is in accordance with the results in Ref. [9]. This conclusion corresponds to the contralateral theory of the body in traditional Chinese medical acupuncture.

In addition, for a whole range of threshold values, the clustering coefficient shows a significant trend to higher values during acupuncture and post-acupuncture periods than the pre-acupuncture control period (Fig. 7), implying that the local connectednesses of networks during and after acupuncture are relatively close. Another important finding is that the characteristic path length shows a significant trend to lower values during acupuncture and post-acupuncture periods than pre-acupuncture control period (Fig. 8). Shorter path lengths have been shown to promote effective interactions between and across cortical regions.[21] Interactions between interconnected areas of the brain are believed to form the basis of cognitive processes.[29] This significant correlation between path length and cognitive function could provide further evidence for the effects of acupuncture in the treatment of disconnection syndrome.

It has also been shown that the brain functional networks for a number of neurological disorders are characterized by a lower C and higher L. For example, Micheloyannis *et al.*[13] found that the functional connectivity of a patient with schizophrenia shows a significantly lower C and significantly higher L than that of a healthy person. Ponten *et al.*[30] proposed that the brain network has an increase of L during seizure activity, which is most prominent in the alpha, theta, and delta frequency bands during and after the seizure.[30] Stam *et al.*[11] found that the characteristic path length L is significantly longer in Alzheimer patients. Interestingly, the results in our study show that acupuncture at ST36 of the right leg can modulate the brain functional network by increasing C and reducing L. Thus, we can infer that acupuncture at ST36 has beneficial effects on brain function, which can alleviate the symptoms of a number of neurological disorders. This result also highlights the therapeutic effects of acupuncturing at ST36, which is widely used in traditional Chinese medical acupuncture treatment.

Previous studies in neuroscience have shown that different types of networks present small-world properties, which are characterized by a higher clustering coefficient and a shorter path length.[11,22] Small-world networks have been shown to efficiently transfer information whilst simultaneously maintaining a local "working group" of neurons.[13] That is, they satisfy the apparently competing needs for functional

integration and functional segregation.[13,19] Our results indicate that the brain functional network shows a typical small-world pattern during acupuncture and post-acupuncture periods, which means the acupuncture stimulus can maintain and even promote the efficient information processing of the brain. This conclusion further highlights that acupuncture can significantly increase the long distance connections between different brain areas. Just because of the increase of these long-distance connections, the brain functional network can maintain a higher clustering coefficient whilst shortening the average path length.

This study is the first to investigate the brain functional network in EEG recordings evoked by manual acupuncture. It provides further support for the presence of small-world characteristics in functional networks in the brain. Graph theoretical analysis can reveal specific patterns of organization of functional connectivity by manual acupuncture, which can provide a new perspective and method for acupuncture analysis. All the results could be regarded as new evidence supporting the functional modulatory effects of manual acupuncture, and highlight the therapeutic effects of acupuncture on brain function. However, these specific modulatory effects need further investigation on clinical patients to verify the findings in this study. The further investigation and confirmation of the mechanisms underlying the effects of acupuncture are also required.

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