WLPVG approach to the analysis of EEG-based functional brain network under manual acupuncture

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Abstract Functional brain network, one of the main methods for brain functional studies, can provide the connectivity information among brain regions. In this research, EEG-based functional brain network is built and analyzed through a new wavelet limited penetrable visibility graph (WLPVG) approach. This approach first decompose EEG into δ , θ , α , β sub-bands, then extracting nonlinear features from single channel signal, in addition forming a functional brain network for each sub-band. Manual acupuncture (MA) as a stimulation to the human nerve system, may evoke varied modulating effects in brain activities. To investigating whether and how this happens, WLPVG approach is used to analyze the EEGs of 15 healthy subjects with MA at acupoint ST36 on the right leg. It is found that MA can influence the complexity of EEG sub-bands in different ways and lead the functional brain networks to obtain higher efficiency and stronger small-world property compared with pre-acupuncture control state.

Keywords Visibility graph · Functional brain network · Manual acupuncture · Small-world property

Introduction

Researching in cognitive neurodynamics can never be isolated from the analysis of neurophysiological data. As one of the most widely used techniques to investigate brain activities (Li et al. 2011; Xiao-Ling and Zong-Lai 2007;

Terry et al. 2004; Qing-Fang et al. 2010), EEG with its high temporal resolution and low spatial resolution (Hong-Rui et al. 2011; Gevins 1998), can not only characterize the cerebral cortex electrical information directly, but also reflect the brain functions and the states of the whole body. Approach to EEG analysis, or more generally, time series analysis, has been always attracting people's persistent attention, from time-domain and frequency-domain analysis to nonlinear analysis, and it has never stopped developing.

Complex network theory is a new branch of statistical physics (Albert and Barabasi 2002). In a complex network, the nodes and edges represent the elements and the relationships between them, respectively. Different from other theory, complex network theory devotes to describe and explain systems in a global view. Just like the complex network method applying in systems analysis, now we have several approaches to use the network theory in time series analysis. Zhang and Small (2006; Zhang et al. 2007, 2008; Xu et al. 2008) firstly considered pseudo-periodic time series and finally found that noisy periodic signals generate random networks, while chaotic time series generate networks with small-world and scale-free features. Lacasa et al. (2008, 2009) proposed the method visibility graph (VG), with which the constructed networks can inherit dynamic characters of the time series. For examples, periodic series convert into regular networks, random series convert into random networks, and fractal series convert into scale-free networks. Based on the visibility graph, Ting-Ting et al. (2012) came up with limited penetrable visibility graph (LPVG), in which defined a limited penetrable distance to get better noise immunity. In this paper, we choose the LPVG to be the center method, and present a new wavelet limited penetrable visibility graph (WLPVG) approach to analyze EEG signals with manual acupuncture (MA).

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Acupuncture has been practiced in China and other Asian countries for thousands of years. It involves stimulating specific points on the body but most often done by inserting thin needles through the skin, to cause a change in the physical functions of the body. There are over 300 acupoints on human body. The reason for us to choose ST36 (Zusanli) is that it is one of the most widely used and basic acupoints in traditional Chinese medical science, which contributes much to treating disease such as gastric diseases, insomnia, dizziness, drug of alcohol addiction. A previous study showed that moxibustion at ST36 increased serum insulin levels (Zhang et al. 1989). Needle-stimulation of the ST36 has been shown to produce some physiological effects including analgesia, increase in immune function, and inhibition of gastric acid secretion (Yang et al. 1989; Yu et al. 1998). Ernst and Lee (1986) find that low-frequency acupuncture stimulation of ST36 induce long-lasting widespread vasodilation against cardiovascular diseases and diseases with hypertensive syndrome. Han et al. (1994), Wu et al. (1999) and Yoshimoto et al. (2001) show that acupuncture at ST36 was capable of suppressing morphine or heroine withdrawal syndromes and suppressed increased alcohol-drinking behaviors in rats. At the same time, many researches have been done to investigate how acupuncture plays a role in adjusting physical condition. Sakai et al. (2007) shows that acupuncture stimulation can reduce gamma sub-band wave and increase alpha and theta sub-bands wave in rabbits. Tanaka et al. (2002) found that acupuncture increased all spectral band power. Furthermore, Chen et al. (2006) investigated the effects of high and low frequency acupuncture stimulation on EEG and found that different frequency acupuncture can induce different effects on EEG theta sub-band wave. But the mechanism of acupuncture is still not clear. In this

research, we try to analysis EEG signals from the view of network, especially highlighting the construction of the functional brain network base on EEG in which considering the electrodes to be the nodes, and finally get some promising results.

Experiment and data

Experiments are performed in 15 right-handed healthy volunteers (with age ranging 23–27, 8 males and 7 females) with no experience of acupuncture. The subjects are acupunctured at ST36 of the right leg (Fig. 1a) by the same licensed acupuncturist who has been in clinical practice for over 25 years. Subjects are required to stay quiet and awake with eyes closed. Experiment procedure is shown in Fig. 1c. After 5 min relaxing, the stainless steel needles are inserted into ST36 as deep as 10–20 mm. In the first 2 min (R1), the needle is kept in a resting state, and then the subjects are acupunctured by using the twirling method for 2 min (M). The twisting is within a range of 90° – 180° with a rate of about 1 Hz. After acupuncture, subjects are kept in resting state for 6 min (R2).

We employed a digital amplifier to acquire EEG, with which the EEGs were amplified and filtered by a high frequency filter of 30 Hz before recorded. In preprocess stage, the EEGs were band-limited between 0 and 30 Hz using a two-way least-squares finite impulse response (FIR) filter with the help of EEGLAB (Delorme and Makeig 2004). It has now become clear that filtered data can occur false suggestions of low-dimensional structure or chaos (Rapp 1993; Rapp et al. 1993), dealing with which we applied not only power of scale-freeness but also graph index complexity approach to dig characteristics of EEG

Fig. 1 The acupuncture experiment. **a** Manual acupuncture is administered to the ST36 acupoint on the right side; **b** electrodes' position in the 10–20 standard system; **c** acupuncture process

under MA in the following research, refraining from an interpretation in terms of dimensions or deterministic chaos (Stam 2005).

According to 10–20 standard system (Fig. 1b), a data set includes 19-channel EEG is obtained. Sampling rate of EEG is 256 Hz. EEG are collected during the whole acupuncture process. Eight-second EEG (2,048 time samples) segments from state R1 (Pre-Acupuncture), M (Acupuncture) and R2 (Post-Acupuncture) are extracted from the EEG recordings (three segments for each subject). Due to insertion of the needle, adaptation to the acupuncture and other possible factors, signal selection must be treated with caution. Intending to extract a typical feature of each MA condition, eight-second EEG segments from the middle of the corresponding experiment state, which may prevent those potential interferences from influencing the final result, were selected. More specifically, EEG segments between 1m0s and 1m8 s (state R1, Pre-Acupuncture), 3m0s–3m8 s (state M, Acupuncture), 7m0s–7m8s (state R2, Post-Acupuncture) of the 10 min' experiment process were extracted to analyze.

Methods

Figure 2 shows a flowchart for the WLPVG approach presented in the paper which includes: wavelet decomposition of the band-limited EEGs into four sub-bands, mapping the EEG sub-bands to their LPVGs. Once the LPVGs obtained, on one hand, compute associated complexities in two different ways, say PSG (Power of Scale-freeness in LPVG) and GIC (Graph Index Complexity), with statistical analysis to extract the influence of acupuncture to each channel respectively. On the other hand, build up brain networks with the method LPVGS (Similarity in LPVG) to investigate how acupuncture affects the connections and the characters of brain-nets.

Wavelet decomposition

Following (Adeli et al. 2007; Ahmadi et al. 2010), a three-level wavelet decomposition is used to decompose the band-limited EEG (0–30 Hz) into four sub-bands: delta (0–4 Hz), theta (4–8 Hz), alpha (8–15 Hz), and beta (15–30 Hz). In this step, 3,420 signals are obtained [(19 channels) × (4 EEG sub-bands) × (15 subjects) × (3 conditions—R1, M, R2)].

Limited penetrable visibility graph (LPVG)

Recently the visibility graph algorithm was introduced as a method of mapping a time series to a graph, called VG (Lacasa et al. 2008). It was shown that the VG of a time

Fig. 2 The flowchart of WLPVG approach

series inherits some dynamical behaviors of the time series (Lacasa et al. 2008, 2009). Based on VG, Ting-Ting et al. (2012) presented LPVG and show that LPVG has an advantage over VG in that it shows good tolerance to noise interference.

The VG and the LPVG method is illustrated in Fig. 3. Figure 3a shows the time series indicated by vertical bars. Each bar is linked with all those that can be seen from the top of the considered one. As a graph, each bar (that is a value of the time series) is a node, and two nodes are connected if visibility exists between the corresponding bars. Visibility, in this case, means that there exists a straight line connecting the series data, but not intersecting any intermediate bar. Consider the i th node of the graph, a_i , corresponds to the i th point of the time series, x_i . Two nodes of the graph, a_m and a_n , are connected via a bidirectional edge if and only if:

$$x_{m+j} < x_n + \left(\frac{n - |m+j|}{n - m} \right) |x_m - x_n|, \quad \forall j \in \mathbb{Z}^+, \quad (1)$$

$$j < n - m.$$

If and only if the points in time series fulfill the expression 1, the nodes in VG can connect together through a bidirectional edge. Some dynamical features of the time series, such as complexity and self-similarity,

Fig. 3 Illustrates the procedure of converting time series **a** to its VG **b** and LPVG (c, $N = 1$). Each *black line* in **a** shows the two connected points can see each other. The *red lines* in **a** are extra connections in LPVG compared with VG. In Fig. 3 N is set to be 1, so each *red line* can go through one bar only. (Color figure online)

hidden in the signal may be revealed in the structure of the resulting graph (Lacasa et al. 2008, 2009).

In LPVG, a limited penetrable distance N is proposed for the sake of diminishing the effect of noise. Here x_m and x_n can see through N bars of the time series to reach each other.

In VG, the connections break easily while noise interfered, and nodes were supposed to gain more neighbor nodes except for those noise peaks. As in Fig. 3, while there are two neighbor nodes for each node at least in VG, the number of neighbor nodes in LPVG becomes $2(N + 1)$. Therefore LPVG may provide against noise partition to some extent in case dynamic characteristics covered by noise. Ting-Ting et al. (2012) applied LPVG to analyzing fractal, periodical and chaotic time series and found that LPVG was more suitable for analyze periodical and chaotic time series containing noise signal than VG. In chaotic signal analysis, for example noise added Rossler and Lorenz system analysis, the networks' clustering coefficient fluctuation rate was reduced from 19.27 to 2.10 % and from 18.38 to 2.01 % respectively by LPVG compared with VG. So in this research we chose LPVG as our center algorithm. On selection of the penetrable distance N , it was set to be 1 in Fig. 3 because it makes the sketch clearer and easier to understand. Besides, in this research N was selected as 2. There are no strict principles for choosing penetrable distance N at present. Ting-Ting et al. (2012) suggested that N should not be too large which may lose too much partial correlation. Additionally, sometimes larger penetrable distance increases the computation amount and time costs in vain. So the penetrable distance N as was set to be 2 just as in Ting-Ting et al.

(2012), in which LPVG performed outstanding in chaotic signal analysis.

Power of scale-freeness in LPVG (PSG)

Same with the VG algorithm, LPVG maps a periodic signal into an ordered graph and a fractal time series into a graph with a scale-free structure. In an ordered graph, all of its nodes connect to the same number of edges. In a scale-free graph, the degree, i.e. the number of edges connected to a node, show a distribution characterized by: $P(k) = k^{-\alpha}$, where k indicates the degree of a node, P is the probability distribution of edges distributed in vertical bars or nodes of a graph, and α is called the power of the scale-freeness. Lacasa et al. (2008, 2009) indicate that the power of the scalefree structure α of the VG is related to the extent of the signal's fractality. While in logarithmic relationship, the $P(k) - k$ in almost linear and the value of the slope of $P(k)$ versus k , known as the PSG, can be a measure of complexity and fractality of the EEG signal (Ahmadlou et al. 2010).

Graph Index Complexity (GIC)

Kim and Wilhelm (2008) propose GIC as a measure of complexity of a graph. Let $\lambda_{\max}$ be the largest eigenvalue of the adjacency matrix of a graph with n nodes. $\lambda_{\max}$, known as GIC is defined as: $C_{\lambda_{\max}} = 4c(1 - c)$, where $c = [\lambda_{\max} - 2 \cos(\pi/(n + 1))]/[\lambda_{\max} - 2 \cos(\pi/(n + 1))][n - 1 - 2 \cos(\pi/(n + 1))]$. The $C_{\lambda_{\max}}$ varies between 0 and 1 in all unweighted bidirectional graphs and larger $C_{\lambda_{\max}}$ indicates that the graph's structure is more complex.

Similarity in LPVG (LPVGS)

Ahmadlou and Adeli (2011) presented visibility graph similarity (VGS) as a method of measuring generalized synchronization. In this research, LPVGS is proposed and it performed just same as VGS, except the part which mapping time series into graphs. LPVGS is investigated in this research for study the change of EEG-based functional connection of brain. To calculate the LPVGS, first, each time series is mapped into a LPVG, i.e. an adjacency matrix. Then, a sequence of degrees of the LPVG, called Degree Sequence (DS), is obtained. Correlation of the DSs of X and Y:

$$S[DS|X], DS|Y = |\text{cov}[DS|X], DS|Y|/\sigma_{DS}[X]\sigma_{DS}[Y]$$

is called LPVGS between X and Y, which is presented as a measurement of similarity of dynamics of a coupled systems. LPVGSs between every two channels is calculated in this research and then a 19×19 weighted adjacency matrix (for one state and one EEG sub-band, averaged among 15 subjects) of brain net is obtained.

Network metrics

Clustering coefficient

The clustering coefficient C_i of a node a_i is defined as the number of edges k_j between its direct neighbors (denoted by subgraph G_i) divided by the total number of all possible edges k_{G_i} in G_i (Luce and Perry 1949; Watts and Strogatz 1998): $c_i = 2 \sum_{G_i} k_j/k_{G_i} |k_{G_i} - 1|$. The clustering coefficient CP of the network is the average clustering coefficient C_i over all N nodes: $CP = \sum_{i=1}^N C_i/N$.

Minimum path length

Consider the i th and the j th node of the graph, a_i and a_j . The minimum path length $L_{i,j}$ between them is the minimum number of edges that needs to be traveled from node a_i to node a_j . The minimum path length of a node a_i is the average of its shortest path lengths to all other node (Watts and Strogatz 1998; Dijkstra 1959): $L_i = \sum_{j=1, j \neq i}^N L_{i,j}/N$. The minimum path length LP of a network is the average of L_i over all N nodes (Watts and Strogatz 1998; Dijkstra 1959): $LP = \sum_{i=1}^N L_i/N$.

Global efficiency

The efficiency $E_{i,j}$ between a node a_i and node a_j is defined as the reciprocal of their minimum path length $L_{i,j}$. The global efficiency E_g is the average of all the $E_{i,j}$: $E_g = \sum_{i,j,i \neq j} E_{i,j} / \sum_{i,j,i \neq j} E_{i,j} N(N-1) = \sum_{i,j,i \neq j} (1/L_{i,j}) / N(N-1)$.

Local efficiency

Let $E_l(i)$ be the global efficiency of subgraph G_i which formed with all the direct neighbors of node a_i . The local efficiency E_l of the network: $E_l = \sum_i^N E_l(i)/N = \sum_i^N E_g(G_i)/N$.

Small-world property (σ)

Small-world property were originally proposed by Watts and Strogatz (1998). A network is defined to have the small-world property when it shows more clustering than a random network and remains similar average minimum path length to that of a random graph (Watts and Strogatz 1998; Humphries et al. 2006): $\gamma = CP_{net}/CP_{random} > 1$, $\lambda = LP_{net}/LP_{random} \approx 1$. These two conditions can also be summarized into a scalar quantitative measurement, the small-worldness, σ : $\sigma = \gamma/\lambda$. The σ of a network is larger than 1 in the case of small-world organization (Humphries et al. 2006; Achard et al. 2006). In this research, a set of 100 comparable random networks with similar degree sequence and symmetric adjacency matrix were formed for each individual functional brain network to obtain the CP_{random} and LP_{random} .

Results

3420 PSGs and 3420 GICs corresponding to 19 (channels) $\times$ 4 (EEG sub-bands) $\times$ 3 (conditions: Pre-Acup, Acup and Post-Acup) $\times$ 15 (subjects) EEGs and 180 19×19 weighted adjacency matrixes each consisted of 361 LPVGS per EEG sub-band per condition per subject are obtained after the wavelet analysis and LPVG computations.

Statistical analysis of single channel

The extracted GICs show no significant discrimination (p value 0.05) between R1 and M, nor between R1 and R2 in delta sub-band. But the one-way ANOVA test showed the ability of GICs in theta, alpha and beta sub-bands to distinguish the condition of Pre-Acup, Acup and Post-Acup. There were 15 samples for each condition, sub-band and channel in ANOVA analysis correlating 15 subjects. Table 1a–c presents discriminative GICs and the corresponding p values (p value 0.05) in theta, alpha and beta sub-bands and their variation trend respectively in which it can be observed that the GICs in alpha are discriminative increased in state M(Acup) and R2(Post-Acup) compared with GICs in R1(Pre-Acup) condition and complicate changes in theta and beta. Figure 4a–c show the loci

Table 1 Discriminating EEG channels obtained from GICs (p value 0.05) and the changing direction of GICs in latter states (M or R2) compared with those in R1 state

Channel	p value	
	R1 versus M	R1 versus R2
(a) Theta frequency sub-band		
Fz	$1.59 \times 10^{-2} \downarrow$	–
C4	–	$9.40 \times 10^{-3} \uparrow$
(b) Alpha frequency sub-band		
F7	$9.60 \times 10^{-3} \uparrow$	$2.61 \times 10^{-2} \uparrow$
F3	$8.90 \times 10^{-3} \uparrow$	–
C3	$2.55 \times 10^{-2} \uparrow$	–
C4	–	$1.21 \times 10^{-2} \uparrow$
T5	–	$4.47 \times 10^{-2} \uparrow$
P4	$9.00 \times 10^{-3} \uparrow$	$5.50 \times 10^{-3} \uparrow$
O1	$3.60 \times 10^{-3} \uparrow$	$2.70 \times 10^{-3} \uparrow$
(c) Beta frequency sub-band		
Cz	–	$3.36 \times 10^{-2} \downarrow$
C4	–	$3.66 \times 10^{-2} \downarrow$
T4	$4.45 \times 10^{-2} \uparrow$	–
P3	$2.84 \times 10^{-2} \downarrow$	–

Bold indicates $p < 0.01$

presented in Table 1a–c, respectively. It is found that MA increased the complexity of EEG through alpha sub-band mostly. According to Fig. 4b, these increase happened more often in left hemisphere which is 2.5 times over that in right hemisphere in number of electrodes.

The extracted PSGs show significant discrimination (p value 0.05) between R1 and M in all EEG sub-bands, and between R1 and R2, too. Table 2 presents discriminative PSGs, the corresponding p values (p value 0.05) and their variation trend, respectively. Table 2a shows that in delta sub-band, increased fractality is only obtained in R2 state. Table 2b shows that PSGs in M and R2 are decreased compared with the ones in R1 in theta sub-band, while discriminative increased PSGs in state M and R2 compared with PSGs in R1 condition are found in Table 2c and d

Table 2 Discriminating EEG channels obtained from PSGs (p value 0.05) and the changing direction of PSGs in latter states (M or R2) compared with those in R1 (Pre-Acup) state

Channel	p value	
	R1 versus M	R1 versus R2
(a) Delta frequency sub-band		
Fp2	–	$2.49 \times 10^{-2} \uparrow$
O1	–	$2.44 \times 10^{-2} \uparrow$
O2	–	$1.82 \times 10^{-2} \uparrow$
(b) Theta frequency sub-band		
F3	$2.03 \times 10^{-2} \downarrow$	$1.56 \times 10^{-2} \downarrow$
Fz	$1.57 \times 10^{-2} \downarrow$	–
O2	$3.65 \times 10^{-2} \downarrow$	–
(c) Alpha frequency sub-band		
C3	–	$4.45 \times 10^{-2} \uparrow$
Cz	$3.39 \times 10^{-2} \uparrow$	$4.13 \times 10^{-2} \uparrow$
C4	–	$4.87 \times 10^{-2} \uparrow$
P3	–	$1.41 \times 10^{-2} \uparrow$
T6	$4.00 \times 10^{-2} \uparrow$	–
O1	–	$7.40 \times 10^{-3} \uparrow$
(d) Beta frequency sub-band		
F8	–	$5.10 \times 10^{-3} \uparrow$
T3	$3.68 \times 10^{-2} \uparrow$	–
C3	–	$4.72 \times 10^{-2} \uparrow$

Bold indicates $p < 0.01$

which represent increased fractality of EEGs during and after acupuncture in alpha and beta sub-bands. Figure 5a–d show the loci of EEG electrodes presented in Table 2 for sub-bands delta, theta, alpha and beta, respectively. Figure 5c indicates that fractality of EEG is mainly influenced in alpha sub-band, same as the result in GICs.

Statistical analysis of brain network

180 brain nets corresponding to 15 (subjects) $\times$ 4 (EEG sub-bands) $\times$ 3 (conditions) EEGs are obtained after the calculation of LPVGS, then averaged among 15 subjects to

Fig. 4 Loci with discriminative GICs of **a** delta sub-band, **b** theta sub-band and **c** alpha sub-band for distinguishing EEGs (p value 0.05) in Pre-Acup and Acup only noted by $\bullet$, Pre-Acup and Post-Acup only noted by $\bullet$, both of these two conditions by $\bullet$

Fig. 5 Location with discriminative PSGs of **a** delta sub-band, **b** theta sub-band, **c** alpha sub-band and **d** beta sub-band for distinguishing EEGs (p value 0.05) in Pre-Acup and Acup only noted by , Pre-Acup and Post-Acup only noted by , both of these two conditions by

Table 3 The mean LPVGSs corresponding each EEG sub-band and state

EEG sub-band	mean LPVGS		
	Pre-acup	Acup	Post-acup
Delta	0.4099 ± 0.0455	0.4148 ± 0.0559	0.4457 ± 0.0578
Theta	0.3160 ± 0.0422	0.3003 ± 0.0408	0.3156 ± 0.0448
Alpha	0.3010 ± 0.0390	0.3015 ± 0.0397	0.2913 ± 0.0410
Beta	0.4316 ± 0.0356	0.4356 ± 0.0296	0.4245 ± 0.0340

12 brain networks corresponding each EEG sub-band and condition.

Brain functional connectivity networks

Table 3 present the mean LPVGSs of the 12 networks. The mean LPVGSs of delta and beta sub-bands are significantly larger than theta and alpha, which indicate that the synchronism of functional connections among delta and beta sub-bands may be stronger than in theta and alpha sub-bands.

Threshold of LPVGS values are selected to obtain the graph of brain network connection. In order to get a clearer view of the connection graphs, threshold LPVGS values are selected respectively as 0.48 for delta sub-band, 0.31 for theta sub-band, 0.28 for alpha sub-band and 0.42 for beta sub-band. For the three conditions in one sub-band, threshold value remain the same. This method of threshold selection is based on the network's sparsity. Each threshold value leaves the network's sparsity at about 30 % so that a distinct network with less weak connection can be obtained. Figure 6 illustrates the connection graphs of delta sub-band in three states. Each connection graph in Fig. 6 represents a mean connection of 15 subjects in one condition and one EEG sub-band. Connection graphs of other sub-bands are left out since they are similar to delta sub-band's. The connections between different brain regions are expressed by different line types, where green line denotes the connections within left brain (LL), red line the

connections between left and right brain (LR), blue line the connections within the right brain (RR), and black line the connections with the midbrain (electrode Pz, Cz, Oz). The width of those lines corresponds to the value of LPVGS, denoting the connection strength of the network.

Statistical analysis of connections

The statistical results of the increased connections in different brain areas over 15 subjects are displayed in Fig. 7. It can be found that connections of the functional brain network are generally increased among all sub-bands during and after acupuncture. We use different color to represent different kind of connections. The green bar denotes connections within left hemisphere (LL in Fig. 7), blue bar the connections within the right hemisphere (RR in Fig. 7) and red bar the connections between left and right hemisphere (LR in Fig. 7).

It can be found that the increased connections are mostly long distance ones between the left and the right hemisphere in all sub-bands except beta. Moreover, the increased connections in the left hemisphere are generally higher than those in the right hemisphere and Fig. 7 also show that there is a significant difference between them in all except beta sub-band during acupuncture and in theta and beta sub-bands after acupuncture. The reason for increased connections in left hemisphere distinguish from those in right may be that the ST36 is stimulated on right leg of subjects.

Network characteristics analysis

Figure 8 shows the statistical result of clustering coefficient and minimum path length.

Except for theta sub-band, those results show that during acupuncture the functional brain network could get bigger clustering coefficients and smaller minimum path length, which may maintain to a certain level over the ones in Pre-Acup state when finish the acupuncture. Clustering coefficient of state Post-Acup in beta sub-band even become

Fig. 6 Brain functional connectivity networks of delta sub-band in condition **a** R1, pre-acupuncture, **b** M, acupuncture, **c** R2, post-acupuncture

Fig. 7 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. The *symbol single asterisks* refers to the case that there is a significant difference between increased connections within right and left hemisphere ($p < 0.05$). The *symbol double asterisks* refers to a significant difference with $p < 0.01$

much higher than in Acup state, and the same situation is found in the result of minimum path length. For delta, theta and alpha sub-bands, minimum path length keep decreasing after acupuncture. This may indicates that MA affect not only in the procedure of simulating in ST32, but also stay effective in a period of time after acupuncture. A larger clustering coefficient and a smaller minimum path length can both make the brain network more efficient in information exchanging. Figure 9 gives a statistical result of global efficiency and local efficiency. It can be found that MA truly inspire subjects performing a more efficient functional brain network, not only in global efficiency but also in local efficiency.

Figure 10 shows the result of the small-world property. Generally, a network is identified as small-world when sigma is larger than 1, and significantly small-world when sigma is larger than 2 (the gray dotted line in Fig. 10). It can be found that for every sub-band, MA lead the

functional brain network to gaining more outstanding small-world property in a range of thresholds, especially in theta and alpha sub-bands. Besides, this small-world property weaken as MA finishing, appear very close to the one before MA, for example, the result shows no significant difference Post-Acup and Pre-Acup in beta sub-band.

Discussion

The main purpose of this study is to investigate how MA influence EEG and the functional connectivity of the whole brain in different sub-bands. To achieve this goal, we acupunctured at acupoint ST36 in right leg to acquire EEG signals and decomposed them into δ , θ , α , β sub-bands through the method wavelet decomposition. Then we systematically investigate the effects of traditional Chinese MA on the activities of different brain rhythms. By the

Fig. 8 Statistical analysis of the minimum path length and clustering coefficient averaged over 15 subjects in different acupuncture states and different sub-bands. The *symbol asterisks* refers to the case that there is a significant difference between two acupuncture states ($p < 0.05$)

Fig. 9 Statistical analysis of the global efficiency and local efficiency averaged over 15 subjects in different acupuncture states and different sub-bands. The *symbol asterisks* refers to the case that there is a significant difference between two acupuncture states ($p < 0.05$)

means of LPVG, every signal was transformed from time series to graph. On one hand, VSGs and GICs were computed to measure the complexity of the signal. On the other hand, we determined the synchronizations between every two EEG channels by computing LPVGS which formed the functional connectivity matrices. Once a threshold was selected, matrices of the synchronization were converted into networks and analyzed in terms of minimum path length, clustering coefficient, global efficiency, local

Fig. 10 Statistical analysis of the small-world property sigma averaged over 15 subjects in different acupuncture states and different sub-bands. The value of the small-world characteristic parameter sigma changes with respect to threshold LPVGS value. The *triangles* on the top of the graphs indicate where the difference between the R1 and M states is significant ($p < 0.05$). The *triangles* below the graphs indicate where the difference between the R1 and R2 states is significant ($p < 0.05$)

efficiency and the small-world property. Besides, the small-world property with respect to a range of thresholds was investigated. This WLPVG approach shown in Fig. 2 is quite general and resultful, which is believed can be further applied to analysis other experiments' EEG and nerve disease influenced EEG in future studies.

By computing the GIC and CGS, it is found that the acupuncture at ST36 can induce obvious changes in different EEG rhythms in healthy subjects, which provide further support for previous hypothesis that the brain plays a key role in acupuncture research (Hong-Rui et al. 2011, b; Xi-Liu et al. 2012; Litscher and Cho 2000; Hui et al. 2000; Guo-Sheng et al. 2013a; b). Our results show that MA at ST36 can decrease the complexity of slow wave of EEG, especially in theta sub-band, and increase the complexity of fast wave of EEG in both alpha and beta sub-band. These changing trends show almost exactly opposite to Ahmadlou et al. (2010), in which illustrated that the EEGs with Alzheimer Disease is more complex in theta sub-band and less complex in alpha sub-band compared with the control group.

Since Friston (1994) carried out the definition of functional connectivity and the study of brain functional network based on functional magnetic resonance imaging (fMRI), series of evidences have been put forward suggesting that somehow the brain functional network is

associated with neurological mechanism and can reflect changes in neurological level. Smit et al. (2008) shown that brain functional network based on EEG got property of small-world. Research of Stam et al. (2009) on brain functional network of Alzheimer's disease patients based on magnetoencephalography (MEG) found that patients obtained larger minimum path length and poorer small-world property in their brain functional networks. The clustering coefficient of functional network during attention task was higher than under no task status with eyes open in Li et al. (2011) study. Micheloyannis et al. (2006) built up functional network of schizophrenia patients, and observed decreases of local clustering coefficient in both resting state and working memory task state compared with healthy people. Xiao-Ling and Zong-Lai (2007) investigated functional network of alcohol addicted patients, which turned out to have significant difference from healthy people in complex network characteristic index, such as distinct smaller information entropy.

By computing the LPVGS, the functional network adjacency matrixs, in others words, the synchronization matrixs were obtained. It is found that acupuncture stimulation can increase the synchronization of the whole brain (shown in table 3), which is in accordance with the previous study by Feng et al. (2011) By investigating the functional networks under MA, it is observed that the increased connections are mainly long distance ones between the left hemisphere and the right hemisphere (Fig. 7). Therefore, it can be hypothesized that acupuncture at ST36 can improve information communicating between the brain's remote areas. What's more, the increased connections in left cerebral hemisphere are higher than those on the right side, which indicate acupuncturing at ST36 on the right leg has greater effects on the left cerebral hemisphere than those of the right side. This verifies the contralateral theory of the body in traditional Chinese medical acupuncture, which also can be proved in Xi-Liu et al. (2012) and Guo-Sheng et al. (2013a).

In addition, the results of network characteristics analysis show a significant trend that the clustering coefficient, global efficiency and local efficiency during acupuncture are higher than those in pre-acupuncture control period except the clustering coefficient of theta sub-band, and the minimum path length during acupuncture is lower than in pre-acupuncture control period (Figs. 8, 9). Larger clustering coefficient is related to more local connection of networks, while shorter minimum path length has been shown to promote more effective interactions between cortical regions (Sporns and Zwi 2004). Global efficiency and local efficiency are measurement of the ability to transmit information, thus it can be investigated that acupuncture at ST36 can promote the information transmitting ability of the whole functional brain network (global

efficiency) and between neighbour nodes of the network (local efficiency).

Since pathological synchronization has been shown to be a main mechanism responsible for a number of neurological disorders (Quiroga et al. 2002; Pereda et al. 2005), we can infer that acupuncture at ST36 might modulate the pathological synchronization state of brain. Besides, it has also been shown that the functional brain networks for a number of neurological disorders are characterized by a lower clustering coefficient and higher minimum path length, for example, schizophrenia (Micheloyannis et al. 2006), seizure (Ponten et al. 2007), Alzheimer Disease (Stam et al. 2007). Interestingly, the results in our study show that acupuncture applied to ST32 led to increasing edges, stronger connections, smaller distance between nodes in functional network, which improve the local as well as global network connectivity so that the information among each potential brain partition may gain more effective communication and interchange. In this study, subjects involved were all healthy and acupuncture affected their brains as above. While applying to patients with neurological disorders, whether there is structurally damage in brain, such as severe shrinkage of brain in advanced Alzheimer's disease, may influence the effect of MA. Recently, Yong Zhang et al. (2014) have found that acupuncture is capable of modulating the functional connectivity of the default mode network in stroke patients. Thus, it can be hypothesized that acupuncture at ST36 may have positive effects on brain function, and further modulate the pathological EEG patterns of a number of neurological disorders. This study offered some proves for the rehabilitation therapy of brain functional diseases and it is worth to continue carrying out experimental and theoretical study.

Small-world networks have been shown more efficient in transferring information and can satisfy the competing needs for functional integration and functional segregation (Micheloyannis et al. 2006; Strogatz 2001). Previous studies in neuroscience presented the small-world property coefficient sigma, which is characterized by a higher clustering coefficient and a shorter path length compared with random networks (Watts and Strogatz 1998; Stam et al. 2007). In our research, terms of sigmas were computed for a range of thresholds (Fig. 10). The results show that the functional brain network during acupuncture is typical small-word, which indicates acupuncture at ST36 can keep and even promote the information processing efficiency of the brain.

Correlating Figs. 7 and 10 beta sub-band has the least increased connections between the left hemisphere and the right hemisphere in Fig. 7, and least significant difference between Pre-Acup and Acup states in Fig. 10, from which we can infer that significantly increased long distance

connections between left hemisphere and right hemisphere may be the main cause of the changes the networks' small-world property. Theoretically, the increase of these long distance connections can maintain the network a higher clustering coefficient and at the same time obviously shorten the minimum path length.

Conclusion

In this paper, we adopt the VG method to analyze EEG signals evoked by acupuncture at ST36 in a brand new way i.e. computing the parameters of signal's visibility graph instead of the signal itself. Differing from other traditional methods, such as time-domain analysis, frequency-domain analysis, nonlinear analysis etc., this approach proposed a simple but effective way to transfer time series into graph, and can analyze not only one single channel of EEG but also the functional connectivity of brain in addition. Here we defined the electrodes to be the nodes of network and LPVGS the connection strength, which is first presented in this research as a new way to make up a functional brain network to the best of authors' knowledge.

Parameters such as power of scale-freeness in LPVG, graph index complexity, similarity in LPVG, minimum path length, clustering coefficient, global efficiency, local efficiency, small-world property were computed in this research to investigate the changes caused by acupuncture quantitatively. Those quantitative methods can be further used to make MA more standardized, or to describe the progress and development of nerve disease.

More over, this study indicates that those parameters above change almost exactly contrary to the ones of subjects with Alzheimer Disease. In that we suppose that MA might have some positive modulating actions to EEG of patients suffer from Alzheimer Disease. To test this hypothesis, further studies is needed and we are working on the relating experiments now.

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