

Magnetic stimulation at *Neiguan* (PC6) acupoint increases connections between cerebral cortex regions

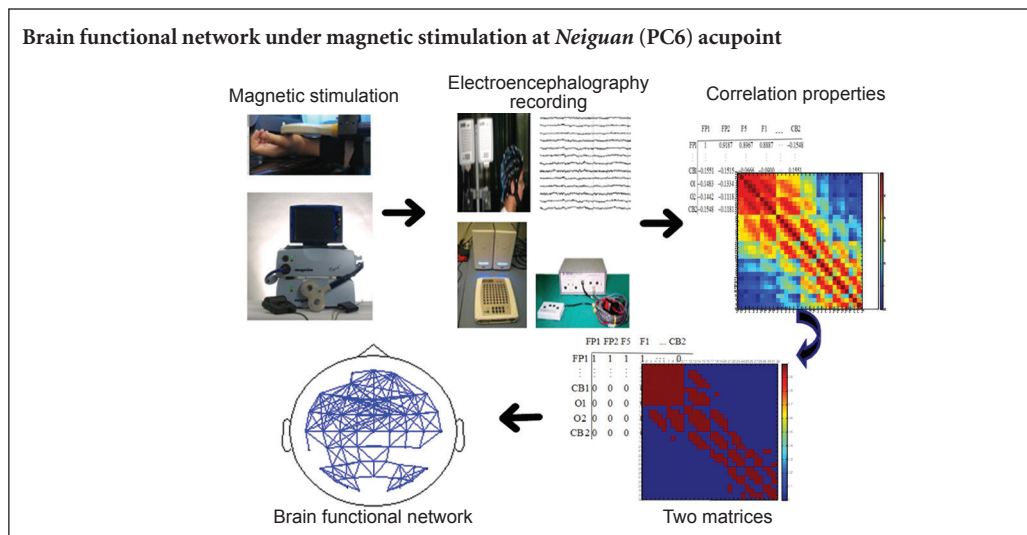
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Graphical Abstract



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Abstract

Stimulation at specific acupoints can activate cortical regions in human subjects. Previous studies have mainly focused on a single brain region. However, the brain is a network and many brain regions participate in the same task. The study of a single brain region alone cannot clearly explain any brain-related issues. Therefore, for the present study, magnetic stimulation was used to stimulate the *Neiguan* (PC6) acupoint, and 32-channel electroencephalography data were recorded before and after stimulation. Brain functional networks were constructed based on electroencephalography data to determine the relationship between magnetic stimulation at the PC6 acupoint and cortical excitability. Results indicated that magnetic stimulation at the PC6 acupoint increased connections between cerebral cortex regions.

Key Words: nerve regeneration; electroencephalography; *Neiguan* (PC6); magnetic stimulation; Pearson's correlation coefficient; complex network; acupuncture effect; brain functional network; neural regeneration

Introduction

Previous work has suggested that various brain regions are usually involved in a single task, and this network organization may be altered in neurodegenerative diseases (Sporns et al., 2004; Greicius, 2008; Bullmore and Sporns, 2009; Behrens and Sporns, 2012). Nevertheless, most previous studies focused on cerebral activation have only emphasized a single cerebral cortex region (Hui et al., 2000; Siedentopf et al., 2002; Wang et al., 2007). The more recently proposed complex network theory provides a new perspective for

large-scale neuronal communication in the human brain (van den Heuvel and Hulshoff Pol, 2010). Functional magnetic resonance imaging, positron emission tomography, and electroencephalography (EEG) were used to investigate the brain functional network under specific tasks or pathological conditions (Sun et al., 2014). Compared with functional magnetic resonance imaging and positron emission tomography, EEG provides better temporal resolution. Moreover, multichannel technology has improved the spatial resolution of EEG (de Vico Fallani et al., 2009; Sakkalis, 2011).

Several studies have shown that various brain regions are activated or de-activated by acupuncture stimulation (Hui et al., 2000, 2010; Siedentopf et al., 2002; Napadow et al., 2005, 2013; Dhondt et al., 2008; Chae et al., 2013). However, very little is known about the relationship between acupoints and the brain. Magnetic stimulation has a similar ionic mechanism to electric stimulation (Gallasch et al., 2015). However, magnetic stimulation is more comfortable and less painful, and can stimulate deep tissues and nerves (Sekino and Ueno, 2002). In this paper, magnetic stimulation was used to stimulate the *Neiguan* (PC6) acupoint, as well as a mock control point. PC6 is one of important acupoints to treat mind-mental disorders and heart diseases in traditional Chinese medicine (Shi, 2002). Multichannel EEG signals were recorded before and after stimulation. This study aimed to investigate the effects of magnetic stimulation at the PC6 acupoint from the perspective of a functional brain network. EEG data was utilized to construct and analyze brain functional networks before and after magnetic stimulation at the acupoint/mock point.

Subjects and Methods

Subjects

A total of 13 (eight males and five females) healthy right-handed volunteers aged 20 to 30 years old were chosen using recruitment advertisement. These volunteers were undergraduate or graduate students of Hebei University of Technology in China. The inclusion and exclusion criteria of volunteers are shown in **Figure 1**. All procedures were performed with the adequate understanding and written consent of the subjects, and the experts of Beichen Hospital of Traditional Chinese Medicine, Tianjin, China guided the experiments.

Magnetic stimulation

The PC6 acupoint was selected in this experiment. According to traditional Chinese medicine, the PC6 acupoint is above the wrist, one sixth of the distance from the distal wrist crease to the cubital crease, between the palmaris longus tendon and the flexor carpi radialis tendon, as shown in **Figure 2**. As a control, a mock point, which is approximately 3 cm away from PC6 acupoint, was chosen.

All experiments were performed at the Bioelectromagnetic Laboratory of Hebei University of Technology in Tianjin, China. The time of experiments was 20:00 to 23:00. During the experiment, the subjects sat in a chair with their eyes closed and earplugs in their ears. The Rapid2 TMS system with a figure-eight coil (Magstim Company Ltd., Whitland, UK) was used as the magnetic pulse generator. Each subject attended two experimental sessions (acupoint stimulation and mock point stimulation) separated by 1 week. Stimulation frequency was 0.5 Hz. Intensity was 1.76 T (80% of the maximum). During each magnetic stimulation trial, the subject was stimulated for 2 minutes (**Figure 3**).

EEG signal recording and preprocessing

The Neuroscan EEG/ERP system (Neuroscan Ltd., Charlotte,

NC, USA) was used to record EEG signals from 32 scalp positions. These 32 positions were selected according to 10–20 international electrode distribution (**Figure 4**). The sample rate was 1,000 Hz, with an amplification factor of 500. A 2-minute before-stimulation EEG and 2-minute after-stimulation EEG were recorded for each trial. After the 0.5–40-Hz band pass filter, the EEG data was preprocessed to reduce artifacts, which consisted of large amplitude ($> \pm 50 \mu\text{V}$), blinks, and slow eye movement. After data preprocessing, EEG signal data were exported and analyzed using Matlab software (Mathworks Inc., Natick, MA, USA).

Data processing

Pearson's correlation coefficient (PCC) for all 13-subject EEG data was calculated and then averaged. The average coefficient matrices were converted to binary matrices for constructing brain functional networks. Small-world properties of these networks before and after stimulation were investigated to determine the effects of magnetic stimulation at the PC6 acupoint/mock point.

PCC

PCC is commonly used to measure dependence of two variables. Compared with other methods, the correlation method better depicts the interaction between brain regions and neurons (Smith et al., 2011). The formula for PCC is:

$$r_{ij} = \frac{\sum_{t=1}^T [x_i(t) - \bar{x}_i] \cdot [x_j(t) - \bar{x}_j]}{\sqrt{\sum_{t=1}^T [x_i(t) - \bar{x}_i]^2 \sum_{t=1}^T [x_j(t) - \bar{x}_j]^2}} \quad (1)$$

where x_i and x_j , respectively, represent EEG signals and sequence acquired from channel i and channel j . \bar{x}_i and \bar{x}_j , respectively, represent the average EEG signal sequence.

Construction of brain functional network

To analyze the different-stage brain functional networks, an undirected graph was drawn, such that the nodes were connected if the PCC between node i and j exceeded a threshold. For this section, the threshold was an important parameter that directly determined construction of the brain functional network and its properties. According to different distribution characteristics, the threshold of positive PCC was different from the negative PCC. For positive PCC, the values were chosen as the threshold, because there were not isolated nodes in the before-stimulation brain functional network. The same threshold value was used for after-stimulation brain network construction. Negative PCC values were small ($-0.25 < \text{negative PCC} < 0$), so we chose 0.5 as the threshold.

Small-world properties of brain functional network

For connectivity of nervous systems, small-world models are attractive (Sporns and Zwi, 2004). It has been demonstrated

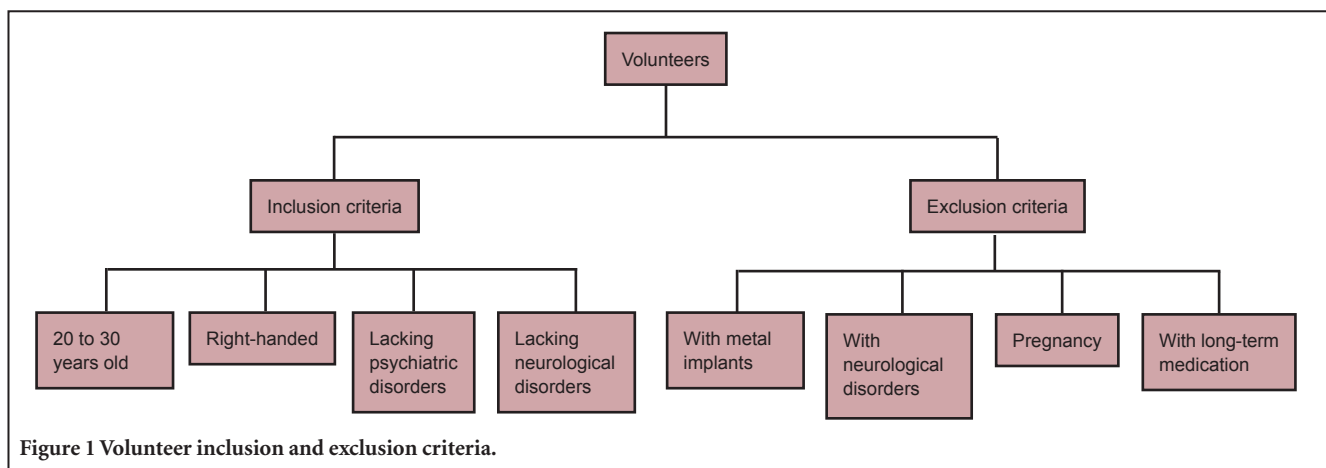


Figure 1 Volunteer inclusion and exclusion criteria.

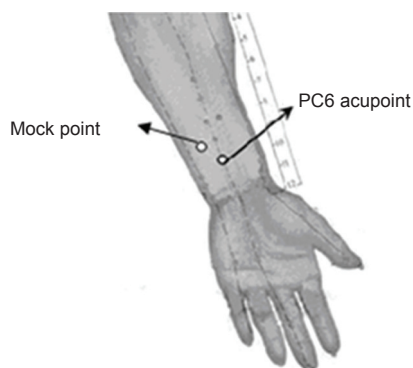


Figure 2 Locations of the Neiguan (PC6) acupoint and the mock point.

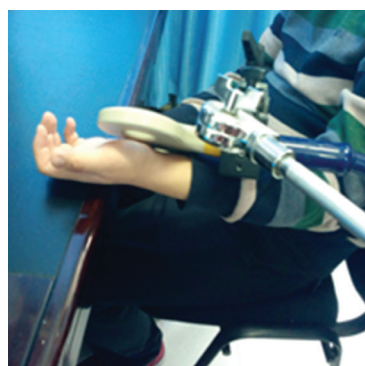


Figure 3 Schematic diagram of magnetic stimulation at the Neiguan (PC6) acupoint.

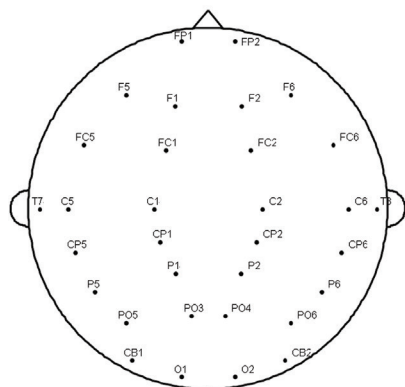


Figure 4 Scalp positions where electroencephalography data were recorded.

These 32 positions were selected according to 10–20 international electrode distribution. F: Frontal lobe; T: temporal lobe; P: parietal lobe; O: occipital lobe; C: cerebral central region.

that monkey and human brain functional networks exhibit small-world properties at a macroscopic scale (Stephan et al., 2000; Stam, 2004). Small-world properties have been described for graph theories, where a network is called “small-world” when it has a high clustering coefficient value and a small characteristic path length. Typically, in a small-world network, the following exists (Watts and Strogatz, 1998; Montoya and SolÉ, 2002):

$$\gamma = C_{net} / C_{rand} > 1, \quad (2)$$

and

$$\lambda = L_{net} / L_{rand} \sim 1, \quad (3)$$

where C_{net} and L_{net} are the clustering coefficient and the characteristic path length of the network, respectively, while C_{rand} and L_{rand} belong to the same-sized random network, respectively. A network has small-world properties when $\sigma = \gamma / \lambda > 1$ (Achard et al., 2006). In this paper, parameter was calculated to investigate whether magnetic stimulation could change the small-world properties of the brain functional network or not.

Results

The distribution of PCC values was similar before and after magnetic stimulation at the PC6 acupoint or at the mock point (Figure 5).

Absolute brain functional networks of positive PCC

First, positive PCC matrices were converted to binary matrices using an appropriate threshold. Before-stimulation EEG data were regarded as the baseline. In this case, the threshold

was 0.69. Brain functional networks under different stages are shown in **Figure 6**. We called this type of network the “absolute brain functional network,” which corresponded to the negative PCC network. Results showed that both magnetic stimulations at PC6 acupoint and at mock point resulted in increased internal connections of some cortical regions, especially in the frontal region.

We also analyzed small-world properties of different-stage brain functional networks. Results showed that $\sigma > 1$ was always true in the different brain networks, as shown in **Table 1**. Here, the clustering coefficient and characteristic path length of the random network were $C_{rand} = 0.26$ and $L_{rand} = 1.97$, respectively. This suggested that the brain functional network exhibited small-world properties. Magnetic stimulation at PC6 acupoint or at the mock point did not change these basic properties of the brain networks.

Relative brain functional networks of negative PCC

Interestingly, after magnetic stimulation at the PC6 acupoint, the negative PCC values between different cerebral cortex regions, such as frontal/occipital regions, frontal/parietal regions, and temporal/occipital regions, were smaller than before stimulation. After magnetic stimulation at the mock point, those values were larger than before stimulation (**Figure 7**).

Generally, negative PCC values were small ($-0.25 < \text{negative PCC} < 0$). To determine how PCC changes under magnetic stimulation at the PC6 acupoint compared with before-stimulation, we subtracted the before-stimulation from the after-stimulation PCC matrix resulting in the relative coefficient matrix. After binary processing (threshold = 0.05), the brain functional network was constructed (**Figure 8**), and this type of network was termed the “relative brain functional network.” Results showed that negative connections between frontal/occipital, frontal/parietal, and temporal/occipital regions increased after magnetic stimulation at the PC6 acupoint.

Discussion

For positive PCC, our results showed that, both after magnetic stimulation at the PC6 acupoint and at the mock point, internal connections of cortical regions, especially the frontal region, increased. Previous studies have shown that PC6 acupuncture activates the frontal cortex, as well as some other regions (Fu et al., 2005; Kim et al., 2008).

A brain functional network was constructed and analyzed at different stages (before stimulation, after stimulation at PC6 acupoint/mock point) based on the positive PCC from EEG data. Results verified that the brain functional networks exhibited small-world properties at the three stages. Previous time-domain analyses have been proposed for the small-world properties of brain interregional correlation (Eguíluz et al., 2005; Salvador et al., 2005). However, our results suggested that magnetic stimulation at the PC6 acupoint/mock point did not alter small-world properties of the brain network.

After magnetic stimulation at the PC6 acupoint, the neg-

ative correlations between frontal/occipital, frontal/parietal, and temporal/occipital regions increased compared with before stimulation. However, after magnetic stimulation at the mock point, those correlations were weakened. According to the positive brain functional network after magnetic stimulation at the PC6 acupoint, results suggested that frontal and temporal regions were activated, while parietal and occipital regions were deactivated. This shows a negative correlation and a special synchronization, and indicates that after magnetic stimulation at the PC6 acupoint, synchronization at each relevant cortex regions increased. Additionally, after magnetic stimulation at the mock point, synchronization decreased. Temporal synchronization of cerebral electrical activity plays an important role in brain function (Tahaei et al., 2012; Guo et al., 2014), and our results demonstrated that magnetic stimulation at the PC6 acupoint increased large-scale connections at different regions in the cerebral cortex compared with the mock point. These results suggested that magnetic stimulation at the PC6 acupoint induced a more complex response to the brain functional networks.

According to traditional Chinese medicine, the PC6 acupoint is associated with the brain and mental functions (Zhang et al., 2012, 2015). Previous studies have reported that various brain regions were significantly correlated with each other after stimulating PC6 (Fu, 2005). Our results confirmed that magnetic stimulation at the PC6 acupoint affects brain functions, although further studies are needed to determine the involved mechanisms. For this study, we carefully designed the experimental paradigms to ensure a consistent experimental process. Nevertheless, there are some uncontrollable factors that could affect the experimental results, such as age, sex, and sensory thresholds of each subject. This study focused on healthy subjects. However, future studies should be conducted on patients with brain-related diseases.

In summary, internal connections of some cortical regions, in particular the frontal region, increased after magnetic stimulation at the PC6 acupoint or at the mock point. However, small-world properties of brain functional networks did not change following magnetic stimulation, magnetic stimulation at the PC6 acupoint increased large-scale connections in different regions of the cerebral cortex compared with the mock point. This multidisciplinary, cross-research study involves various aspects of Chinese medicine, electromagnetic techniques, information technology, and neuroscience, and has important academic and clinical values.

Author contributions: HLY, GZX and LG conceived and designed the study. HLY wrote the paper. LDF and SY performed the experiments and data analysis. SS and HL reviewed and edited the paper. All authors approved the final version of the paper.

Conflicts of interest: None declared.

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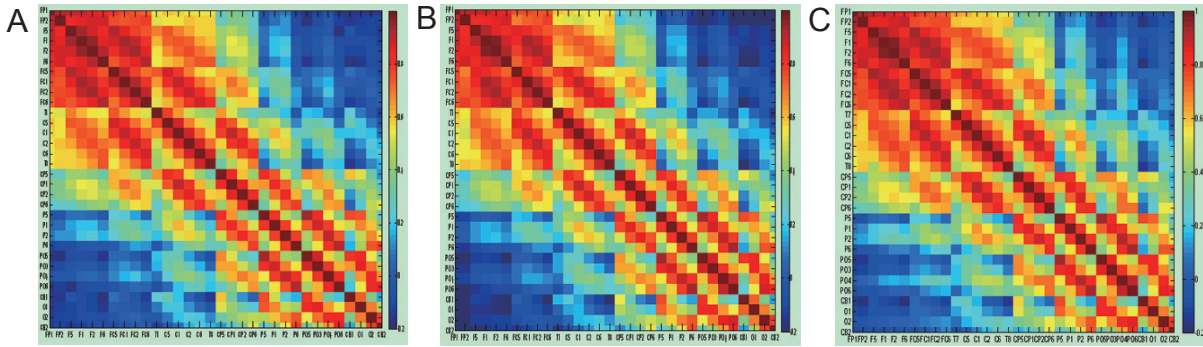


Figure 5 Similar Pearson's correlation coefficient matrices before stimulation (A), after acupoint stimulation (B), and after mock-point stimulation (C).

Table 1 Characteristic parameters of brain functional networks at different stages

States	Clustering coefficient (C_{net})	Average distance (L_{net})	γ	λ	σ
Before stimulation	0.69	2.43	2.65	1.23	2.15
After acupoint stimulation	0.68	2.49	2.62	1.26	2.08
After mock-point stimulation	0.68	2.33	2.62	1.18	2.22

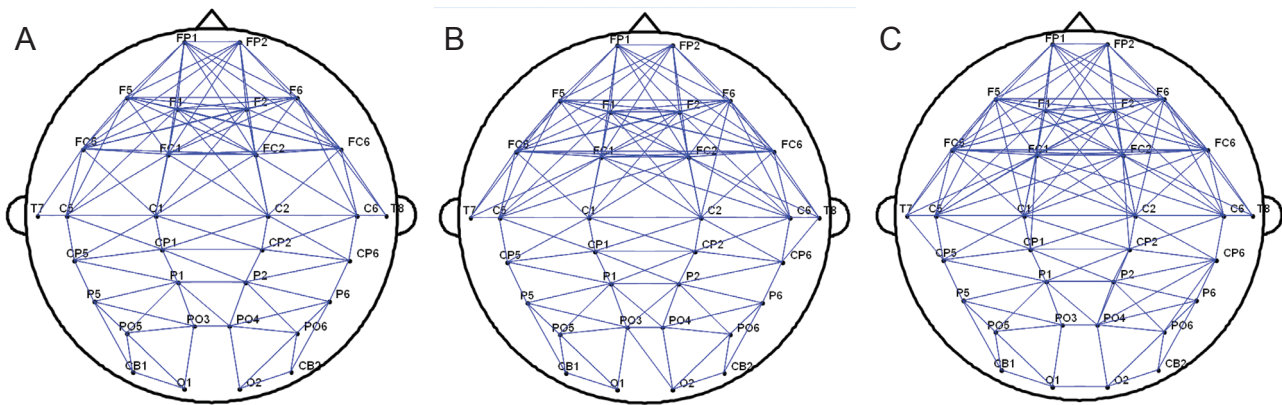


Figure 6 Brain functional networks under different stages. (A) Before stimulation; (B) after acupoint stimulation; (C) after mock-point stimulation. When positive Pearson's correlation coefficient is > 0.69 , there is a connection. Magnetic stimulations at the *Neiguan* (PC6) acupoint and at the mock point increase internal connections, respectively. F: Frontal lobe; T: temporal lobe; P: parietal lobe; O: occipital lobe; C: cerebral central region.

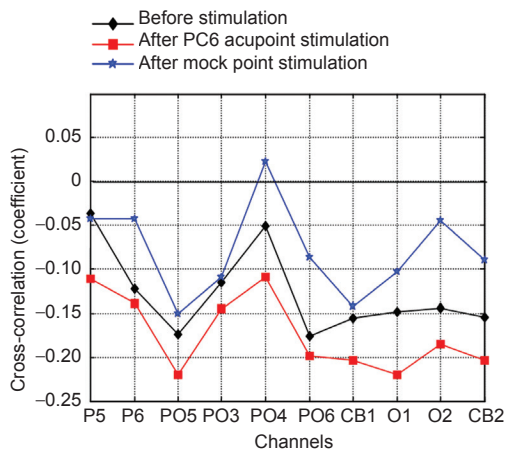


Figure 7 PCC values between EEG signals of FP1 channel. PCC: Pearson's correlation coefficient; EEG: electroencephalography; F: frontal lobe; T: temporal lobe; P: parietal lobe; O: occipital lobe; C: cerebral central region.

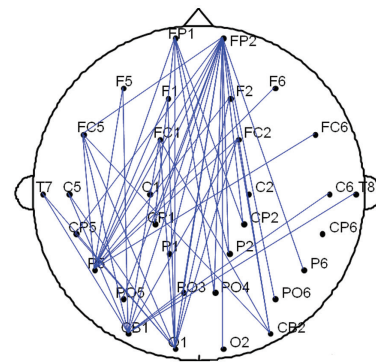


Figure 8 Negative-correlation brain network induced by magnetic stimulation at the *Neiguan* (PC6) acupoint. When negative Pearson's correlation coefficient is < 0.05 , there is a connection. Magnetic stimulation at the PC6 acupoint increases internal connections. F: Frontal lobe, T: temporal lobe, P: parietal lobe, O: occipital lobe, C: cerebral central region.