Analysis of the effect of repeated-pulse transcranial magnetic stimulation at the Guangming point on electroencephalograms

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Abstract

Here, we administered repeated-pulse transcranial magnetic stimulation to healthy people at the left Guangming (GB37) and a mock point, and calculated the sample entropy of electroencephalogram signals using nonlinear dynamics. Additionally, we compared electroencephalogram sample entropy of signals in response to visual stimulation before, during, and after repeated-pulse transcranial magnetic stimulation at the Guangming point. Results showed that electroencephalogram sample entropy at left (F3) and right (FP2) frontal electrodes were significantly different depending on where the magnetic stimulation was administered. Additionally, compared with the mock point, electroencephalogram sample entropy was higher after stimulating the Guangming point. When visual stimulation at Guangming was given before repeated-pulse transcranial magnetic stimulation, significant differences in sample entropy were found at five electrodes (C3, Cz, C4, P3, T8) in parietal cortex, the central gyrus, and the right temporal region compared with when it was given after repeated-pulse transcranial magnetic stimulation, indicating that repeated-pulse transcranial magnetic stimulation at Guangming can affect visual function. Analysis of electroencephalogram revealed that when visual stimulation preceded repeated pulse transcranial magnetic stimulation, sample entropy values were higher at the C3, C4, and P3 electrodes and lower at the Cz and T8 electrodes than visual stimulation followed preceded repeated pulse transcranial magnetic stimulation. The findings indicate that repeated-pulse transcranial magnetic stimulation at the Guangming evokes different patterns of electroencephalogram signals than repeated-pulse transcranial magnetic stimulation at other nearby points on the body surface, and that repeated-pulse transcranial magnetic stimulation at the Guangming is associated with changes in the complexity of visually evoked electroencephalogram signals in parietal regions, central gyrus, and temporal regions.

Key Words: nerve regeneration; brain injury; acupuncture; magnetic stimulation; acupuncture point; mock point; Guangming point; brain function; electroencephalogram signals; complexity; sample entropy; nonlinear dynamics; NSFC grant; neural regeneration

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Introduction

Acupuncture therapy is an in vitro means of regulating the body’s functions and treating diseases through stimulation. Acupoints are nerve-sensitive regions found in Chinese medicine (NIH, 1997; Maciocia, 2005). As modern scientific techniques in meridian research develop, accumulated evidence has begun to reveal mechanisms underlying acupuncture therapy, and explain the essence of acupuncture points and meridians in a modern scientific and intuitive way (Li et al., 2008; You et al., 2011; Jiang et al., 2013; Zhang et al., 2013). Stimulation at acupoints has a positive impact on nerve regeneration. For example, electric acupuncture promoted the regeneration of different fiber components in tibial nerves (Kong et al., 1993), and significantly up-regulated the expression of nerve growth factor in mimetic muscle tissue during facial nerve regeneration (Ya et al., 2000).

Magnetic stimulation is a relatively new acupuncture stimulation-therapy with incomparable advantages (Yu et al., 2011). Growing evidence has focused on cell physiology and transmission of neural signals under a magnetic field. Static magnetic fields are thought to be a potential non-invasive treatment for Parkinson’s disease and other neurological disorders. For instance, a 100–1,000 mT static magnetic field reproduced the cellular effects of ZM241385 (a candidate drug for Parkinson’s disease) in cultured rat-PC12 cells (Wang et al., 2010). Moderate-strength static magnetic fields can also...
cause apparent alterations in action potentials, half-activation voltage, and slope factor. As evidenced by the reduction of neural rectifier-potassium channels (Li et al., 2009; Li et al., 2010). Using independent component analysis, Spasic et al. (2011) analyzed the sources of fractal complexity in activity induced by a 2.7 mT static magnetic field in snail Br neurons and found two opposite intrinsic mechanisms underlying the neuronal responses. Prina-Mello et al. (2005) found that static magnetic fields were associated with cell differentiation and found a certain association between cell differentiation and a constant magnetic field. Jiang et al. (2004) investigated the correlation between the excitation functions and external conditions. They found that the magnetic field elicited a negative excitation function value at a fixed point of a nerve fiber membrane, which can evoke an action potential. Additionally, according to a previous study addressing the effect of magnetic acupoint stimulation on free radical metabolism in rabbits, acupoint stimulation enhanced superoxide dismutase activity, inhibited free radicals, lowered serum lipid peroxide, and strengthened antioxidant capacity (Zhao et al., 2000). Therefore, the effects of magnetic fields on neurons as well as the role of acupuncture points have been verified in the fields of the cytology, cell biology, and biophysics. In an effort to further understand the mechanisms and applicability of magnetic stimulation, this study measured electroencephalogram (EEG) signals in response to repeated-pulse transcranial magnetic stimulation (rTMS).

Emerging, non-classical methods in EEG signal analysis and nonlinear-dynamics theory have attracted widespread interest. The complexity of an EEG signal reflects the degree of signal randomization. Previous studies addressing the complexity of EEG signals at different sleep stages showed that deeper sleep is accompanied by less complex EEG signals, indicating that brain activity is largely convergent during deep sleep (Meng et al., 1998). Thus, signal complexity is a measurable indicator for evaluating neural excitability. The vast majority of stimulation studies that attempt to regulate nerve function employ functional magnetic resonance imaging (fMRI), while those that analyze EEG signals are virtually nonexistent. If we find statistically significant differences after comparing changes in EEG signals after magnetic stimulation at true and sham acupoints, we can illustrate that magnetic stimulation at acupoints has a specific impact on EEG signals. Depending on the research purpose, different EEG indicators are appropriate. For example power spectrum analysis is a simple and reliable method for smooth EEG signals, nonlinear-dynamics indicators are necessary for analyzing non-stationary signals, and sample entropy is a simple, reliable, highly precise algorithm for complexity that has been effectively applied to the classification of EEG signals.

The Guāngming (GB37) point is a vision-related acupoint that is related to blepharitis, refractive error, night blindness, optic atrophy, and other diseases (WHO, 2008). Previous hemodynamic studies demonstrated that occipital cortex responds to stimulation at the Guāngming (Cho et al., 1998; Gareus et al., 2002; Kong et al., 2009; Li et al., 2003). Because this phenomenon is known and apparently reliable, we selected Guāngming as the target for magnetic stimulation. Thus, to search for scientific evidence that magnetic stimulation alters brain activity, we conducted several experiments. First, we examined changes in sample entropy (SampEn; a nonlinear dynamics index) of EEG signals after magnetic stimulation was given at the Guāngming and mock points in healthy people. Next, we looked at differences in SampEn values after visual stimulation given either before or after magnetic stimulation at the Guāngming point. We studied EEGs under these conditions because in Chinese medicine the Guāngming point is known to be related to visual function, and thus changes in visually-evoked EEG signals after magnetic stimulation at Guāngming could verify whether magnetic stimulation there can affect visual function.

**Subjects and Methods**

**Subjects and facilities**

The experiment was performed at Province-Ministry Joint Key Laboratory of Electromagnetic Field and Electrical Apparatus Reliability at Hebei University of Technology in Tianjin, China from March to June, 2013. Ten right-handed subjects (6 male; average age: 26.5 years; range: 20–33 years) participated in the study and comprised students and faculty. They were recruited through a network registration and volunteered to participate in this experiment. All subjects signed informed consent prior to the experimentation. Subjects were included only if they were (1) in good physical and mental health, (2) were well rested, and (3) had no history of mental illness.

The experiment was performed without environmental stimuli or interference. The laboratory was in a quiet, temperature-controlled environment with good ventilation. Subjects indicated they were relaxed and not anxious before the experiment. Sweating and noise were avoided.

**Guāngming and mock points**

Guāngming is a vision-related point, belonging to the Gall Bladder Channel of Foot-Shaoyang, and is located laterally on human legs, 5 cm above the lateral malleolus tip, at the anterior margin of the fibula (Maciocia, 2005). The control condition consisted of magnetic stimulation at a mock point (a non-acupoint lateral location on the leg). Figure 1 shows the location of the Guāngming and mock points.

**Magnetic stimulator**

rTMS was conducted with a Magstim Rapid2 magnetic stimulator (Magstim Company, Whitland, Carmarthenshire, UK) with the coil arranged in a figure-eight formation to achieve accurate positioning (Yang et al., 2010). The inner and outer diameters of the 8-shaped coil were 53 and 73 mm, respectively. rTMS (2.0 T) was administered at an intensity of 0.8 times the maximum central intensity at a fixed frequency of 1 Hz.

**EEG system**

A 128-lead EEG recording and analysis system (NeuroScan Company, Charlotte, NC, USA) was used to record 64 lead EEG signals, with post aereum and mastoid (M1, M2) as a reference electrode, and a sampling frequency of 1,000 Hz. We selected 64-channel data, and distributed 19 channels evenly over the scalp (Figure 2). We chose 19 channels to meet the requirements of Scan 4.3.2 software (NeuroScan, Charlotte, NC, USA) and the EEGLab Toolkit (MathWorks, Natick, MA, USA), which were used to plot the EEG signal sample-entropy map.
EEG data were recorded under the following five conditions. (A) Magnetic stimulation at the Guangming point: the coil was placed 1 cm above the left Guangming point and stimulation was given for 3 minutes. (B) Magnetic stimulation at the mock point: the coil was placed 1 cm above the left mock point and stimulation was given for 3 minutes. (C) Visual stimulation before magnetic stimulation at the Guangming: participants were first subjected to a checkerboard reversal-stimulation for 3 minutes during which EEG data were recorded. (D) Visual stimulation during magnetic stimulation at the Guangming: The 3-minute checkerboard reversal-stimulation was conducted simultaneously with magnetic stimulation at the Guangming. (E) Visual stimulation after magnetic stimulation at the Guangming: The 3-minute checkerboard reversal-stimulation was carried out following magnetic stimulation at the Guangming, and EEG data were recorded. The interval between each condition was about 5 minutes. A longer interval was not possible because the conductive gel being used would dry and impact signal acquisition. All subjects participated in each of the five conditions.

EEG signal analysis
EEG signal preprocessing
Scan4.3.2 software provided with the NeuroScan 128-channel EEG analyzer was used for offline data preprocessing. The processing flowchart is shown in Figure 3.

Sample entropy analysis
In 1991, approximate entropy was introduced to quantify the amount of regularity and unpredictability in time-series fluctuations (Pincus, 1991). Approximate entropy requires small amounts of data and is less influenced by system noise, thus it is suitable for both random and stable signals. However, because both deterministic and random components are involved in biological signals, a bias in approximate entropy can be caused by self-matches. Sample entropy is a refinement of approximate entropy, a measurement introduced by Richman et al. (2000), which does not include self-matches. It is simpler and more accurate than approximate entropy, and requires approximately one-half the amount of time to calculate. A smaller sample-entropy value indicates lower complexity and more self-similarity. Practically, a relatively small data set is required for a rough estimate of sample entropy. In terms of analyzing mixed signals that consist of random and deterministic components, sample entropy is better than simple descriptive statistics such as the mean, variance, and standard deviation. Additionally, sample entropy does not require coarse-grained original signals, and is thereby particularly suitable for the analysis of biological signals (Zhang et al., 2009). In this study, we selected the value of sample entropy as eigenvalues of EEG data obtained from magnetic stimulation at the Guangming, and drew a sample entropy diagram based on EEG mapping to compare brain activity under magnetic stimulation at different points.

We processed the data as follows: For a time series of N points, the initial data were set as follows.

Step 1: A group of m dimensional vectors were formed such that:

\[ X_m(i) = [u(i), u(i+1), ..., u(i+m-1)] \]

where \( X_m(i) \) represents the vector of \( m \) data points from \( u(i) \) to \( u(i+m-1) \).

Step 2: The distance between \( X_m(i) \) and \( X_m(j) \) was defined as

\[ d[X_m(i), X_m(j)] = \max_i |u(i+k) - u(j+k)| \]

Step 3: Tolerance for accepting matches was defined as \( \tau \), the function for \( i \leq N-m+1 \) was defined as

\[ B^\tau_m(r) = \{ d[X_m(i), X_m(j)] < \tau \} \]

and the mean value corresponding to \( i \) was calculated as

\[ B^{\tau} = m \cdot \sum_{i=m+1}^{N-m+1} B^\tau_m(r) \]

Step 4: To form a group of \( m+1 \) dimensional vectors, steps 2 and 3 were repeated to obtain \( B^{m+1}(r) \). Then, the mean value corresponding to all “i”s was calculated to obtain \( B^{m+1}(r) \).

Step 5: Theoretically, we defined sample entropy as

\[ SampEnt(m, r) = \lim_{N \to \infty} \left\{ -\ln \left[ B^{m+1}(r) / B^{m}(r) \right] \right\} \]
Table 1 Differences in mean sample entropy values of electroencephalogram signals between conditions

| Channel | A    | B    | C    | D    | E    | P (A vs. B) | P (C vs. D) | P (C vs. E) | P (D vs. E) |
|---------|------|------|------|------|------|-------------|-------------|-------------|-------------|
| FP1     | 0.212±0.033 | 0.202±0.019 | 0.215±0.062 | 0.292±0.116 | 0.206±0.053 | 0.524          | 0.017*       | 0.796       | 0.138       |
| FP2     | 0.217±0.044 | 0.195±0.042 | 0.217±0.049 | 0.267±0.060 | 0.210±0.045 | 0.038*       | 0.060        | 0.800       | 0.085       |
| F7      | 0.239±0.032 | 0.217±0.036 | 0.235±0.063 | 0.303±0.108 | 0.231±0.022 | 0.259          | 0.019*       | 0.856       | 0.099       |
| F3      | 0.213±0.026 | 0.187±0.015 | 0.206±0.056 | 0.266±0.087 | 0.194±0.016 | 0.025*       | 0.100        | 0.638       | 0.080       |
| FZ      | 0.206±0.031 | 0.202±0.045 | 0.193±0.064 | 0.262±0.095 | 0.188±0.018 | 0.895          | 0.039*       | 0.703       | 0.015*       |
| F4      | 0.208±0.045 | 0.189±0.012 | 0.214±0.011 | 0.266±0.091 | 0.203±0.026 | 0.312          | 0.07         | 0.545       | 0.749       |
| F8      | 0.209±0.052 | 0.201±0.024 | 0.229±0.110 | 0.288±0.091 | 0.226±0.050 | 0.691          | 0.017*       | 0.908       | 0.034*       |
| T7      | 0.234±0.048 | 0.235±0.049 | 0.267±0.086 | 0.336±0.101 | 0.247±0.046 | 0.542          | 0.025*       | 0.096       | 0.515       |
| C3      | 0.227±0.032 | 0.218±0.063 | 0.211±0.075 | 0.289±0.112 | 0.209±0.029 | 0.784          | 0.005*       | 0.005*      | 0.039*       |
| CZ      | 0.213±0.035 | 0.198±0.031 | 0.195±0.059 | 0.293±0.112 | 0.198±0.035 | 0.757          | 0.940        | 0.002*      | 0.022*       |
| C4      | 0.231±0.074 | 0.198±0.044 | 0.237±0.125 | 0.278±0.065 | 0.229±0.058 | 0.753          | 0.100        | 0.001*      | 0.006*       |
| T8      | 0.239±0.073 | 0.236±0.050 | 0.270±0.092 | 0.328±0.144 | 0.275±0.124 | 0.642          | 0.024*       | 0.006*      | 0.002*       |
| P7      | 0.230±0.073 | 0.218±0.036 | 0.233±0.086 | 0.297±0.121 | 0.215±0.022 | 0.705          | 0.009*       | 0.137       | 0.246       |
| P3      | 0.231±0.069 | 0.220±0.033 | 0.218±0.091 | 0.292±0.116 | 0.203±0.019 | 0.732          | 0.030*       | 0.015*      | 0.109       |
| PZ      | 0.237±0.055 | 0.214±0.028 | 0.196±0.063 | 0.279±0.107 | 0.185±0.015 | 0.360          | 0.007*       | 0.329       | 0.455       |
| P4      | 0.248±0.065 | 0.233±0.039 | 0.205±0.065 | 0.278±0.084 | 0.197±0.024 | 0.620          | 0.016*       | 0.071       | 0.037*       |
| P8      | 0.248±0.065 | 0.231±0.041 | 0.217±0.069 | 0.286±0.082 | 0.206±0.025 | 0.384          | 0.220        | 0.121       | 0.089       |
| O1      | 0.274±0.071 | 0.248±0.024 | 0.241±0.110 | 0.354±0.137 | 0.221±0.026 | 0.519          | 0.057        | 0.410       | 0.953       |
| O2      | 0.246±0.076 | 0.240±0.039 | 0.223±0.084 | 0.291±0.123 | 0.208±0.034 | 0.818          | 0.000*       | 0.116       | 0.046*       |

EEG data were recorded under the following conditions, (A) magnetic stimulation at the Guangming, (B) magnetic stimulation at mock point, (C) visual stimulation before magnetic stimulation at the Guangming, (D) visual stimulation during magnetic stimulation at the Guangming, and (E) visual stimulation after magnetic stimulation at the Guangming. *P < 0.05. Data are represented as mean ± SD, n = 8, paired t-test.

When N is a finite value, we can obtain the estimated value of SampEn, that is,

\[ \text{SampEn}(m, r, N) = -1 \ln \left[ B^m(r)/B^{m+1}(r) \right]. \]  

(6)

Where m is an embedding dimension, which is the length of sequences to be compared. Generally, m and r in the sample entropy algorithm are the same as those in approximate entropy, that is, \( m = 1 \) or \( m = 2 \), and r ranges from 0.1 to 0.25 of \( \delta \) (\( \delta \) is the standard deviation of the raw data). N is the data length, and often ranges from 100 to 5,000 to reduce artifacts. In this study, we used the value of \( m = 2 \), \( r = 0.2\delta \), and \( N = 5,000 \).

**Statistical analysis**

Measurement data are expressed as mean ± SD and all data were normally distributed. Statistical analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Paired t-tests were conducted to test for statistical differences between sample entropy under different conditions. A \( P < 0.05 \) was considered significant difference.

**Results**

**Quantitative analysis of subjects**

EEG raw data extracted from 10 experimental subjects were preprocessed as described above (removal of electrooculogram artifacts, baseline correction, and filtering). Data from two subjects were excluded because data from two channels were identical. This may have been because of excess injection of the conductive paste. Thus, data from eight subjects were used for statistical analysis.

**Comparison of sample entropy values between conditions A and B**

Mean sample entropy values from the eight subjects under conditions A and B were calculated, and then differences were statistically analyzed using SPSS 13.0 software. The data from 19 channels are shown in Table 1. Sample entropy values obtained from the right frontal electrode (FP2) and left frontal electrode (F3) were significantly higher under condition A than under condition B (\( P < 0.05 \)), while no differences were found at other channels (Figure 4, upper left).

**Comparison of sample entropy values between conditions C, D, and E**

After data preprocessing, there were 80 cycles of single-channel time series for each condition, and each cycle was 80 s long (totally 80,000 data points). Sample entropy values from the EEG signals of eight subjects were calculated under conditions C, D, and E. Five-thousand data points were de-
In this study, we compared sample entropy values of EEGs generated after magnetically stimulating the Guangming (condition A) or a mock point (condition B), and sample entropy values of visually-evoked EEGs before magnetic stimulation at Guangming (condition C), during magnetic stimulation at Guangming (condition D), and after magnetic stimulation at Guangming (condition E). Comparison between conditions A and B allowed us to examine differences in EEG complexity after magnetic stimulation at the Guangming or a mock point. We eliminated the impact of somatosensation, and found differences in EEG complexity that can be attributed solely to the location of stimulation. Significant differences between conditions A and B were seen in channels FP2 and F3, which are located in the frontal cortex. This suggests that brain activity in frontal regions underwent changes after magnetic stimulation that were specific to the location of stimulation. This result is consistent with findings described previously (Guan et al., 2008), showing that acupuncture at Guangming or a mock point led to differential activation of the frontal lobe. The sample entropy values in channels FP2 and F3 were generally higher during stimulation at the Guangming than at the mock point, indicating higher EEG complexity after acupoint stimulation. Magnetic stimulation at Guangming may therefore raise cortical neuronal activity.

Under conditions C, D, and E, we compared the changes in EEG signals when magnetic stimulation was delivered at the Guangming before, during, or after visual stimulation. Here, despite an uneven distribution, many channels differed significantly depending on whether visual stimulation was before or during magnetic stimulation. Similarly, many channels differed significantly depending on whether visual stimulation occurred during or after magnetic stimulation. Many channels exhibited significant differences that were widely distributed on the scalp. A focus of this study was to compare EEG signal complexity before and after magnetic stimulation at Guangming (between conditions C and E). The comparison between conditions C and E allows us to explore how visually evoked EEG signals are affected by magnetic stimulation at Guangming. While most $P_{CE}$ values were not significant, those at channels C3, C4, CZ, T8, and P3 were. This suggests that in response to visual stimulation, EEG complexity in the parietal cortex, central gyrus, and right temporal lobe is affected by prior magnetic stimulation at the Guangming, and that the effect may be delayed. Although the Guangming is an acupoint related to visual function, magnetic stimulation at Guangming in this study did not alter EEG patterns in primary visual (occipital) cortex. Effects observed in the parietal cortex, central gyrus, and the right temporal lobe, suggest that magnetic stimulation at the Guangming primarily affects somatosensory and auditory functions (Gareus et al., 2002). This may be why subjects complained of numbness and hearing voices during the stimulation. This study did not find evidence that magnetic stimulation at Guangming can evoke changes in EEGs, which is somewhat similar to fMRI study of acupuncture stimulation at Guangming (Gareus et al., 2002).

In this study, we explored the effects of magnetic stimu-
luation at Guangming on the sample entropy of EEG signals, and found that magnetic stimulation at Guangming had an impact on EEG complexity. Variation in sample entropy is a suitable measure to distinguish EEG signals under different physiological conditions. We selected points related to visual function as the target of stimulation because changes in EEG activity after visual stimulation are well known and easy to observe. Furthermore, understanding vision-related acupuncture points will help understand visually evoked EEG signals.

To date, many studies have confirmed the association between acupuncture and activity of specific brain regions, but experimental conclusions are controversial. Improvement in experimental and analytic methods is therefore necessary. Experimental findings will guide the application of magnetic stimulation in clinical treatment, nerve rehabilitation, and neural regeneration. In this study, a weak spasm that differed individually was visible after magnetic stimulation at the mock point. This phenomenon indicates that magnetic stimulation at a mock point can activate motor muscles, and efforts to eliminate this phenomenon should be undertaken to improve experimental methods.

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Author contributions: Zhang X collected and analyzed experimental data. Fu LD designed and implemented the study. Geng YH was responsible for the study concept and design. Zhai X performed data analysis. Liu YH was responsible for statistical analysis. All authors approved the final version of the manuscript.

Conflicts of interest: None declared.

Peer review: This study aims to observe the effect of repeated-pulse transcranial magnetic stimulation on healthy people through a sample entropy analysis of electroencephalogram signals. Additionally, we compared electroencephalogram signals in response to visual stimulation before, during, and after repeated-pulse transcranial magnetic stimulation at the Guangming using nonlinear dynamics. The results showed that, repeated-pulse transcranial magnetic stimulation at the Guangming has an apparent effect on the electroencephalogram signals, which confirms the contribution of magnetic stimulation.

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