Amplitude of Low Frequency Fluctuation (ALFF) study of the spontaneous brain activities of patients with phantom limb pain

J.-G. DU, H. XIAO, Y.-X. ZUO

[1]Department of Anesthesia, The People's hospital of SND, Suzhou, China
[2]Department of Anesthesiology West China Hospital Sichuan University, Chengdu, China

Abstract. – OBJECTIVE: Our objective was to use Amplitude of Low-Frequency Fluctuation (ALFF) method to investigate the changes in spontaneous brain activity in HM patients and their relationships with clinical features.

PATIENTS AND METHODS: This study was set out to observe, using Functional magnetic resonance imaging (fMRI), the changes in spontaneous brain activity in patients with phantom limb pain (PLP). Eleven amputees with PLP closely matched in age, sex, and education in a right side lower limb were scanned using fMRI to measure the amplitude of low-frequency fluctuation (ALFF) and functional connectivity (FC) in the resting state of the brain (G_{PLP}) before use of prosthetic. They were then scanned again after recovering from PLP (G_{PLPr}) after use of artificial limbs. Eleven healthy volunteers (G_C) were also scanned.

RESULTS: When compared to G_C, G_{PLP} exhibited decreased ALFF in the left inferior parietal lobule, and G_{PLPr} exhibited decreased ALFF in the left precuneus. When compared to G_{PLP}, G_C showed positive FC in the part regions of the limbic system structure. When compared to G_C, the positive FC in G_{PLPr} was significantly decreased in the midbrain. Finally, when compared to G_{PLPr}, G_{PLP} showed significantly decreased positive FC in the right precuneus and inferior parietal lobe. The central nervous system shows functional changes in the resting state of the brain in patients with PLP, which may indicate the presence of neurobiological changes. The recovery time of the changes may be longer than the pain symptoms of patients.

CONCLUSIONS: The technique of fMRI of the resting network of the brain in patients with PLP may be able to be used to monitor clinical therapeutic effects.

Key Words:
Functional magnetic resonance imaging, Phantom limb pain, Artificial limbs, Amplitude of low-frequency fluctuation, Functional connectivity.

Introduction

PLP refers to the pain experienced in a missing limb, and recognized as a kind of neuropathic pain. Currently, there are many clinical treatments for phantom limb pain[1-4] that aim to alleviate pain and improve quality of life, but the effects in different patients show no consistently satisfactory results. With the emergence of nuclear magnetic resonance technology, research on the central nervous system has developed rapidly[5-7]. One of these technologies, fMRI, provides an excellent way to study the mechanisms of chronic pain. Researches have shown that patients with various kinds of chronic pain exhibit changes in the structure and function of the related brain areas[8,9], and there are similar changes in subcortical structures[10,11]. A similar reorganization has been found in the brains of patients with PLP[12,13], and so it has been observed that cortical reorganization may be a central mechanism in PLP[14]. The default mode network[15] suggests that the organized spontaneous activity in the resting state of the brain may be related to nerve function to consolidate memories, predict the future, and stay alert. The ALFF of the blood oxygen level-dependent[16] signal wave detected by fMRI reflects the spontaneous nerve activity of the brain of the patient in the resting state. The ALFF has been widely applied to detect the brain function within a specific frequency range (0.01-0.08 Hz)[17,18]. We wanted to address the question of how changes in the central neural spontaneous activity and functional connectivity between the resting state of patients suffering from PLP and those recovering from the clinical symptoms of PLP, and whether the changes disappear with the disappearance of pain symp-

Corresponding Author: Yunxia Zuo, MD; e-mail: 1043545263@qq.com

toms. This study looked at the spontaneous electrical activities and functional connectivity in the resting state of the central nervous system of the control group, the PLP group, and the PLP group to explore the possible mechanisms of PLP and to provide a reference standard for imaging to use in the clinical cure of PLP.

Patients and Methods

Patients

Eleven patients with right lower limb amputations with PLP were recruited from hospitals and Prosthetics and Orthotics Centers between January 2009 and December 2010. All amputees used artificial limbs to promote the rehabilitation. A fMRI of their brains was obtained before use of prosthetic and after use of prosthetic with VAS below 2 (defined the pain has been restored). In addition, fMRIs of 11 healthy volunteers were obtained as a control. Inclusion criteria: right lower limb amputees who still had PLP and with no mental disability were included.

Exclusion criteria: left lower limb amputees with PLP who had claustrophobia, who had a metal implant in their body and who had not signed the informed consent form were excluded.

The experimental procedures: questionnaires based on McGill pain questionnaire (MPQ) were completed by all patients before the day of the fMRI, covering basic information, and the period over which the PLP had evolved (using VAS). The fMRIs in the resting state of the brain were then obtained. The Ethics Committee of West China Hospital of Sichuan University approved the study. All participants signed an informed consent form.

Image Acquisition

MRI scanning was performed on a 3 TMR scanner (Trio; Siemens, Munich, Germany). The functional data were obtained with spoiled gradient-recalled echo sequence with the parameters (repetition time = 1,900 ms, echo time = 2.26 ms, thickness =1.0 mm, gap = 0.5 mm, acquisition matrix = 256 × 256, field of view = 250 × 250 mm, flip angle = 9°). We also obtained 240 functional images (repetition time = 2,000 ms, echo time=30 ms, thickness = 4.0 mm, gap = 1.2 mm, acquisition matrix = 64 × 64, flip angle = 90°, field of view = 220 × 220 mm, 29 axial).

All subjects were scanned using a 3T MR system (GE Excite, Milwaukee, WI, USA) with an 8-channel phased array head coil. During the MRI examination, subjects were instructed to relax with their eyes closed but not to fall asleep. MR images sensitized to changes in BOLD signal levels (TR/TE = 2,000/30 ms; flip angle = 90°) were obtained by a gradient-echo echo-planar imaging (EPI) sequence. There were five dummy scans collected before fMRI scans, and the first five volumes of fMRI time series were discarded for magnetization stabilization. The slice thickness was 5 mm (no slice gap) with a matrix size of 64 × 64 and a field of view of 240 × 240 mm^2, resulting in a voxel size of 3.75 × 3.75 × 5 mm^3. Each brain volume comprised 30 axial slices, and each functional run contained 200 image volumes.

Data Pre-processing

Functional image preprocessing and statistical analyses were carried out by using Statistical Parameter Map (SPM8). For each participant, echo-planar imaging (EPI) images were slice-time-corrected, realigned to the first image, and unwarped to correct for susceptibility-by-movement interaction. All of the realigned images were spatially normalized to the Montreal Neurological Institute (MNI) template, and each voxel was resampled to 3 × 3 × 3 mm^3 without spatial filtering.

Amplitude of Low-Frequency Fluctuation (ALFF) Calculation

ALFF is thought to reflect spontaneous neural activity in humans [16]. After the preprocessing, resulting data were further temporally band-pass filtered (0.01-0. 0.08 Hz) to reduce the effects of low-frequency drift and high-frequency physiological noises and linear-trend removal, the time series were transformed to the frequency domain by using Fast Fourier Transform (FFT), and the power spectrum was obtained. Next, the power spectrum obtained by FFT was square root-transformed and averaged across 0.01-0.08 Hz at each voxel. The averaged square root of activity in this frequency band was taken as the ALFF. For standardization purposes, the ALFF of each voxel was divided by the global mean ALFF values. ALFF and Functional Connectivity (FC) values were calculated by using REST (State Key Laboratory of Cognitive Neuroscience and Learning in Beijing Normal University; http://resting-fmri.sourceforge.net, Beijing, China).

Functional Connectivity Analysis

Functional connectivity was examined by using a seed voxel correlation approach. The left precuneus was selected as seed region based on our ALFF findings and previous neuroimaging studies of patients with phantom limb pain that revealed abnormal function in the region associated with pain processing. In SPM5, after band-pass filtering (0.01-0.08 Hz) and linear-trend removal, a reference time series for the seed was extracted by averaging the fMRI time series of voxels. Then, correlations were computed between the seed reference and the rest of the brain in a voxelwise manner. Finally, individual relativity value (r-value) map was produced and the correlation coefficients in each voxel were transformed to z values by using the Fisher r-to-z transformation to improve normality before performing the random effect *t*-tests. By using SPM5, we removed components with high correlation to CSF or white matter, or with low correlation to gray matter, which are thought to be associated with artifacts, such as cardiac-induced or respiratory-induced variations, rather than representing neural activity.

Statistical Analysis

ALFF maps in the G_{PLP}, G_{PLPr}, and G_C were compared by using one-sample *t*-test pair *t*-test between PLP and PLPr, and two-sample *t*-test between controls and PLP, PLPr, respectively in SPM8. Statistical analysis was performed with a general linear model analysis using the Statistical Parametric Mapping 8 (SPM8). Statistical threshold of $p < 0.01$ without correction was used for an exploratory whole-brain analysis. Functional connectivity maps in the G_{PLP}, G_{PLPr}, and G_C were compared by using pair *t*-test between G_{PLP} and G_{PLPr}, and two-sample *t*-test between G_C and G_{PLP}, G_{PLPr}, respectively in SPM5. Statistical threshold $p < 0.005$ without correction was considered as significant.

Results

Clinical Features of Participants

Eleven patients with right lower limb amputations with PLP were recruited from hospitals and prosthetics and orthotics centers. Analysis of clinical features revealed no statistically significant differences between amputation patients and healthy volunteers (Table I).

Changes in ALFF

Between the PLP group and control group, a final binary mask image showing voxels in the brain exhibited significant decreased low-frequency fluctuations in left inferior parietal lobule in the PLP groups at the specified statistical threshold ($p < 0.001$) (Figure 1).

Between the PLPr group and control group, a final binary mask image showing voxels in the brain exhibited significant decreased low-frequency fluctuations in left precuneus in the PLPr groups at the specified statistical threshold ($p < 0.001$) (Figure 2).

Changes in FC Pattern

Between the PLP group and control group, the difference of functional connectivity exhibited positive functional connectivity in the control group in the inferior parietal lobule (L), temporal lobe (L), parahippocampal gyrus (L), lingual gyrus (L), at the specified statistical threshold ($p < 0.001$) (Figure 3). Between the PLPr group and control group, in midbrain (a key region of pain matrix) there was significant decreased positive functional connectivity in the PLPr group at the specified statistical threshold ($p < 0.005$) (Figure 4). Between the PLPr group and PLP group, there was significant decreased positive functional connectivity in right precuneus (a key region of pain matrix) and in right inferior parietal lobule in PLP group at the specified statistical threshold ($p < 0.005$) (Figure 5). The changes of ALFF and FC in all patients showed in Table II.

Table I. Clinical features of participants (N = 11).

Feature	GPLP-G_{PLPr}	G_C	*p*-value
Age (mean ± SD), years	45 ± 12	38 ± 9	0.56
Male/Female	8/3	7/4	0.25
Education, years	7.50 ± 3.30	7.52 ± 2.91	0.71
Amputation level	All above knee		
VAS (mean ± SD)	6.24 ± 0.38		
T (mean ± SD), days	92.83 ± 40.81		

VAS = visual analogue scale before the use of prosthetic; T = Time from the first use of prosthetic to visual analogue scale below 2.

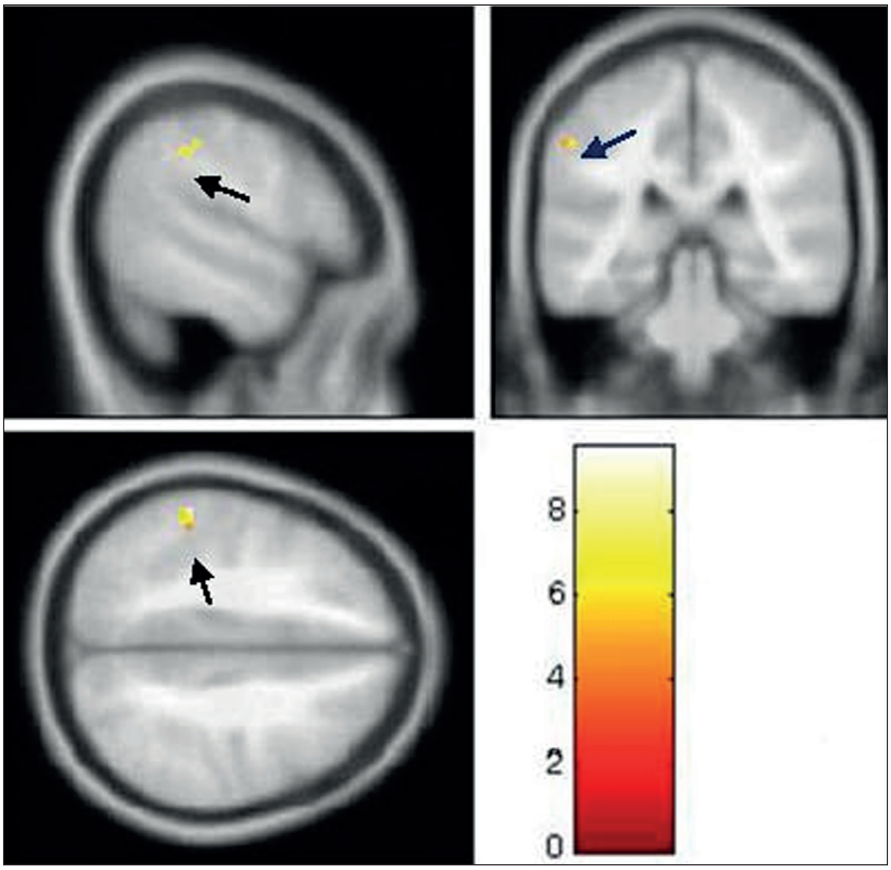

Figure 1. Region showing differences in ALFF between the PLP patients and controls during rest with eyes closed. In the left inferior Parietal Lobule the PLP group exhibited decreased ALFF ($p < 0.001$). ALFF: Amplitude of Low-Frequency Fluctuation; PLP: Phantom Limb Pain.

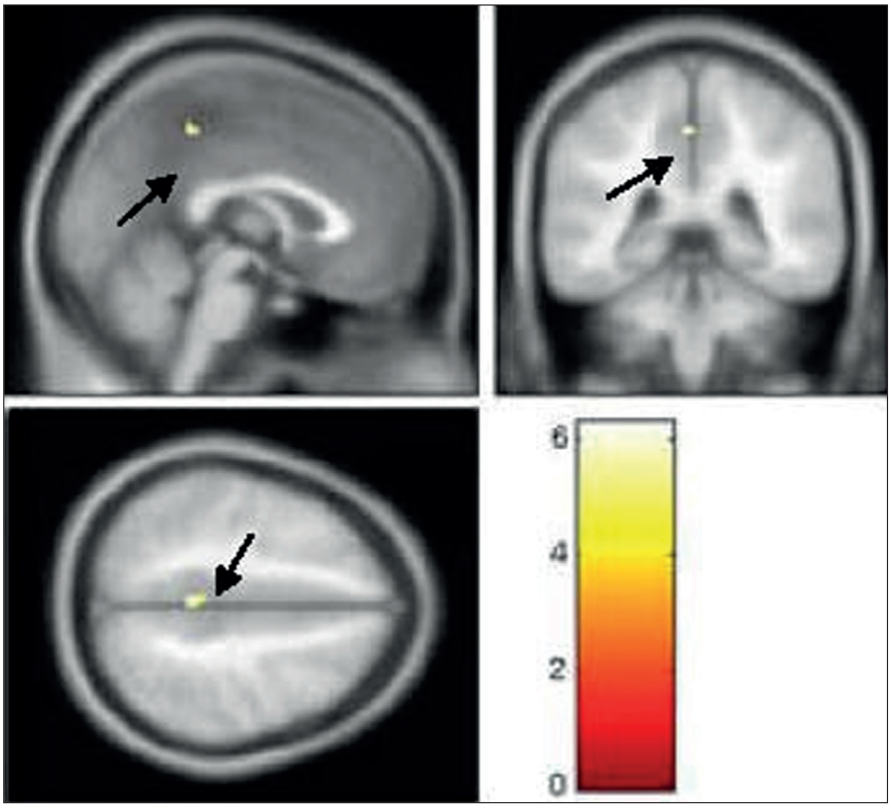

Figure 2. Region showing differences in ALFF between the PLPr patients and controls during rest with eyes closed. In the left Precuneus the PLPr group exhibited increased ALFF ($p < 0.001$). ALFF: Amplitude of Low-Frequency Fluctuation. PLPr: Recovery from Phantom Limb Pain.

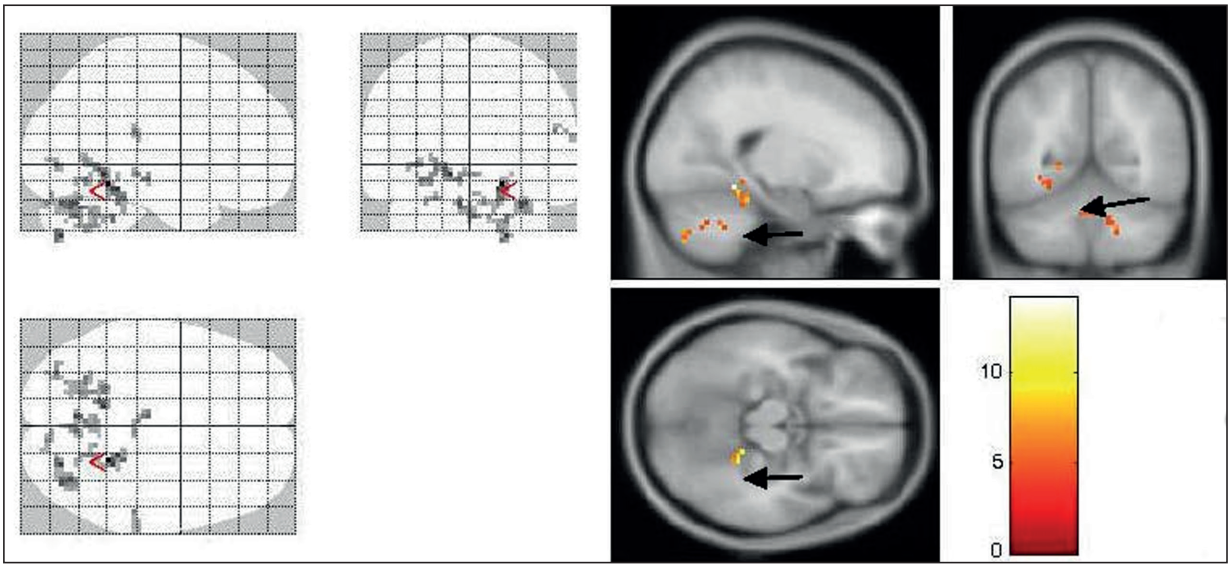

Figure 3. Regions showing differences in functional connectivity with the left precuneus (region of interesting, ROI) between the PLP patients and controls. The control group showed the positive functional connectivity in the left Inferior parietal lobule, temporal lobe, parahippocampal gyrus, lingual gyrus ($p < 0.005$). PLP: Phantom Limb Pain

Discussion

Currently PLP in patients after amputation still has no satisfactory treatment, so there is an urgent need to clarify the mechanism. Researches[11,17] show changes of FC in a task state of the brain using different methods in amputees. This raises some of questions as to whether there are changes of FC in the resting brain in patients with PLP, and whether the reorganization of the central nervous system returns to normal when the patients recover from PLP. To address these questions, this trial compared the ALFF and FC in the resting-state brain between G_{PLP}, G_{PLPr}, and G_C. The research found that ALFF in the left inferior parietal lobule were decreased in G_{PLP}.

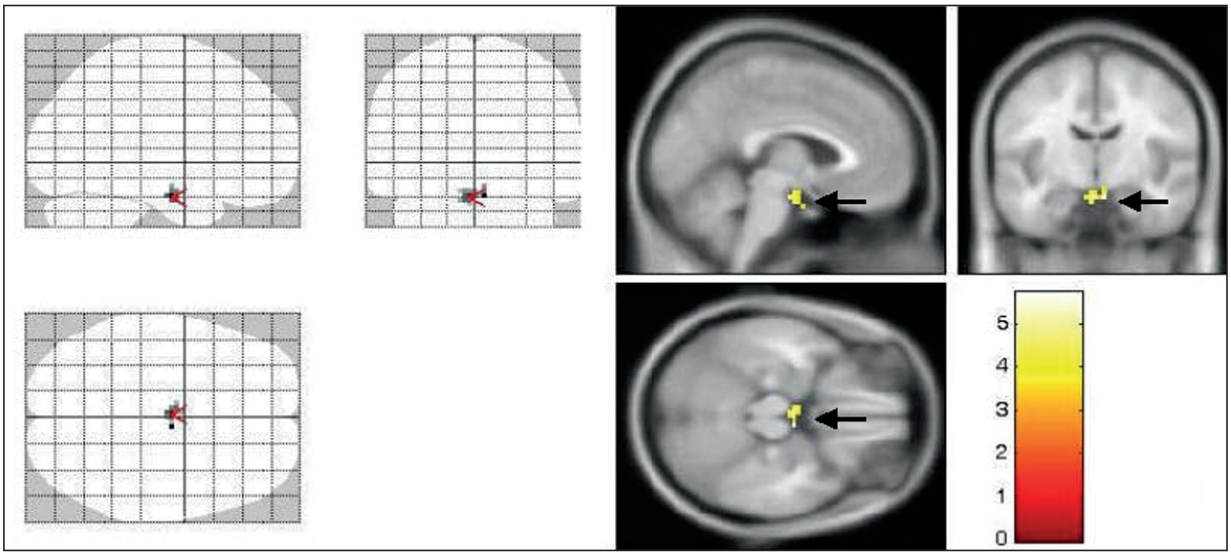

Figure 4. Region showing differences in functional connectivity with the left precuneus (region of interesting, ROI) between the PLPr patients and controls. The positive functional connectivity in the PLPr group was significantly decreased in the midbrain ($p < 0.005$). PLPr: Recovery from Phantom Limb Pain.

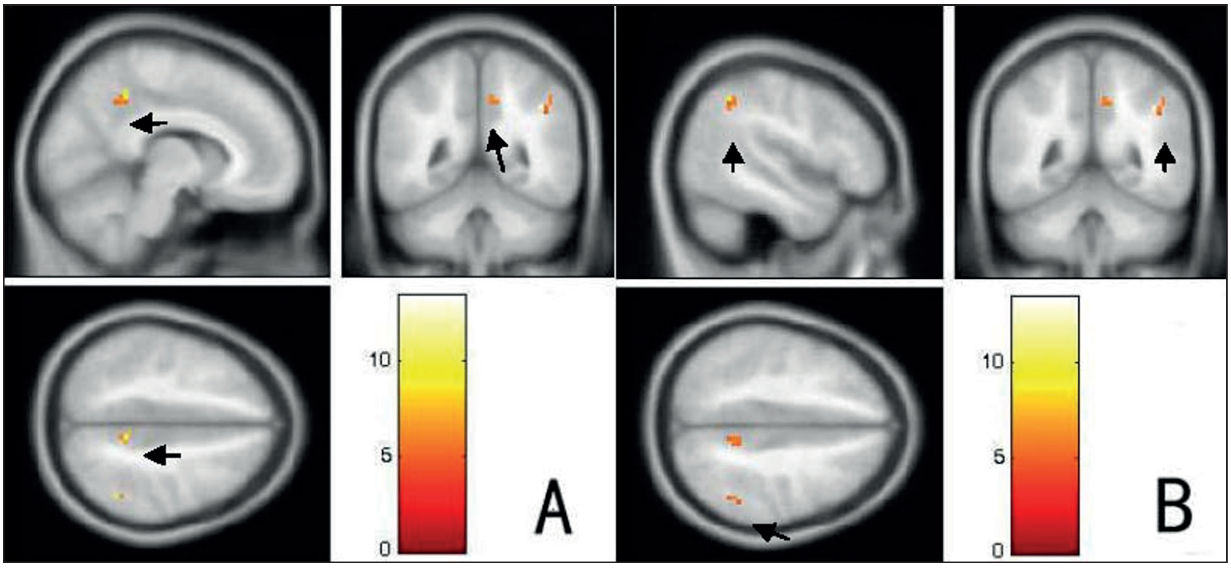

Figure 5. Regions showing differences in functional connectivity with the left precuneus (region of interesting, ROI) between the PLPr and PLP patients. The PLP group showed significantly decreased positive functional connectivity in the right precuneus ***(A)*** and inferior parietal lobe ***(B)*** ($p < 0.005$). PLP: Phantom Limb Pain. PLPr: Recovery from Phantom Limb Pain.

Because the left inferior parietal lobule is related to the visual body image, this result may be the central mechanisms of body image disorder due to the lack of the right leg. The control group showed positive connection in the relevant regions compared to G_{PLP}, which are all part of the limbic system and are associated with memories and emotions of pain. So the results may reflect neural functional connectivity decreased in the corresponding regions, and may indicate the mental and psychological mechanisms in the central nervous system in patients with PLP. The results are similar to the results from previous investigations[19,20] of functional connections in the task state of the brain. In the left precuneus showed decreased ALFF and in the midbrain (a key region of the pain matrix) showed a significant decreased positive functional connectivity in G_{PLPr} compared to the G_C. As we know, the precuneus and the midbrain are pain-related brain regions. The results indicate that, after recovery from pain symptoms in patients with PLP, the neurological functional connections of related parts of the brain are not restored to the level of healthy people, and show abnormal central nervous activity in the resting state of brain areas related to pain. In the past, research has suggested that cortical reorganization occurred in all patients with or without PLP post-amputation[21], but there were differences in the degree of reorganization between patients with phantom limb sense and phantom limb pain[22]. Other works[23,24] also showed that patients born with no limb and patients with phantom limb sense have no cortical reorganization. Whether the above differences between the recovery group patients and the control group shown in this trial were the result of the aftermath of pain or were caused by the absence of afferent neurons[25] needs further study to confirm. In the right precuneus (a key region of the pain matrix) and in the right inferior parietal lobule in PLP patients showed a significant decreased positive functional connectivity. The results may suggest that the changes related to visual psychology impaired in these areas are a certain degree of recovery in G_{PLPr}. The experiment showed differences in amplitude of low-frequency fluctuation and functional connectivity in the resting brain between G_{PLP}, G_{PLPr}, and G_C, which suggest that the recovery of phantom limb pain may be a long process. Even if the clinical symptoms of pain disappear, changes in the relevant parts of the central nervous system need a longer recovery process. From the point of view of treating PLP, how to prevent and reverse cortical reorganization[26,27] may be more direct and thorough treatment for patients with phantom limb pain. In this study, the ALFF and functional connectivity in the resting state of the brain showed differences between G_{PLP}, G_{PLPr}, and G_C suggest that the central nervous exhibits a functional reorganization in patients with PLP. The recovery time of the reorganization of the central nervous system in patients with PLP may be longer than the recovery time from pain symptoms.

Conclusions

The technique of fMRI of the resting network of the brain in patients with PLP may be able to be used to monitor clinical therapeutic effects, but this conclusion needs to be further confirmed by expanding the sample size.

Acknowledgements
We would like to thank the staff of the Hospitals and Prosthetics and Orthotics Centers for their support. This study was supported by the National Natural Science Foundation (Grant No. 30872435).

Conflict of Interest
The Authors declare that they have no conflict of interests.

References

1) Flor H. The modification of cortical reorganization and chronic pain by sensory feedback. Appl Psychophysiol Biofeedback 2002; 27: 215-227.
2) Dahl E, Cohen SP. Perineural injection of etanercept as a treatment for postamputation pain. Clin J Pain 2008; 24: 172-175.
3) Modirian E, Shojaei H, Soroush MR, Masoumi M. Phantom pain in bilateral upper limb amputation. Disabil Rehabil 2009; 31: 1878-1881.
4) de Roos C, Veenstra AC, de Jongh A, den Hollander-Gijsman M, van der Wee NJ, Zitman FG, van Rood YR. Treatment of chronic phantom limb pain using a trauma-focused psychological approach. Pain Res Manag 2010; 15: 65-71.
5) Wang SY, Wang M, Wang XX, Chen W, Sheng C, Gong ZK. Study on the clinical application of the MRS in the cognitive assessment after stroke. Eur Rev Med Pharmacol Sci 2017; 21: 2437-2442.
6) Inci Kenar AN, Unal GA, Kiroglu Y, Herken H. Effects of methylphenidate treatment on the cerebellum in adult attention-deficit hyperactivity disorder: a magnetic resonance spectroscopy study. Eur Rev Med Pharmacol Sci 2017; 21: 383-388.
7) Wang N, Huang HL, Zhou H, Yu CY. Cognitive impairment and quality of life in patients with migraine-associated vertigo. Eur Rev Med Pharmacol Sci 2016; 20: 4913-4917.
8) Wrigley PJ, Press SR, Gustin SM, Macefield VG, Gandevia SC, Cousins MJ, Middleton JW, Henderson LA, Siddall PJ. Neuropathic pain and primary somatosensory cortex reorganization following spinal cord injury. Pain 2009; 141: 52-59.
9) Unlu S, Dogan M, Kapicioglu Y, Kamisli S, Oner S, Yildirim IO, Ozturk M. CSF flow patterns in the brain in patients with neuro-Behcet disease and Behcet disease. Eur Rev Med Pharmacol Sci 2017; 21: 3906-3910.
10) Soros P, Knecht S, Bantel C, Imai T, Wusten R, Pantev C, Lutkenhoner B, Burkle H, Henningsen H. Functional reorganization of the human primary somatosensory cortex after acute pain demonstrated by magnetoencephalography. Neurosci Lett 2001; 298: 195-198.
11) Roux FE, Ibarrola D, Lazorthes Y, Berry I. Virtual movements activate primary sensorimotor areas in amputees: report of three cases. Neurosurgery 2001; 49: 736-741; discussion 741-732.
12) Karl A, Birbaumer N, Lutzenberger W, Cohen LG, Flor H. Reorganization of motor and somatosensory cortex in upper extremity amputees with phantom limb pain. J Neurosci 2001; 21: 3609-3618.
13) Draganski B, Moser T, Lummel N, Ganssbauer S, Bogdahn U, Haas F, May A. Decrease of thalamic gray matter following limb amputation. Neuroimage 2006; 31: 951-957.
14) Wu CL, Tella P, Staats PS, Vaslav R, Kazim DA, Wesselmann U, Raja SN. Analgesic effects of intravenous lidocaine and morphine on postamputation pain: a randomized double-blind, active placebo-controlled, crossover trial. Anesthesiology 2002; 96: 841-848.
15) Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001; 98: 676-682.
16) Laufs H, Kleinschmidt A, Beyerle A, Eger E, Salek-Haddadi A, Preibisch C, Krakow K. EEG-correlated fMRI of human alpha activity. Neuroimage 2003; 19: 1463-1476.
17) Lotze M, Flor H, Grodd W, Larbig W, Birbaumer N. Phantom movements and pain. An fMRI study in upper limb amputees. Brain 2001; 124: 2268-2277.
18) Yu HL, Liu WB, Wang T, Huang PY, Jie LY, Sun JZ, Wang C, Qian W, Xuan M, Gu QQ, Liu H, Zhang FL, Zhang MM. Difference in resting-state fractional amplitude of low-frequency fluctuation between bipolar depression and unipolar depression patients. Eur Rev Med Pharmacol Sci 2017; 21: 1541-1550.
19) Rosen G, Hugdahl K, Ersland L, Lundervold A, Smievoll AI, Barndon R, Sundberg H, Thomsen T, Roscher BE, Tjolsen A, Engelsen B. Different brain areas activated during imagery of painful and non-painful 'finger movements' in a subject with an amputated arm. Neurocase 2001; 7: 255-260.

20) Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumer N, Larbig W, Taub E. Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. Nature 1995; 375: 482-484.

21) Maruno N, Kaminaga T, Mikami M, Furui S. Activation of supplementary motor area during imaginary movement of phantom toes. Neurorehabil Neural Repair 2000; 14: 345-349.

22) Willoch F, Rosen G, Tolle TR, Oye I, Wester HJ, Berner N, Schwaiger M, Bartenstein P. Phantom limb pain in the human brain: unraveling neural circuitries of phantom limb sensations using positron emission tomography. Ann Neurol 2000; 48: 842-849.

23) Flor H, Elbert T, Muhlnickel W, Pantev C, Wienbruch C, Taub E. Cortical reorganization and phantom phenomena in congenital and traumatic upper-extremity amputees. Exp Brain Res 1998; 119: 205-212.

24) Montoya P, Ritter K, Huse E, Larbig W, Braun C, Topfner S, Lutzenberger W, Grodd W, Flor H, Birbaumer N. The cortical somatotopic map and phantom phenomena in subjects with congenital limb atrophy and traumatic amputees with phantom limb pain. Eur J Neurosci 1998; 10: 1095-1102.

25) Radhakrishnan V, Tsoukatos J, Davis KD, Tasker RR, Lozano AM, Dostrovsky JO. A comparison of the burst activity of lateral thalamic neurons in chronic pain and non-pain patients. Pain 1999; 80: 567-575.

26) Diers M, Christmann C, Koeppe C, Ruf M, Flor H. Mirrored, imagined and executed movements differentially activate sensorimotor cortex in amputees with and without phantom limb pain. Pain 2010; 149: 296-304.

27) Giuffrida O, Simpson L, Halligan PW. Contralateral stimulation, using TENS, of phantom limb pain: two confirmatory cases. Pain Med 2010; 11: 133-141.