Longitudinal Spatial Patterns of Intrinsic Brain Activity and Functional Connectivity in Upper Limb Amputees Patients

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Abstract

Background: Amputation in adults is a serious condition and most patients were associated with the remapping of representations in motor and sensory brain network.

Methods: The present study includes 8 healthy volunteers and 16 patients with amputation. We use resting-state fMRI to investigate the local and extent brain plasticity in patients suffering from amputation simultaneously. Both the amplitude of low-frequency fluctuations (ALFF) and degree centrality (DC) were used for the assessment of neuroplasticity in central level.

Results: We described changes in spatial patterns of intrinsic brain activity and functional connectivity in amputees in the present study and we found that not only the sensory and motor cortex, but also the related brain regions involved in the functional plasticity after upper extremity deafferentation.

Conclusion: Our findings showed local and extensive cortical changes in the sensorimotor and cognitive-related brain regions, which may imply the dysfunction in not only sensory and motor function, but also sensorimotor integration and motor plan. The activation and intrinsic connectivity in the brain changed a lot showed correlation with the deafferentation status.

Background

Amputation in adults is a serious condition and most patients were associated with the remapping of representations in motor and sensory brain network. Alterations in the relative sensorimotor cortex in amputees have been investigated by using various electrophysiological and neuroimaging techniques [1–5]. Almost ninety percent of patients suffering from amputation described abnormal “phantom sensations”, which means that the patients feel the perception that the missing upper or lower extremity is still intact, with the abnormal phantom sensations like intractable pain [6–9]. According to the literature, phantom pain may correlate to a maladaptive plasticity in bilateral sensory and motor cortex [10–11]. However, the widely accepted theory that maladaptive plasticity is the cause of phantom pain in amputees has been challenged recently. An unusual increased activity in the affected sensorimotor brain regions of the amputated upper extremity was found to be responsible to the phantom pain [12–13]. These results may indicate that a more complex and multifactorial cause of the phantom pain was at play. Accordingly, cerebral plasticity has also been investigated in amputation patients without phantom pain [5, 14–15], and the results indicated that the alteration in primary somatosensory cortex were not consistently related to the pain symptoms [1].

Structural changes were also found in the patients suffering from amputation. The reduction of gray matter in amputated upper extremities were observed, as well as the white matter changes in the corpus callosum [12, 15]. Thus, previous studies showed both the functional and structure changes in amputee's brain and this may indicate a maladaptive functional reorganization after the deafferentation of the neural signal from the amputated extremity [16].
Nowadays, neuroimaging techniques such as functional magnetic resonance imaging, positron emission tomography have become the most useful tool to detecting the relate functional and anatomical changes in the brain. Different from the structural magnetic resonance imaging, functional magnetic resonance imaging detects changes in blood oxygenation (i.e., blood oxygen level–dependent signal) [17–18] because of small distortions in the magnetic field as a consequence of unbound iron to oxygen deoxyhemoglobin.

In the present study, we use resting-state fMRI to investigate the local and extent brain plasticity in amputation patients suffering simultaneously. Both the amplitude of low-frequency fluctuations (ALFF) and degree centrality (DC) were used for the assessment of central mechanisms.

**Methods**

**Participants**

The present study includes 8 healthy volunteers of 4 men and 2 women; mean age: 37.9 years old, and 16 patients with amputation (11 men and 5 women; mean age: 41.2 years old. All the patients were assessed by this study was approved by the Committee for Medical Ethics of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from all study participants.

**Data Preprocessing**

Data Preprocessing Functional images of each subject were preprocessed by using Data Processing Assistant for Resting-State fMRI (DPARSF) [19] which is based on Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm) and Resting-State fMRI Data Analysis Toolkit [20]. Functional images preprocessing was executed as follows:(1) the first ten volumes were removed to make subjects adapted to the scanning noise and to allow for scanner stabilization. And the number of time point is not less than 230. (2) slice scan time correction. (3) head movement correction. (4) spatial normalization: individual structural images of subjects were segmented to functional images after co-registered, and functional images were resampled to Montreal Neurological Institute (MNI) space (each voxel was resampled to 3*3*3mm3) applying the unified segmentation parameters. (5) nuisance variables including white matter (WM), head motion parameter measured by Friston-24 model and cerebrospinal fluid (CSF) signals were regressed out. (6) spatial smoothing using a 6 mm FWHM Gaussian kernel. (7) removal of linear trends.

**ALFF Calculation**

The amplitude of low frequency fluctuations (ALFF) is the blood oxygen level dependent (BOLD) signal of every single voxel [21]. ALFF calculation was identical with the study of Professor Zang [22–23]. ALFF was calculated for the traditional low frequency band (0.01–0.08 Hz).

**DC Calculation**
Degree centrality captures the relationship with the whole brain network in the voxel level and represents the node characteristic of large-scale brain intrinsic connectivity networks [24]. Compared to binary version, weighted DC provides a more precise centrality characterization of functional brain networks, therefore, we used weighted DC [25]. The Pearson correlation was performed between the time course of each voxel with that of every other voxel in the entire brain. The correlation coefficients with $r > 0.2$ were summed up for each voxel and then a weighted DC was obtained for each voxel. 0.2 was used as threshold to eliminate counting voxels that had low temporal correlation and it has been proved that different threshold selections did not qualitatively change the results [26]. Spatial smoothing (FWHM = 6 mm) was carried out after DC calculation, since spatial smoothing may lead into possible artificial local correlations.

\[
D = \sum a_{ij}
\]

Where $j = 1...N, i \neq j, a_{ij} = \begin{cases} 0, & a_{ij} < 0.2 \\ a_{ij}, & a_{ij} \geq 0.2 \end{cases}$

According to previous fMRI studies, we removed negative correlation [27]. Since the physiological basis of the negative correlations was uncertain, it was not calculated respectively [28–30].

**Statistical Analysis**

We compared ALFF and DC maps between patient group and control group. Two-sample t-tests were used on two groups separately. The results were corrected for multiple comparisons with a combined threshold of single voxel ($p < 0.05$) with GRF correction.

**Results**

**ALFF:**

In the comparison of ALFF value between amputation patients and control normal, we found that the ALFF value increased in Cingulate_Ant, ParaHippocampal, Hippocampus, Cingulate_Mid, Supp_Motor_Area, Insula, Putamen, Postcentral, Insula, Caudate, Hippocampus; decreased in Precuneus, Precentral, Occipital_Mid, Postcentral, Supp_Motor_Area, Paracentral_Lobule at 2 months.(Fig. 1, Table 1)
Table 1
Brain region information of ALFF comparison between amputation patients and control normal at 2 months:

| Contrast Name       | Region Label | Extent | t-value | x  | y  | z  |
|---------------------|--------------|--------|---------|----|----|----|
| Positive            | Cingulate_Ant_R | 100    | 3.295   | 3  | 27 | 0  |
|                     | ParaHippocampal_R | 49     | 4.258   | 18 | 0  | -21|
|                     | Hippocampus_L   | 189    | 4.257   | -21| -27| -12|
|                     | ParaHippocampal_L | 189   | 3.452   | -21| -9 | -30|
|                     | Supp_Motor/Area_L | 8      | 3.729   | -3 | 21 | 54 |
|                     | Insula_R        | 19     | 3.583   | 42 | -9 | -9 |
|                     | Putamen_L       | 34     | 3.489   | -27| -15| 3  |
|                     | Postcentral_L   | 11     | 3.414   | -63| -21| 18 |
|                     | Putamen_R       | 16     | 3.334   | 27 | 3  | -3 |
| Negative            | Precuneus_R     | 51     | -4.492  | 6  | -66| 54 |
|                     | Precentral_R    | 97     | -4.332  | 42 | -12| 57 |
|                     | Precentral_L    | 51     | -4.128  | -27| 0  | 60 |
|                     | Postcentral_R   | 44     | -3.836  | 27 | -42| 63 |
|                     | Postcentral_L   | 19     | -3.758  | -21| -45| 69 |
|                     | Supp_Motor/Area_R | 42    | -3.705  | 3  | -15| 72 |

The ALFF value increased in Putamen, Caudate, Hippocampus, Supp_Motor_Area, Lingual, ParaHippocampal, Precuneus, Cingulate_Mid, Cingulate_Ant, Parietal_Inf, Caudate; decreased in
Paracentral_Lobule, Supp_Motor_Area, Precentral, Cuneus, Precuneus, Fusiform, Postcentral at 6 months. (Fig. 2, Table 2)

Table 2
Brain region information of ALFF comparison between amputation patients and control normal at 6 months:

| Contrast Name | Region Label          | Extent | t-value | x   | y   | z   |
|---------------|-----------------------|--------|---------|-----|-----|-----|
| Positive      | Putamen_L             | 88     | 4.993   | -21 | -3  | 3   |
|               | Caudate_R             | 17     | 3.924   | 15  | 15  | 3   |
|               | Hippocampus_L         | 36     | 3.804   | -30 | -21 | -21 |
|               | Supp_Motor_Area_L     | 10     | 3.521   | -3  | 24  | 54  |
|               | Putamen_R             | 11     | 3.386   | 24  | 6   | -6  |
| Negative      | Paracentral_Lobule_L  | 903    | -6.797  | -15 | -36 | 63  |
|               | Supp_Motor_Area_R     | 903    | -5.979  | 6   | -6  | 60  |
|               | Precentral_R          | 30     | -5.233  | 48  | -3  | 42  |
|               | Lingual_R             | 428    | -5.184  | 6   | -72 | -9  |
|               | Cuneus_R              | 428    | -4.449  | 9   | -81 | 24  |
|               | Precuneus_R           | 86     | -5.020  | 15  | -66 | 39  |
|               | Fusiform_R            | 47     | -3.948  | 24  | 9   | -45 |
|               | Temporal_Inf_R        | 27     | -3.210  | 42  | -57 | -9  |

The ALFF value increased in Cingulate_Ant, Cingulate_Mid, Caudate, Precuneus, Supp_Motor_Area, Frontal_Sup_Media, Insula, Thalamus, Putamen; decreased in Precentral, Postcentral, Supp_Motor_Area, Precuneus, Paracentral_Lobule. (Fig. 3, Table 3)
| Contrast Name       | Region Label         | Extent | t-value | x  | y  | z   |
|---------------------|----------------------|--------|---------|----|----|-----|
| Positive            | Cingulate_Mid_R      | 30     | 6.424   | 12 | -33| 42  |
|                     | Caudate_R            | 18     | 5.593   | 21 | -3 | 18  |
|                     | Precuneus_L          | 28     | 4.674   | -9 | -69| 60  |
|                     | Caudate_R            | 202    | 3.910   | 15 | 12 | 3   |
|                     | Rectus_L             | 202    | 3.835   | 3  | 30 | -18 |
|                     | Pallidum_L           | 25     | 4.531   | -21| -3 | 0   |
|                     | Supp_Motor/Area_L    | 16     | 4.509   | -3 | 21 | 54  |
|                     | Cingulate_Mid_L      | 95     | 4.198   | -12| -42| 42  |
|                     | Hippocampus_R        | 14     | 4.155   | 36 | -30| 6   |
|                     | Frontal_Sup_Medial_R | 41     | 3.936   | 6  | 57 | 7   |
|                     | Insula_R             | 10     | 3.873   | 39 | -18| 0   |
|                     | Cingulate_Ant_R      | 69     | 3.100   | 3  | 18 | 21  |
|                     | Thalamus_R           | 30     | 3.805   | 15 | -9 | 3   |
|                     | Occipital_Mid_R      | 24     | 3.614   | 51 | -66| 24  |
|                     | Putamen_L            | 12     | 3.368   | -27| -15| 3   |
|                     | ParaHippocampal_L    | 32     | 3.362   | -27| -39| -12 |
| Negative            | Precentral_R         | 29     | -6.454  | 30 | -21| 63  |
|                     | Lingual_R            | 476    | -6.267  | 18 | -63| -6  |
| Contrast Name                  | MNI Coordinates |
|-------------------------------|-----------------|
| Postcentral _R               | 41 -6.032       |
| Supp_Motor_Area_R            | 25 -5.477       |
| Fusiform_R                   | 102 -5.381      |
| Lingual_L                    | 67 -3.192       |
| Precuneus_ R                 | 55 -4.646       |
| Precentral_ L                | 26 -4.588       |
| Paracentral _Lobule_L        | 13 -3.299       |

In the comparison of DC between amputation patients and control normal, we found that the DC value increased in Rolandic_Oper, Postcentral, Calcarine, Parietal, Cingulate_Mid, Frontal_Med_Orb, Frontal_Sup_Medial; decreased in Precuneus, ParaHippocampal, Caudate, Frontal_Inf_Tri, Precentral, Postcentral at 2months. (Fig. 4, Table 4)
Table 4
Brain region information of DC comparison between amputation patients and control normal at 2 months:

| Contrast Name | Region Label       | Extent | MNI Coordinates |
|---------------|--------------------|--------|-----------------|
|               |                    |        | x   y   z       |
| Positive      | Rolandic_Oper_L    | 11     | 4.459 63 3 12   |
|               | Postcentral_L      | 10     | 3.603 63 21 27  |
|               | Cingulate_Mid_L    | 27     | 3.373 6 12 39   |
|               | Frontal_Med_Orb_R  | 12     | 3.290 9 42 -12  |
| Negative      | Precuneus_R        | 105    | -4.736 15 -48 21|
|               | Precuneus_R        | 105    | -3.788 18 -39 -6|
|               | Caudate_R          | 23     | -4.711 21 24 -6|
|               | Occipital_Mid_L    | 18     | -4.159 27 -78 0 |
|               | Frontal_Inf_Tri_R  | 10     | -3.876 51 24 6  |
|               | Precuneus_L        | 23     | -3.808 -9 -60 15|
|               | Precentral_R       | 10     | -3.426 60 9 15  |

The DC value increased in Cuneus, Calcarine, Postcentral, Parietal_Inf, ParaHippocampal; decreased in Cingulate_Mid, Precuneus, Hippocampus, Precuneus, ParaHippocampal, Caudate, Postcentral at 6 months. (Fig. 5, Table 5)
Table 5
Brain region information of DC comparison between amputation patients and control normal at 6 months:

| Contrast Name | Region Label   | Extent | t-value | x   | y   | z   |
|---------------|----------------|--------|---------|-----|-----|-----|
| Positive      | Cuneus_L       | 19     | 6.229   | 0   | -90 | 33  |
|               | Calcarine_R    | 103    | 5.182   | 18  | -99 | 0   |
|               | Postcentral_L  | 9      | 4.074   | -63 | -21 | 24  |
|               | ParaHippocampal_L | 14   | 3.290   | -18 | 3   | -33 |
|               | Postcentral_R  | 6      | 3.138   | 45  | -42 | 63  |
| Negative      | Cingulate_Mid_R | 14    | -5.296  | 12  | -6  | 39  |
|               | Precuneus_R    | 31     | -4.937  | 21  | -51 | 18  |
|               | Hippocampus_R  | 15     | -4.685  | 27  | -21 | -15 |
|               | Precuneus_L    | 15     | -4.310  | -3  | -54 | 69  |
|               | ParaHippocampal_R | 27  | -4.193  | 21  | -39 | -6  |
|               | Caudate_R      | 5      | -4.040  | 21  | 24  | -6  |
|               | Cingulate_Mid_L | 11    | -3.602  | -12 | -33 | 42  |
|               | Postcentral_L  | 6      | -3.025  | -30 | -33 | 54  |

The DC value increased in Lingual, Cuneus, Caudate, Fusiform, Postcentral, Frontal_Med_Or; decreased in ParaHippocampal, Precuneus, Cingulate_Post, Precuneus, Frontal_Inf_Tri, Frontal_Inf_Oper, Frontal_Med_Orb, Fusiform, Lingual, Putamen, Lingual at 12 months. (Fig. 6, Table 6)
Table 6
Brain region information of DC comparison between amputation patients and control normal at 12 months:

| Contrast Name | Region Label | Extent | t-value | x    | y    | z    |
|---------------|--------------|--------|---------|------|------|------|
| Positive      | Lingual_R    | 188    | 6.163   | 24   | -93  | -15  |
|               | Cuneus_R     | 188    | 5.813   | 18   | -99  | 9    |
|               | Caudate_L    | 12     | 4.725   | -12  | -3   | 15   |
|               | Fusiform_L   | 23     | 4.308   | -21  | 3    | -45  |
|               | Postcentral_L | 10    | 4.030   | -21  | -30  | 78   |
|               | Parietal.Inf_R | 21  | 3.916   | 45   | -39  | 54   |
| Negative      | Parahippo. campal_R | 162 | -8.290  | 21   | -39  | -6   |
|               | Precuneus_ R  | 162    | -4.969  | 12   | -63  | 24   |
|               | Cingulate_ Post_L  | 56  | -6.593  | -6   | -39  | 12   |
|               | Precuneus_ L  | 25     | -5.595  | -6   | -60  | 15   |
|               | Frontal.Inf_ Tri_R | 16  | -5.115  | 51   | 24   | 6    |
|               | Fusiform_R   | 47     | -4.827  | 39   | -33  | -18  |
|               | Frontal.Inf_ Oper_R | 20   | -4.427  | 36   | 6    | 27   |
|               | Putamen_R    | 12     | -3.446  | 30   | -6   | 6    |

Discussion
In the present study, we described changes in spatial patterns of intrinsic brain activity and functional connectivity in amputees. We found that not only the sensory and motor cortex, but also the related brain regions involved in the functional plasticity after upper extremity deafferentation.
As a drastic upper extremity injury, amputation in human beings may change the primary motor cortex and primary sensory cortex of the deafferent hemisphere. The sensory and motor brain networks of the human brain are somatotopically organized. Denervation that is due to amputation or nerve injure breaks up normal sensorimotor function. It is reported that cortical reorganization in the sensorimotor area where intact body parts ‘invade’ areas associated with the missing limb is appeared subsequently in numerous animal studies [31–33].

Similarly, sensorimotor reorganization is found in some transcranial magnetic stimulation studies in amputees, in their studies, increased excitability of motor areas contralateral to the amputated limb is described, where stump muscles demonstrate higher response amplitudes that can be induced from a larger scalp area than responses in the intact arm [34, 35]. In addition, a shift of lip [36], chin [37], and shoulder [38] representation into the deafferented cortical hand area has been reported in magnetoencephalography (MEG) and functional MRI studies with upper limb amputees.

Denervation does not lead in a complete loss of the affected limb representation, since the sensorimotor cortex still seem to be working on so-called ‘attempted movement’. Therefore, when the amputees try to move their phantom limb, the corresponding sensorimotor areas present functional MRI activation which is similar to executed movements in able-bodied subjects [36, 39–40].

It has been shown that, meanwhile, the persistent representation is relatively detailed for postcentral and parietal regions. For example, trajectories and movement goals have been successfully decoded from posterior parietal cortex by intracranial recordings in a tetraplegic patient [41]. A persistent hand representation in S1 was also reported in a long-term spinal cord injury patient by micro-stimulation [42]. And an individual finger topography of the phantom hand has been shown in the somatosensory cortex in amputees [43].

ALFF measures the amplitude of time series fluctuation at each voxel and DC represents the large-scale brain intrinsic connectivity in the voxel level. These measures of fMRI probe into the brain activity from different aspects. In our investigation, we found that there is an overlapping between ALFF and Degree Centrality, which mainly located in the Frontal_Sup_L. This area showed correlation with the depression status of the patients.

ALFF measures the amplitude of time series fluctuation at each voxel. We found that the primary motor cortex and primary sensory cortex decreased at 2, 6, 12 months after the amputation. Meanwhile, the brain regions of sensorimotor integration included the putamen, caudate and precuneus were increased, which could be the compensation pattern. DC represents the large-scale brain intrinsic connectivity in the voxel level. The results showed that DC value decreased in precuneus, caudate and post central gyrus, which may indicate that the sensorimotor integration function impaired. In light of these clinical results, we found that the amputation patients showed significant brain function alteration in the local and extensive brain regions.
Conclusion

Our findings showed local and extensive cortical changes in the sensorimotor and cognitive-related brain regions, which may imply the dysfunction in not only sensory and motor function, but also sensorimotor integration and motor plan. The activation and intrinsic connectivity in the brain changed a lot showed correlation with the deafferentation status. Further studies are needed to assess the brain networks of sensorimotor network and emotion network in the larger cohort studies.

Abbreviations

ALFF: amplitude of low-frequency fluctuations; DC: degree centrality; MNI: montreal neurological institute; WM: white matter; CSF: cerebrospinal fluid.

Declarations

Abbreviations: ALFF: amplitude of low-frequency fluctuations; DC: degree centrality; MNI: montreal neurological institute; WM: white matter; CSF: cerebrospinal fluid.

Ethics approval and consent to participate: This study was approved by the Committee for Medical Ethics of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All participants or families of amputations provided informed consent before participation.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Xianyou Zheng was responsible for study design and manuscript writing. Bingbo Bao and Xuyun Hua were responsible for data collection and analysis. Bingbo Bao was responsible for the revision of the manuscript. Bingbo Bao and Xuyun Hua contributed equally to this manuscript. All the authors critically reviewed the content of the manuscript. All authors read and approved the final manuscript.

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**Figures**
Figure 1

ALFF comparison between amputation patients and control normal at 2 months
Figure 2

ALFF comparison between amputation patients and control normal at 6 months
Figure 3

ALFF comparison between amputation patients and control normal at 12 months.
Figure 4

DC comparison between amputation patients and control normal at 2 months.
Figure 5

DC comparison between amputation patients and control normal at 6 months.
Figure 6

DC comparison between amputation patients and control normal at 12 months.