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 previous studies suggested a remapping of representations in motor and sensory brain networks. However, little is known about the longitudinal reorganizing pattern in upper limb amputees' patients.

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

Results: We described changes in spatial patterns of intrinsic brain activity and functional connectivity in amputees; and we found that not only the sensory and motor cortex, but also the cognitive-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 changes in activation and intrinsic connectivity in the brain showed correlation with the deafferentation status.

Background

Amputation in adults is a serious condition and previous studies suggested a remapping of representations in motor and sensory brain network. Alterations in the 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 "phantom sensations", which means that the patients can still perceive the missing upper or lower extremities and, in some cases, experience severe pain from the missing limb [6-9]. According to the literature, phantom pain may correlate to a maladaptive plasticity in sensorimotor network in the brain [10-11]. However, the widely accepted theory that maladaptive plasticity is the cause of phantom pain 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 amputee 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 and positron emission tomography have become the most useful tools to detect 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), for assessing the functional alteration in specific brain regions [17-18].

In the present study, we use resting-state fMRI to investigate the local and global-scale brain plasticity in amputee patients suffering. 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 procedures used in this study were 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 calculated from the blood oxygen level dependent (BOLD) time course at each 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.

$$D = \sum a_{ij}$$

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

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

Two-sample t-tests were used on the two measures (ALFF and DC) 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. (Figure 1, Table 1)

Table 1: Brain region information of ALFF comparison between amputation patients and control normal at 2 months:

Contrast Name				MNI Coordinates		
	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. (Figure 2, Table 2)

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

Contrast Name				MNI Coordinates		
	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. (Figure 3, Table 3)

Table 3: Brain region information of ALFF comparison between amputation patients and control normal at 12 months:

Contrast Name				MNI Coordinates		
	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	27
	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
	Postcentral_R	41	-6.032	27	-42	63
	Supp_Motor_Area_R	25	-5.477	3	-9	57
	Fusiform_R	102	-5.381	24	3	-45
	Lingual_L	67	-3.192	-12	-75	-6
	Precuneus_R	55	-4.646	24	-45	6
	Precentral_L	26	-4.588	-21	-18	63
	Paracentral_Lobule_L	13	-3.299	-18	-30	66

DC:

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. (Figure 4, Table 4)

Table 4: Brain region information of DC comparison between amputation patients and control normal at 2 months:

Contrast Name				MNI Coordinates		
	Region Label	Extent	t-value	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
	ParaHippocampal_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. (Figure 5, Table 5)

Table 5: Brain region information of DC comparison between amputation patients and control normal at 6 months:

Contrast Name				MNI Coordinates		
	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. (Figure 6, Table 6)

Table 6: Brain region information of DC comparison between amputation patients and control normal at 12 months:

Contrast Name				MNI Coordinates		
	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	ParaHippocampal_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 could 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 injury 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 measurements of fMRI probe into the brain function 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 may correlate with the depression status of the patients.

ALFF measures the amplitude of time series fluctuation at each voxel. We found that the ALFF value in 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

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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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.