Altered intra- and inter-network brain functional connectivity in upper-limb amputees revealed through independent component analysis

Introduction

The human brain continuously adapts to physical and environmental changes, ranging from small local functional changes to large structural changes (Edde et al., 2021). It is also now clear that the occurrence and development of many diseases are accompanied by changes in brain function and brain structure. Therefore, understanding the processes underlying disease-related changes in brain function is not only useful for understanding a disease, but also for identifying new diagnostic criteria, prevention strategies, and treatment methods for diseases that manifest in the brain (Heneka et al., 2018; Barth and Ray, 2019; Hortobágyi et al., 2021).

Amputation is a treatment option for severe limb trauma, limb tumor, or limb vascular disease (Qureshi et al., 2020). Although amputations can help patients survive, amputees still face many complications, including phantom limb sensation, phantom limb pain (PLP), and residual limb pain (RLP) (Stankevicius et al., 2021). In addition, many amputees experience varying degrees of mood disorders, including anxiety and depression, which can also interfere with their ability to return to work and engage in social activities (McKechnie and John, 2014). From a lay point of view, it may seem that amputation “cures” the patient’s disease. However, amputation and the resulting limb denervation inevitably lead to local or global changes in brain function, which may manifest as pain and/or changes in emotion and cognition, for example (Makin and Flor, 2020). Therefore, amputees are faced not only with the loss of the limb itself, but also with changes in brain function caused by the loss of that limb.

Various imaging techniques, such as functional magnetic resonance imaging (fMRI), electroencephalography, magnetoencephalography, and positron emission computed tomography, have been used to assess brain function abnormalities after amputation (Collins et al., 2018). One widely used, noninvasive method for assessing the function of regional and local neural...
In the present study, we quantified large-scale functional reorganization in the brains of ULAs by using ICA-based RSN analysis methods and by investigating intra-network and inter-network connectivity. We hypothesized that intra-network and inter-network FC is appreciably altered in ULAs compared with that in healthy controls (HCs). Our aim was to determine whether changes in intra-network and inter-network resting-state connectivity underlie neurobehavioral symptoms experienced by ULAs, in particular PLP. We also investigated correlations between clinical behavioral measures and altered resting-state FC. To our knowledge, this rs-ICA study in ULAs is the first of its kind to use an ICA-based approach to elucidate the mechanisms underlying PLP.

Participants and Methods

Participants
Participants in the ULA group comprised 40 individuals (32 male and 8 female; mean age ± SD: 45.23 ± 9.05 years) that had undergone unilateral upper-limb amputation (22 patients with dominant right-side amputations) at the Shanghai Jiao Tong University Affiliated Sixth People’s Hospital between October 2020 and May 2021. Of the 40 amputations, 23 were below the elbow, and 17 were above the elbow. All potential participants were screened according to the following inclusion criteria: (1) aged 18–60 years (this age setting was determined by our trial design, which took into account the potential limitations of brain remodeling in older subjects); and (2) unilateral upper-limb amputation due to traumatic injury. The exclusion criteria were as follows: (1) upper-limb amputation accompanied by other limb injuries; (2) history of neurological disease; (3) pre-existing psychiatric or neurological disorders; (4) history of using psychotropic drugs; (5) left-handed; and (6) history of neurological disease; (3) pre-existing psychiatric or neurological disorders; (4) history of using psychotropic drugs; (5) left-handed; and (6) history of neurological disease.

Independent component analysis
Participants’ data were analyzed by ICA using GIFT software (Version 4.0b, http://mialab.mrn.org/software/gift/). GIFT software automatically estimates the number of independent components (N = 23) using the minimum description length criteria. The data are then broken down by spatial ICA into mixtures of components that are spatially independent and that display unique time course profiles. In the present study, we accomplished this decomposition by reducing the data in two steps. First, subject-specific data were reduced into 39 principal components using principal component analysis, and these components were linked (concatenated) across subjects and then reduced (decomposed) further into 23 independent components using the infomax algorithm. We repeated the infomax algorithm 100 times in ICASSO (http://research.isi.tkk.fi/ica/casso/) to ensure estimate stability, and then selected the most stable run for further analysis. Second, we applied the group ICA back-reconstruction algorithm to compile spatial maps and time courses for each participant. From these analyses, we were able to identify several independent components, i.e., functional components that peak activations in gray matter; low spatial overlap with known artifacts (e.g., vascular, motion, susceptibility); and mainly low-frequency power. This method identified 11 functional networks from the 23 independent components derived from the second decomposition step.

Data analysis
Analysis of demographic and clinical characteristics
After determining that our data were normally distributed, we evaluated demographic and clinical data of ULAs and HCs using two-tailed two-sample t-tests and chi-square test (only the sex variable was tested) (SPSS 24.0; IBM, Armonk, NY, USA). P < 0.05 was designated as significant.

Analysis of intra-network functional connectivity
We created a component map and a network mask for each subject to adapt to the scanner environment. Second, we manually set the anterior commissure as a reference point, and then aligned the remaining 230 images of each subject with their anatomical data sets. Third, to correct for head motion during scanning, we calculated the subject’s head motion parameters by estimating the translation in x, y, and z directions and the angular rotation at each axis for each volume. We excluded data from subjects whose heads moved > 2.0 mm in any direction and rotated > 2.0° (Yang et al., 2014). Fourth, we normalized each subject’s structural images with their mean functional image, which was divided into gray matter, white matter, and cerebrospinal fluid. Fifth, we spatially normalized the functional images according to the Montreal Neurological Institute (MNI) space-coordinate system using the deformation parameters estimated above, and then resampled them into a 3-mm cubic voxel. Finally, to decrease spatial noise, we spatially normalized and smoothed all data sets using the full width at half maximum of 6 mm of the Gaussian kernel.

Ethical approval and informed consent
Ethical approval for the study was obtained from the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital of China (approval No. 2017-034), registered with the Chinese Clinical Trial Registry (registration no. ChiCTR1900025882), and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave informed consent prior to inclusion in this study.

MRI scanning and image acquisition
All images were acquired using a Siemens 3.0-Tesla MRI scanner (MAGNETOM Prisma; Siemens Healthcare GmbH, Erlangen, Germany) equipped with a 64-channel phased-array head coil by a senior radiologist in our hospital. During image acquisition, subjects were instructed to relax but remain awake, to close their eyes during the entire scanning period, and to try not to think of anything specific. To reduce head movement artifacts, the subject’s head was placed in a padded foam collar, and the subject wore earplugs to minimize loud startling noise from the scanner during image acquisition. A three-dimensional magnetization-prepared, rapid gradient echo sequence was used to record structural images following the following acquisition parameters: repetition time = 2300 ms; echo time = 2.46 ms; flip angle = 8°; matrix = 256 × 256 mm2; slice thickness = 1.0 mm; voxel size = 1.0 × 1.0 × 1.0 mm3. A total of 176 slices were acquired for each subject session. An echo-planar imaging sequence was used to record functional images with the following acquisition parameters: repetition time = 1200 ms; echo time = 39 ms; flip angle = 52°; matrix = 88 × 88; field of view = 210 mm; slice thickness = 2.4 mm; 56 slices, voxel size = 2.4 mm × 2.4 mm × 3.0 mm. After a scanning time of 288 seconds, we detected 240 volumes. None of the subjects mentioned experiencing any significant discomfort during or after scanning. The radiologist was well aware of the trial, but not of the patient’s details, such as the timing of the amputation or the degree of pain.

Data preprocessing
Preprocessing was performed using Statistical Parametric Mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB R2013b (MathWorks Inc., Natick, MA, USA). Data preprocessing comprised six steps. First, we discarded the first 10 volumes of each subject’s data set; this number was sufficient to allow the scanner to reach magnetization equilibrium and for the subject to adapt to the scanner environment. Second, we manually set the anterior commissure as a reference point, and then aligned the remaining 230 images of each subject with their anatomical data sets. Third, to correct for head motion during scanning, we calculated the subject’s head motion parameters by estimating the translation in x, y, and z directions and the angular rotation at each axis for each volume. We excluded data from subjects whose heads moved > 2.0 mm in any direction and rotated > 2.0° (Yang et al., 2014). Fourth, we normalized each subject’s structural images with their mean functional image, which was divided into gray matter, white matter, and cerebrospinal fluid. Fifth, we spatially normalized the functional images according to the Montreal Neurological Institute (MNI) space-coordinate system using the deformation parameters estimated above, and then resampled them into a 3-mm cubic voxel. Finally, to decrease spatial noise, we spatially normalized and smoothed all data sets using the full width at half maximum of 6 mm of the Gaussian kernel.
of each pair of the 11 RSNs to compute the temporal correlation, namely functional network connectivity. The resulting functional network connectivity
correlation results were then normalized using the Fisher r-to-z transformation to
maintain a normal distribution and obtain an 11 × 11 matrix inter-network FC within
RSNs. Finally, to evaluate functional network connectivity differences between
ULAs and HCs, we subjected the matrix to a two-sample t-test; FDR (P < 0.05)
was used to correct the multiple comparison results.

\textbf{Correlation analysis}
Correlation analyses were conducted to determine and quantify the
relationship between intra-network FC, inter-network FC, and clinical variables.
We used the following data in our analyses: intra-network FC, inter-
network FC, RLP scores, PLP scores, amputation location, and ULAs’
daily activity hours. After controlling for certain variables (e.g., age, sex,
educational level, and amputation time), we performed a two-tailed partial
correlation analysis; P < 0.05 was considered statistically significant.

\textbf{Results}

\textbf{Clinical and demographic characteristics of participants}
Clinical and demographic clinical characteristics of participants (ULAs and
HCs) are shown in Table 1. Statistical analysis of participants’ clinical and
demographic data failed to identify any significant between-group differences
in age, sex, or education (P > 0.05).

\textbf{Resting-state network components}
We identified various RSN components in line with previously described
methods (Smith et al., 2009). The following 11 independent components
were identified: anterior default mode network, posterior default mode
network (aDMN); dorsal attention network (DAN); ventral sensorimotor network (vSMN);
left frontoparietal network, right frontoparietal network (rFPN); and medial
visual network, posterior visual network, auditory network (Figure 1).

\textbf{Altered FC within resting-state networks}
In ULAs, multiple RSNs displayed significant FC differences compared with
those in HCs (P < 0.001, AlphaSim-corrected; Figure 2 and Table 2). In the
DAN, the left precuneus (precuneus gyrus) showed decreased FC. In the
vSMN, the left Parietal_Infer (inferior parietal, but supramarginal and angular
gyrus) showed increased FC. In the ventral attention network, the right
Cerebelum_Crus2 (crus II of cerebellum) and left Temporal_Mid (middle
temporal gyrus) showed increased FC. In auditory network, the left precentral
(precentral gyrus) showed decreased FC, but the left Rolandic_Oper (rolandic
operculum) showed increased FC.

\textbf{Altered inter-network functional connectivity}
In ULAs, the three following pairs of networks showed decreased inter-
network FC compared with that in HCs: dSMN and vSMN, dSMN and rFPN,
and dSMN and DAN (P < 0.05, FDR-corrected; Figure 3).

\textbf{Correlation analysis results}
In the ULA group, dSMN-vSMN FC and RLP scores were negatively correlated
(r = –0.655, P < 0.001; Figure 4A), and dSMN–vSMN FC and PLP scores were
negatively correlated (r = –0.695, P < 0.001; Figure 4B). In addition, in the
ULA group, dSMN-vSMN FC and daily activity hours of the stump limb were
positively correlated (r = 0.718, P < 0.001; Figure 4C).

Table 1 | Demographic and clinical characteristics of participants

| Characteristics | ULA (n = 40) | HC (n = 40) | t/\text{P}^{|P|} |
|-----------------|-------------|-------------|-----------------|
| Age (years)     | 45.23 ± 9.05 | 47.23 ± 8.84 | 1 0.768 |
| Education (years) | 7.85 ± 4.73 | 7.98 ± 4.53 | 0.121 0.703 |
| Sex (female/male) | 8/32 | 11/29 | 0.623 0.6 |
| Hand dominance (left/right) | 0/40 | 0/40 | – |
| Amputation side (left/right) | 18/22 | – | – |
| Amputation location: above/below elbow | 17/23 | – | – |
| Time since amputation (months) | 73.85 ± 24.46 | – | – |
| Age at amputation (years) | 39.35 ± 9.52 | – | – |
| Peak T value | (21–59) | – | – |
| RLP scores | 4.58 ± 2.46 | – | – |
| PLP scores | 5.23 ± 2.76 | – | – |
| Stump limb daily activity hours | 4.40 ± 3.05 | – | – |

Values are expressed as the mean ± SD, mean ± SD (range) or number/number. HC: Healthy control; PLP: phantom limb pain; RLP: residual limb pain; ULA: upper limb amputee.

Table 2 | Brain regions with significant differences in intra-network FC between ULAs and HCs

| Regions | Side | Peak T value | Cluster size | X | Y | Z |
|---------|------|-------------|--------------|----|----|----|
| DAN     | L    | –3.951      | 19           | –12 | –66 | 51 |
| Precuneus | L    | –4.211      | 16           | –51 | –39 | 45 |
| vSMN    | L    | –3.951      | 19           | –60 | –15 | 0  |
| Parietal_Infer | L    | –4.211      | 20           | –51 | –39 | 45 |
| VAN     | R    | –4.309      | 20           | –57 | 3   | 6  |
| Cerebelum_Crus2 | L    | –4.543      | 24           | –36 | –9  | 54 |
| Temporal_Mid | L    | –4.543      | 24           | –36 | –9  | 54 |
| AN      | L    | –4.543      | 24           | –36 | –9  | 54 |
| Rolandic_Oper | R    | –4.543      | 24           | –36 | –9  | 54 |

\(n = 40\) in ULA and HC, respectively. AN: Auditory network; Crus2: crus II of cerebellum; DAN: dorsal attention network; L: left; MID: middle temporal gyrus; VAN: ventral attention network; vSMN: ventral sensorimotor network.

Figure 1 | Spatial maps of 11 selected functional networks in upper-limb amputees and healthy controls using independent component analysis.
The colored bars indicate the t-values of the one-sample t-test of all subjects (P < 0.05, false discovery rate-corrected). dSMN: Anterior default mode network; AN: auditory network; DAN: dorsal attention network; dSMN: dorsal sensorimotor network; rFPN: left frontoparietal network; L: left; mVN: medial visual network; pDMN: posterior default mode network; pVN: posterior visual network; R: right; fFPN: right frontoparietal network; VAN: ventral attention network; vSMN: ventral sensorimotor network.
Brain regions with significant differences in intra-network FC between upper-limb amputees and healthy controls by two-sample t-tests

Warm and cold colors indicate regions with higher or lower intra-network FC in upper-limb amputees compared with healthy controls ($P < 0.001$, AlphaSim-corrected). The colored bars indicate the $t$-values of the two-sample $t$-tests. AN: Auditory network; DAN: dorsal attention network; FC: functional connectivity; VAN: ventral attention network; vSMN: ventral sensorimotor network.

Figure 2

A

Heat map showing inter-network FC between pairs of regions in upper-limb amputees. Warm and cold colors indicate regions with higher or lower inter-network FC. (B) Three-dimensional renderings of the brain showing the statistical significance networks of inter-network FC. FC was decreased between the dSMN and the vSMN, rFPN, and DAN ($P < 0.05$, FDR-corrected). (C) Dot plots showing inter-network FC in healthy controls (HCs) and upper-limb amputees (ULAs) using two-sample t-tests. dDMN: Anterior default mode network; AN: auditory network; DAN: dorsal attention network; dSMN: dorsal sensorimotor network; FC: functional connectivity; FDR: false discovery rate; IFPN: left frontoparietal network; mVN: medial visual network; pDMN: posterior default mode network; pVN: posterior visual network; rFPN: right frontoparietal network; vSMN: ventral sensorimotor network.

Discussion

To our knowledge, this is the first exploratory study to analyze intra- and inter-network FC and their correlations in ULAs. Depending on the ICA approach, in ULAs, we found that 4 of the 11 well-known RSNs were abnormal, including attention, sensorimotor, and auditory networks. At the brain-network level in ULAs, we observed decreased inter-network FC in sensorimotor and high attention networks. Correlation analysis revealed that this decrease in brain-network FC was correlated with certain clinical manifestations, such as the ULAs’ RLP scores, PLP scores, and daily activity hours of the stump limb. Together, these findings support our hypothesis that intra-network and inter-network FC in ULAs is altered compared with that in HCs. The present findings also enhance and clarify our current understanding of the processes involved in pathophysiological changes that occur following amputation.

Most importantly, we found that amputation induces reorganization in sensorimotor networks, which disrupts related networks that underlie sensorimotor behavior. This supports the notion that there is a common mechanism underlying network FC alternations following amputations.

A large percentage of limb amputees suffer from PLP and phantom limb sensation after amputation (Sherman et al., 1984), which considerably impairs their quality of life. Of 2750 respondents in a survey of military veteran amputees, 78% reported PLP (Limakatso et al., 2020). In a systematic review of all-cause amputations, a PLP prevalence rate of 64% was estimated (Limakatso et al., 2020; Wheaton, 2017). Our correlation results may provide a starting point; further research is needed to determine whether active stump activity maintains FC in sensorimotor areas.
The brain as a whole can be viewed as comprising functional networks that interact continuously to maintain complex behavioral routines. Similarly, brain functional changes after amputation are not confined to local circumscribed brain areas that correspond to the amputated limb. Recent animal and human studies have shown that sensorimotor areas or networks are coupled to other brain regions or networks. This can explain many of the clinical manifestations after amputation, including pain (Kikkeri et al., 2018), emotional impairment (Armstrong et al., 2019), and social impairment (Makin and Flor, 2020). We found a decrease in fC between the dSMN and rFPN, as well as the DAN. The rFPN has been shown to be important in pain processing, somatosensory perception, and activity inhibition, as well as working memory and cognition (Corbetta and Shulman, 2011). The DAN is also called the dorsal attention network, and underlies hierarchical attention orientation (Vossel et al., 2014). As our knowledge of brain plasticity after amputation deepens, an increasing number of studies have proposed new therapeutic strategies for improving remapping processes to help alleviate postoperative complications, including mirror therapy (Wang et al., 2021), virtual reality (Ambrón et al., 2021), and transcranial magnetic stimulation (Pacheco-Barrios et al., 2020).

Plasticity-based therapy has been found to alleviate pain for amputees to some extent; however, the mechanism underlying plasticity-based therapy remains unclear. In the present study, the sensorimotor networks were isolated by ICA of fMRI signals. Significant changes in functional connections were found between the vSMN and dSMN, which further demonstrates the diversity of brain remodeling after amputation. This new understanding has potential implications for neurorehabilitation. Clearly, for the rehabilitation of amputees, we not only need to pay attention to the treatment of local cortical areas that have become deafferented by the trauma, but we also need to consider that abnormal changes may occur in brain networks that are distant to the locally affected area. This new understanding of global network changes could inform more comprehensive strategies for amputee rehabilitation. For example, stimulating cortical regions that have become remote due to plastic alterations can exacerbate functional changes after amputation and may also play a role in the recovery of amputees.

The present study has some limitations. First, the sample of ULA subjects was small, because it is difficult to recruit these kinds of patients. In future studies, we aim to recruit more ULA subjects to more reliably control for the time since and level of amputation. Second, the sample size may be affected by bias because the ULAs volunteered for the trial; thus, these amputees might have had more pronounced clinical manifestations and were willing to come to the hospital for treatment solutions. Third, to better understand the pathophysiologic processes that underlie long-term functional changes in multiple networks after an amputation, we have not examined the cross-sectional design of this study means that we were unable to do so. Last, the plasticity process is affected by many factors, including time since amputation and prosthesis use, the effects of which cannot be affected based on the current data. Given these limitations, further studies of upper-limb amputations need to be conducted with larger groups of subjects and over longer periods of time.

Conclusion
Brain plasticity in ULAs is not restricted to local remapping; rather, plasticity occurs at the network level. Inter-network FC alterations are not only observed in the sensorimotor network but also occur in other brain networks following upper-limb amputation. These findings shed further light on brain plasticity after upper-limb amputation and the mechanisms underlying post-amputation pain.

Author contributions: XYZ was responsible for study design and manuscript revision. BBB, HWF, and HYZ were responsible for data collection and analysis. BBB, JL, BZW, and YHL were responsible for fMRI data collection. XYH and MXZ were responsible for the interpretation of the data. All the authors critically reviewed, read, and approved the final manuscript.

Conflicts of interest: The authors declare that they have no conflicts of interests.

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