Structural Damage and Functional Reorganization in Ipsilesional M1 in Well-Recovered Patients With Subcortical Stroke

Jing Zhang, MD; Liangliang Meng, MS; Wen Qin, PhD; Ningning Liu, MS; Fu-Dong Shi, MD; Chunshui Yu, MD

Background and Purpose—Both structural atrophy and functional reorganization of the primary motor cortex (M1) have been reported in patients with subcortical infarctions affecting the motor pathway. However, the relationship between structural impairment and functional reorganization in M1 remains unclear.

Methods—Twenty-six patients exhibiting significant recovery after subcortical infarctions were investigated using multimodal MRI techniques. Structural impairment was assessed via cortical thickness, and functional reorganization was analyzed using task-evoked activation, amplitude of low-frequency fluctuation, and resting-state functional connectivity.

Results—Compared with healthy controls, patients with stroke exhibited reduced cortical thickness in the ipsilesional M1; however, this region exhibited increased task-evoked activation, amplitude of low-frequency fluctuation, and resting-state functional connectivity in these patients. Patients with stroke demonstrated increased task-evoked activation in another ipsilesional M1 region, in which increased amplitude of low-frequency fluctuation and resting-state functional connectivity were observed. The structural and functional changes in M1 were located selectively in the ipsilesional hemisphere.

Conclusions—We provide convincing evidence that indicates extensive functional reorganization in the ipsilesional M1 of patients with chronic subcortical infarctions, including the structurally impaired M1 region. (Stroke. 2014;45:788-793.)

Key Words: cerebral infarction • functional neuroimaging • magnetic resonance imaging • motor cortex • neuronal plasticity

Spontaneous motor recovery after stroke may occur even in patients who have never received any form of rehabilitative therapy and has been associated with cortical reorganization.1 In patients with stroke, movement of the affected hand initially activates extensive brain regions.2,3 However, this initial widespread activation does not predict functional recovery; rather, only normalization of ipsilesional primary motor cortex (M1) activation is associated with good outcomes.4 In these patients, the resting-state functional connectivity (rsFC) between the ipsilesional M1 and the contralesional motor cortex initially decreases, and then it gradually increases during the recovery process, until it is finally restored to close to or greater than normal levels.5,6 However, patients with subcortical stroke exhibit decreased cortical thickness and gray matter volume in the ipsilesional M1.6,7 It is clinically important to determine the functionality of the structurally damaged M1 region because it may be useful for planning rehabilitative strategies. If there is no potential for functional reorganization of this region, stimulating this M1 region may not benefit motor recovery.

We hypothesize that the structurally damaged M1 region has the potential for functional reorganization and that the M1 region, exhibiting functional reorganization during hand movement task, also demonstrates functional reorganization during rest in patients with stroke. Structural impairment was assessed by cortical thickness analysis. Functional reorganization was evaluated by 3 measures: task-evoked activation measures the responsiveness to external stimuli; the amplitude of low-frequency fluctuation (ALFF) reflects resting-state brain activity levels,10 and the rsFC detects large-scale functional integration between brain regions. The data-driven analyses within the bilateral M1 were used to identify the M1 regions exhibiting significant structural or functional change in patients with stroke and to demonstrate the extent to which the observed changes are selective for the ipsilesional M1. The hypothesis-driven analyses were performed to validate our hypotheses. The combination of these measures may provide new insight into the relationship between structural impairment and functional reorganization in M1.

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Methods

Subjects

Twenty-six well-recovered patients with subcortical infarcts were recruited. The inclusion criteria were as follows: first-onset ischemic stroke, single subcortical lesion involving the motor pathway, right-handed before the stroke, amount of time poststroke onset >6 months, significant recovery in global motor function with upper extremity Fugl–Meyer Assessment >60 and whole extremity Fugl–Meyer Assessment >90, and capable of completing neurological and MRI examinations. The exclusion criteria were as follows: recurrent stroke, any other brain abnormalities, and a history of drug dependency or psychiatric disorders. According to the degree of motor recovery, patients with stroke were further divided into complete recovery (CR; Fugl–Meyer Assessment=100) and partial recovery (PR; Fugl–Meyer Assessment<100) subgroups. Twenty-five age-matched healthy right-handed volunteers were also included as controls. The key demographic and clinical features are presented in Table 1 (for details, please see Table I in the online-only Data Supplement). The lesion incidence map is shown in Figure I in the online-only Data Supplement. The experimental protocol was approved by the Medical Research Ethics Committee of Tianjin Medical University, and written informed consent was obtained from all participants before enrollment.

Task Design

Subjects performed a unilateral voluntary hand-grasping task with a frequency of 2 Hz. Cycles consisting of a 20-s resting block (baseline) followed by a 20-s unilateral hand-grasping block were repeated 4x. Patients with stroke performed the motor task using the affected hand, whereas healthy controls used their left hand. Potential mirror movements of the unaffected hand were monitored carefully by the experimenters. Two patients with observable mirror movements were excluded from the activation analysis.

MRI Examinations

MR images were acquired using a Signa HDx 3.0 Tesla MR scanner (General Electric, Milwaukee, WI). Tight but comfortable foam padding was used to minimize head movement, and earplugs were used to reduce scanner noise. Resting-state functional MRI data were obtained using a gradient-echo single-shot echo-planar imaging sequence with the following imaging parameters: repetition time/echo time, 2000/30 ms; field of view, 240 mm×240 mm; matrix, 64×64; flip angle, 90°; slice thickness, 3 mm; 1-mm gap; 38 interleaved transversal slices; and 180 volumes. During the resting-state functional MRI scans, all subjects were instructed to keep their eyes closed, to stay as still as possible, to think of nothing in particular, and to not fall asleep. The same scan parameters were used in the task functional MRI with the exception that 80 volumes were acquired. Sagittal 3-dimensional (3D) T1-weighted images were acquired by a brain volume sequence (repetition time/echo time, 8.1/3.1 ms; inversion time, 450 ms; flip angle, 13°; field of view, 256 mm×256 mm; matrix, 256×256; slice thickness, 1 mm; no gap; and 176 slices). After flipping the imaging data from left to right along the midline for patients with left hemispheric lesions, the right side of the image corresponded to the ipsilesional hemisphere and the left side corresponded to the contralesional hemisphere.

M1 Definition

The cortical surface was reconstructed from high-resolution structural images using Freesurfer software (http://surfer.nmr.mgh.harvard.edu/). The bilateral M1 masks for each subject were manually delineated in the individual native space. The M1 range included the posterior 2/3 of the precentral gyrus and anterior bank of the central sulcus. The M1 maps were then coregistered to the Montreal Neurological Institute space, and the M1 probability map was calculated. Finally, a probability of 20% was used to generate a bilateral M1 mask that included most M1 voxels and excluded most non-M1 voxels (Figure II in the online-only Data Supplement).

Multimodal MRI Analyses

Detailed descriptions of our preprocessing procedures and methods for analyzing cortical thickness, task-evoked activation, ALFF, and rsFC appear in the online-only Data Supplement. First, we performed vertex- or voxel-wise comparisons between patients with stroke and healthy controls in terms of the cortical thickness, task-evoked activation, and ALFF within the bilateral M1 mask. The general linear model was used to test group differences in these measures using age and sex as nuisance variables. Multiple comparisons were corrected using the Monte Carlo simulation, resulting in a corrected threshold of \( P < 0.05 \). These data-driven analyses were used to identify M1 regions exhibiting significant structural or functional changes in patients with stroke; they were also used to demonstrate the extent to which the observed changes are selective for the ipsilesional M1. Second, we extracted M1 regions with significant group differences in these measures as regions of interest (ROIs). We then computed the mean values of these measures for these ROIs in each subject. A 1-way ANCOVA \( (P < 0.05) \) controlling for age and sex was used to test group differences in each measure of each ROI among the CR, PR, and control groups. The ROI analyses were used to determine

Table 1. Demographic and Clinical Information of Patients with Stroke

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
<th>PR</th>
<th>Total</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.8±8.2 (42–72)</td>
<td>58.1±7.6 (49–72)</td>
<td>56.8±7.9 (42–72)</td>
<td>56.8±6.9 (47–74)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>11 (73.6%)</td>
<td>5 (41.7%)</td>
<td>16 (61.5%)</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>Women</td>
<td>3 (21.4%)</td>
<td>7 (58.3%)</td>
<td>10 (38.5%)</td>
<td>14 (56%)</td>
</tr>
<tr>
<td>Duration, mo</td>
<td>23.3±12.3 (11–52)</td>
<td>40.6±25.2 (11–96)</td>
<td>31.3±20.9 (11–96)</td>
<td></td>
</tr>
<tr>
<td>Lesion location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left hemisphere</td>
<td>9 (64.3%)</td>
<td>4 (33.3%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>Right hemisphere</td>
<td>5 (35.7%)</td>
<td>8 (66.7%)</td>
<td>13 (50%)</td>
<td></td>
</tr>
<tr>
<td>FMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper extremity</td>
<td>66±0.0 (66)</td>
<td>64.5±1.3 (62–66)</td>
<td>65.3±1.2 (62–66)</td>
<td></td>
</tr>
<tr>
<td>Whole extremity</td>
<td>100±0.0 (100)</td>
<td>97.1±2.1 (93–99)</td>
<td>98.7±2.0 (93–100)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD (range) for continuous data and n (%) for categorical data. CR indicates complete recovery; FMA, Fugl–Meyer Assessment; and PR, partial recovery.
whether CR and PR patients exhibit similar structural and functional changes. Third, a 1-way ANCOVA \((P<0.05)\) controlling for age and sex was used to test group differences in activation, ALFF, and rsFC in the ROI with reduced cortical thickness, which may explain whether the structurally damaged M1 region maintains the potential for functional reorganization in patients with stroke. Finally, the same analysis was also applied to test group differences in the ALFF and rsFC in the ROI, exhibiting increased task-evoked activation; this may improve the understanding of functional reorganization from different perspectives.

**Results**

**Cortical Thickness Analysis**

Vertex-wise comparisons were performed between the cortical thickness of patients with stroke and healthy controls within the bilateral M1 mask. Patients with stroke demonstrated reduced \((P<0.05, \text{corrected})\) cortical thickness only in the ipsilesional M1 (peak Montreal Neurological Institute coordinate=32, -17, 44; peak \(z\) score=4.00; cluster size=414 mm\(^3\)) compared with healthy controls (Figure 1A). We also compared the mean cortical thickness of this cluster and found a significant difference among the CR, PR, and control groups \((F=8.93; P=0.001)\). Post hoc comparisons revealed significantly decreased cortical thickness in CR \((P=0.005)\) and PR patients \((P<0.001)\) than in healthy controls (Figure 1B).

**Figure 1.** Cortical thickness difference between patients with stroke and healthy controls as well as functionality of the region with significant difference in cortical thickness. A, Brain region with significant group difference in cortical thickness is shown in the pial surface (left) and white matter surface (right) of the brain. B, Regions of interest–based comparisons reveal that both complete recovery (CR) and partial recovery (PR) patients exhibit decreased cortical thickness compared with healthy controls. C, Group comparisons of activation difference in the primary motor area (M1) region with significant difference in cortical thickness. The activation is expressed as the percentage of blood oxygen–dependent (BOLD) signal change. D, Group comparisons of amplitude of low-frequency fluctuation (ALFF) difference in the M1 region with significant difference in cortical thickness. ALFF values are given relative to the global mean ALFF of the brain. NC indicates normal controls.

**Activation Analysis**

The motor-evoked activation of the affected hand was compared between the patients and controls within the bilateral M1 mask. Only activation of the ipsilesional M1 (peak Montreal Neurological Institute coordinate=39, -12, 63; peak \(z\) score=3.98; cluster size=67 voxels) was significantly increased \((P<0.05, \text{corrected})\) in patients with stroke than in healthy controls (Figure 2A). We also compared the mean activation strength of this cluster and found a significant difference among the 3 groups \((F=11.71; P<0.001)\). Specifically, both the CR \((P=0.008)\) and PR patients \((P<0.001)\) demonstrated greater activation than healthy controls; in addition, PR patients exhibited a trend toward greater activation \((P=0.068)\) than CR patients (Figure 2B).

**ALFF Analysis**

Voxel-wise comparisons of the ALFF in the bilateral M1 mask were performed between patients and controls. Patients with stroke exhibited increased \((P<0.05, \text{corrected})\) ALFF only in the ipsilesional M1 (peak Montreal Neurological Institute coordinate=42, -9, 36; peak \(z\) score=4.98; cluster size=143 voxels) when compared with healthy controls (Figure 3A). We further compared the mean ALFF of this cluster and found a significant difference among the 3 groups \((F=11.91; P<0.001)\). Specifically, both the CR \((P<0.001)\) and PR patients \((P=0.005)\) demonstrated greater ALFF than healthy controls (Figure 3B).

**Functionality of the M1 Region With Structural Damage**

To determine the functional reorganization potential in the thinned M1 region, we compared group differences in activation, ALFF, and rsFC of this cluster across the 3 groups. We found a trend approaching significance in activation difference \((F=2.94; P=0.066)\). Specifically, PR patients exhibited increased activation compared with healthy controls \((P=0.020; \text{Figure 1C})\). Moreover, we found a significant difference in ALFF \((F=7.66; P=0.002)\): CR patients demonstrated greater ALFF compared with PR patients \((P=0.025)\) and healthy controls \((P<0.001; \text{Figure 1D})\). The rsFC analysis was performed across the entire brain. The rsFC pattern of the M1 cluster in each group is presented in Figure IIIA and IIIB in the online-only Data Supplement. This cluster demonstrated increased \((P<0.05, \text{corrected})\) rsFC with the bilateral sensorimotor cortices in patients with stroke (Figure 4A; Table 2). Specifically, patients with stroke exhibited stronger rsFC compared with healthy controls \((P<0.05; \text{Figure IV in the online-only Data Supplement})\).

We also performed partial correlation analyses between structural (cortical thickness) and functional (activation, ALFF, and rsFC) changes in this M1 region in patients with stroke with age and sex as nuisance covariates. We did not find any significant \((P<0.05)\) correlations between structural and functional changes, suggesting that functional changes are not directly related to the structural damage in this M1 region. Using the similar analysis, we tested correlations among the 3 functional measures in this M1 region in patients with stroke and only found a significant (partial correlation
coefficient=0.653; \(P=0.011\) positive correlation between the ALFF and rsFC with the nearby ipsilesional M1.

Resting-State Functional Changes in the M1 Region With Increased Activation

We investigated the resting-state functional changes in the M1 region in which increased activation was observed to further understand functional reorganization from another perspective. We found significant differences in the ALFF of this M1 cluster (\(F=6.13; P=0.005\)). Post hoc comparisons revealed that CR patients exhibited greater ALFF compared with PR patients (\(P=0.022\)) and healthy controls (\(P=0.001\); Figure 2C). The rsFC pattern of this M1 cluster in each group is presented in Figure IIIC and IIID in the online-only Data Supplement. This cluster showed enhanced (\(P<0.05\), corrected) rsFCs with the nearby ipsilesional M1 and supplementary motor areas in patients with stroke (Figure 4B; Table 2). Specifically, both the CR and PR groups exhibited stronger rsFC compared with the control group (\(P<0.05\); Figure IV in the online-only Data Supplement). We tested correlations among the 3 functional measures in this M1 region in patients with stroke and only found a significant (partial correlation coefficient=0.748; \(P=0.001\)) positive correlation between the ALFF and rsFC with the nearby ipsilesional M1.

Changes in the Contralesional M1

Data-driven analyses of the cortical thickness, activation, and ALFF within the bilateral M1 mask did not reveal any significant changes in these measures in the contralesional M1 of patients with stroke. We also extracted the contralesional M1 to collect homologous measures from this region, which were compared between the stroke and control groups. We did not find any significant group differences in any of these measures.

![Figure 2. Motor task–evoked activation difference between patients with stroke and healthy controls as well as amplitude of low-frequency fluctuation (ALFF) difference in the region exhibiting significant activation difference. A, The primary motor area (M1) region with increased activation in patients with stroke compared with healthy controls. B, Comparisons of activation strength across groups. Activation is expressed as the percent blood oxygen–dependent (BOLD) signal change. C, Group comparisons of ALFF difference in the M1 region with significant activation difference. ALFF values are given relative to the global mean ALFF of the brain. CL indicates contralesional; CR, complete recovery; IL, ipsilesional; NC, normal controls; and PR, partial recovery.](image)

![Figure 3. Amplitude of low-frequency fluctuation (ALFF) difference between patients with stroke and healthy controls. A, The primary motor area (M1) region with increased ALFF in patients with stroke compared with healthy controls. B, Comparisons of ALFF values across groups. ALFF values are given relative to the global mean ALFF of the brain. CL indicates contralesional; CR, complete recovery; IL, ipsilesional; NC, normal controls; and PR, partial recovery.](image)

![Figure 4. Voxel-based comparisons reveal brain regions with resting-state functional connectivity (rsFC) differences between patients with stroke and healthy controls. A, Brain regions with significant differences in the rsFC to the primary motor area (M1) region with decreased cortical thickness in patients with stroke. B, Brain regions with significant differences in the rsFC to the M1 region with increased activation in patients with stroke. CL indicates contralesional; IL, ipsilesional; PostCG, postcentral gyrus; PreCG, precentral gyrus; and SMA, supplementary motor area.](image)
(P>0.05; Table II in the online-only Data Supplement), suggesting that the observed changes are selective for the ipsilesional M1 in stroke.

### Discussion

In the present study, we investigated the relationship between structural damage and functional reorganization of M1 in well-recovered patients with chronic subcortical infarcts involving the motor pathway. Patients with stroke exhibited decreased cortical thickness in the ipsilesional M1, which despite cortical thinning, retained the potential for functional reorganization. Patients with stroke demonstrated increased activation in another ipsilesional M1 region; the region also exhibited increased functionality during rest. We found that the functional reorganization was restricted to the ipsilesional M1, indicating that this region is highly important in motor recovery after stroke.

We found that patients with stroke exhibited decreased cortical thickness in the ipsilesional M1. This result is consistent with previous findings indicating focal thinning of the ipsilesional M1 in patients with incident subcortical infarcts, and volumetric atrophy of the ipsilesional M1 in patients with stroke with damage to subcortical areas or the middle cerebral artery territory. More importantly, structural impairments of the ipsilesional M1 have been associated with the magnitude of residual motor deficits and the extent of motor improvement gained from constraint-induced movement therapy. Although the exact mechanisms for structural impairment of the ipsilesional M1 in subcortical stroke remain unclear, it is possible that retrograde degeneration may account for this finding, especially considering that the corticospinal tract was partially damaged in a large number of our patients. The integrity of the corticospinal tract potentially associated with the extent of focal cortical thinning in patients with incident subcortical infarcts. In addition, retrograde trans-synaptic degeneration in the retinal nerve fiber layer has been observed in patients with occipital lobe infarction. Cortical thickness analysis confirmed that M1 cortical thinning is present even in patients with stroke with CR of global motor function, suggesting that motor recovery in these patients may be mainly associated with functional reorganization.

Patients with subcortical stroke exhibited gradually increased activation in the ipsilesional M1; this overactivation gradually decreased to the near normal levels in patients with CR. However, patients with relatively poor outcomes maintain overactivation of the ipsilesional M1 in the chronic stage of recovery. These findings are consistent with our results that indicated significant overactivation of the ipsilesional M1 in PR patients and near normal activation in CR patients.

We found increased ALFF in the ipsilesional M1 of patients with stroke, with more greatly increased values in patients with CR. This suggests that increased ALFF in the M1 region may represent functional reorganization. However, the exact neurophysiological basis of enhanced ALFF values remains unclear. Enhanced levels may be related to other factors such as altered vasomotor activity and neurovascular coupling. Further studies are required to elucidate the neurophysiological basis of enhanced ALFF and determine whether the ALFF of the ipsilesional M1 can be used to monitor poststroke motor recovery.

Determining the potential for functional reorganization of the structurally damaged M1 region is of clinical significance. If the damaged M1 region has no potential for functional reorganization, rehabilitative strategies aimed to recover functionality, such as transcranial magnetic stimulation to facilitate functional reorganization, may not benefit motor recovery. We found that the structurally damaged M1 region retains the potential for functional reorganization (increased activation, ALFF, and rsFC), suggesting that stimulation of the M1 region may facilitate motor recovery. Unlike a previous study that reported increased cortical thickness in regions with increased activation, we did not find a similar association between structural and functional changes. Many factors, such as patients’ heterogeneous clinical characteristics and differences in experimental tasks and analysis methods, may account for this discrepancy. Whether the M1 region with increased activation exhibits functional reorganization during rest remains unclear. We found that the M1 region exhibited increased ALFF and rsFC in patients with stroke, which may improve our understanding of functional reorganization from different perspectives.

We found that both structurally damaged and functionally reorganized M1 regions exhibited increased rsFC with other sensorimotor-related areas; this suggests large-scale functional reorganization of the M1 region. These findings are consistent with previous studies that reported increased rsFC of the ipsilesional M1 during the chronic stage of subcortical stroke. As suggested by other authors, rsFC of the ipsilesional M1 may be used to predict and monitor poststroke motor recovery. Our seed-based rsFC analysis revealed that functionally connected regions partially overlapped the seed regions that were connected to the structurally damaged regions, consistent with previous findings indicating focal thinning of the ipsilesional M1, which may account for this discrepancy. Whether the M1 region with increased activation exhibits functional reorganization during rest remains unclear.
M1 regions, which may reflect a limitation of this method. However, the relatively large nonoverlapping areas may reflect increased short-range functional connectivity.

Although task-evoked activation, ALFF, and rsFC are measures of cortical function, they provide a different message. The task-evoked activation measures the responsiveness of a brain region to external stimuli; the ALFF reflects the level of resting-state brain activation of a region; and the rsFC detects temporal synchronisation of resting-state brain activity across regions. In patients with stroke, we analyzed correlations among the 3 functional measures in the ROIs, exhibiting reduced cortical thickness and increased activation in these patients. We did not find any correlation between activation and resting-state measures (ALFF and rsFC), suggesting that they measure different aspects of cortical function. The ALFF of each ROI was positively correlated with the rsFC between the ROI and its nearby region but not with the rsFC between the ROI and its remote region. This finding suggests that the ALFF and rsFC also have different functional implications, although they both reflect intrinsic functional reorganization.

A major limitation of the present study is that patients with stroke performed a motor task with their affected hand, whereas healthy controls were asked to use their left hand only. We are uncertain whether brain function during right-hand movement after stroke can be compared with brain function during left-hand movement because there are important differences between right and left brain motor control. Future studies are encouraged to collect functional MRI data from healthy controls during right-hand movement to validate our task-evoked activation results. Another limitation of this study is the lack of information on the degree of initial loss of motor function. This precludes the assessment of the extent of functional recovery during the chronic stage of stroke recovery.

Conclusions
Although both structural damage and functional reorganization were present in the ipsilesional M1 of patients with subcortical stroke with significant motor recovery, the structurally impaired M1 region retained the potential for functional reorganization. These findings improve our understanding of the role played by the ipsilesional M1 in post-stroke motor recovery. This may provide a theoretical basis for designing rehabilitation strategies to restore ipsilesional M1 function after stroke.

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Disclosures
None.

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SUPPLEMENTAL MATERIAL

Methods

Task fMRI data preprocessing

The task fMRI data were preprocessed using Statistical Parametric Mapping software (SPM8, Wellcome Department of Imaging Neuroscience, London, UK). Eighty volumes were firstly corrected for acquisition time delay between different slices. Head motion parameters were then estimated and each volume was realigned to the mean map of the whole volumes to correct for geometric displacement using a six-parameter rigid-body transformation. Two patients and one healthy control were excluded from further analysis due to maximum displacement in any of the orthogonal directions (x, y, z) greater than 2 mm, or maximum rotation (x, y, z) greater than 2.0°. All data were spatially normalized to the standard EPI template, and resampled to $3 \times 3 \times 3 \text{mm}^3$ voxels. Normalized data were smoothed using a Gaussian kernel set to a 6-mm full width at half maximum (FWHM). In total, 20 patients and 21 healthy controls were included in the task fMRI analysis.

Cortical thickness calculation

A total of 23 patients and 25 healthy controls were included in the cortical thickness analysis. Cortical thickness was calculated using Freesurfer V.5.1.0 (http://surfer.nmr.mgh.harvard.edu/). All procedures were performed using the automated surface-based pipeline with the default Freesurfer parameters, which mainly included segmentation, surface reconstruction, and surface-based spatial registration. First, the 3D T1 structural images were registered to the Talairach atlas,
and the intensity variation in the white matter was removed by intensity normalization. Skull stripping was applied with a deformable template model. White matter was then segmented based on intensity and neighbor constraints. Thereafter, the gray/white surface was obtained via tessellation of the gray/white matter boundary and topology correction. The pial surface was generated by nudging the gray/white matter surface along the T1 intensity gradients to reach the gray matter/cerebrospinal fluid boundary. Both surfaces were represented by vertices. The distance between each pair of vertices on the gray/white matter surface and the corresponding pial surface was defined as the cortical thickness between the vertex pair. To compare cortical thickness between groups, the cortical surface of each subject was transformed into an average surface space (fsaverage, provided in Freesurfer package) using a spherical registration method. Cortical thickness maps were smoothed with a Gaussian kernel of 15 mm FWHM. To make the structural and functional results comparable, we converted the coordinates of the cortical thickness maps from the Talairach space to the MNI space.

ALFF preprocessing
The resting-state fMRI data were preprocessed using the Data Processing Assistant for Resting-State fMRI (DPARSF). The first ten volumes of each subject were discarded to allow the signal to reach equilibrium and the participants to adapt to the scanning noise. The remaining 170 volumes were first corrected for the acquisition time delay between different slices. To correct for geometrical displacements, head motion parameters were estimated and each volume was realigned to the mean map of
the total volumes using a six-parameter rigid-body transformation. Ten subjects were excluded from further analysis due to maximum displacement in any of the orthogonal directions (x, y, z) greater than 2 mm, or maximum rotation (x, y, z) greater than 2.0°. Thus, 23 patients and 18 healthy controls were included in the resting-state fMRI analyses. All of the data were spatially normalized to the standard EPI template, and resampled to 3 x 3 x 3 mm³ voxels.

**ALFF calculation**

The ALFF was computed using REST software (downloaded from http://restfmri.net, version 1.3). Because the ALFF represents the low-frequency band, linear-trend removing and temporal band-pass filtering (0.01-0.08 Hz) were performed on the time series of each voxel to reduce very-low-frequency drift and high-frequency noise. The time series of each voxel was then transformed to the frequency domain using fast Fourier transform (parameters: taper percent = 0, length = shortest), and the power spectrum was obtained. The square root of the power spectrum was calculated at each frequency and averaged across 0.01-0.08 Hz for each voxel. The averaged square root was taken as the ALFF. For standardization purposes, the ALFF of each voxel was divided by the global mean ALFF within the brain tissue mask. The standardized ALFF of each voxel should thus have a value of approximately 1. Finally, spatial smoothing was conducted on the standardized ALFF map of each subject using an isotropic Gaussian kernel of 6 mm of FWHM.

**rsFC preprocessing**

Most of the preprocessing steps for rsFC analysis were similar to those used in the
ALFF analysis, including discarding the first ten volumes, slice timing, realignment, normalization to the MNI template, and resampling to $3 \times 3 \times 3$ mm$^3$ voxels. After resampling, the images were smoothed with a Gaussian kernel of $6 \times 6 \times 6$ mm$^3$ FWHM. Several sources of spurious variances including estimated motion parameters, linear drift, global average blood oxygenation level dependent (BOLD) signals, and average BOLD signals in ventricular and white matter regions were removed from the data through linear regression. Finally, temporal band-pass filtering (0.01-0.08 Hz) was performed on the time series of each voxel to reduce low-frequency drift and high-frequency noise.

**rsFC calculation**

In the rsFC analysis, correlation coefficients were calculated for the mean time series of each ROI and each voxel of the whole brain for each subject. To improve normality, these values were converted to z-values using Fisher’s r-to-z transformation. For each group, individuals’ z-values were entered into a random effect one-sample t-test to determine brain regions that were significantly positively correlated with the seed ROIs. Corrections for multiple comparisons were performed by the family-wise error (FWE) method at $P < 0.05$. The individuals’ z-values were then entered into a GLM to test group differences in the rsFC with age and sex as nuisance variables.
<table>
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<th>Age (years)</th>
<th>Duration (months)</th>
<th>Lesion location</th>
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<th>FMA WE</th>
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<td>11</td>
<td>Left CR, IC, BG</td>
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<td>99</td>
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</tbody>
</table>

BG indicates basal ganglia; CR, corona radiata; F, female; FMA, Fugl-Meyer Assessment; IC, internal capsule; M, male; Thal, thalamus; UE, upper extremities; WE, whole extremities.
Table II. Comparisons of bilateral M1 MRI measure between stroke patients and healthy controls.

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<th></th>
<th>PR</th>
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<td><strong>Task-evoked activation</strong></td>
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<td>Ipsilesional M1</td>
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<td>0.90±0.23</td>
<td>0.70±0.07</td>
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<td>0.85±0.12</td>
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</table>

ALFF indicates amplitude of low frequency fluctuation; CR, patients with complete recovery in global motor function; M1, primary motor area; NC, normal controls; and PR, patients with partial recovery in global motor function.
Figure I. The stroke patient lesion incidence map.
Figure II. The probability map of the primary motor cortex (M1). Only voxels with a probability > 20% of belonging to M1 are shown. Color bar represents the overlapping percentage of the M1 in all subjects.
**Figure III.** The resting-state functional connectivity (rsFC) map of each seed ROI in each group. A (stroke group) and B (control group) show the rsFC maps of the M1 ROI that showed decreased cortical thickness in stroke patients. C (stroke group) and D (control group) demonstrate the rsFC maps of the M1 ROI that showed increased BOLD activation in stroke patients.
Figure IV. ROI-based comparisons in the rsFC of the ipsilesional M1 between stroke patients and healthy controls. (A) The rsFC of the M1 region with decreased cortical thickness in stroke patients. (B) The rsFC of the M1 region with increased activation in stroke patients. CR indicates patients with complete recovery in global motor function; M1, primary motor area; NC, normal controls; PostCG, postcentral gyrus; PR, patients with partial recovery in global motor function; PreCG, precentral gyrus; ROI, region of interest; rsFC, resting-state functional connectivity; and SMA, supplementary motor area.
References

