Enhanced Interhemispheric Functional Connectivity Compensates for Anatomical Connection Damages in Subcortical Stroke

Jingchun Liu, MS; Wen Qin, PhD; Jing Zhang, MD; Xuejun Zhang, PhD; Chunshui Yu, MD

Background and Purpose—Motor recovery after stroke has been shown to be correlated with both the fractional anisotropy (FA) of the affected corticospinal tract (CST) and the interhemispheric resting-state functional connectivity (rsFC) of the primary motor cortex (M1). However, the role of the restoration or enhancement of the M1–M1 rsFC in motor recovery remains largely unknown. We aimed to clarify this issue by investigating the correlations between the M1–M1 rsFC and the integrity of the M1–M1 anatomic connection and the affected CST in chronic subcortical stroke patients with good motor outcomes.

Methods—Twenty patients and 16 healthy controls underwent multimodal magnetic resonance imaging examinations. Diffusion tensor imaging was used to reconstruct the M1–M1 anatomic connection and bilateral CSTs. White matter integrity of these tracts was assessed using FA. Resting-state functional magnetic resonance imaging was used to calculate M1–M1 rsFC. Group differences in these measures were compared. Correlations between M1–M1 rsFC and FA of the M1–M1 anatomic connection and the affected CST were analyzed in patients with stroke.

Results—Compared with healthy controls, patients with stroke exhibited significantly reduced FA in the affected CST and the M1–M1 anatomic connection and a significantly increased M1–M1 rsFC. The FA values of the affected CST were positively correlated with the M1–M1 anatomic connection, and these FA values were negatively correlated with the M1–M1 rsFC in these patients.

Conclusions—Our findings suggest that the M1–M1 anatomic connection impairment is secondary to CST damage, and the M1–M1 rsFC enhancement may reflect compensatory or reactive neural plasticity in stroke patients with CST impairment. (Stroke. 2015;46:1045-1051. DOI: 10.1161/STROKEAHA.114.007044.)

Key Words: cerebral infarction ▪ diffusion tensor imaging ▪ functional neuroimaging ▪ magnetic resonance imaging ▪ motor cortex ▪ neuronal plasticity
and some patients do not exhibit any increase within 1 year.\textsuperscript{12} At a final stable stage (=1 year post stroke), the rsFC returns to a normal level in some patients,\textsuperscript{13,14} reaches a greater than normal level in some other patients, and remains at a lower level in other ones.\textsuperscript{12} Moreover, the M1-M1 rsFC has been associated with motor recovery in patients with subcortical stroke.\textsuperscript{10,15} However, these studies cannot answer whether the restoration or enhancement of the rsFC plays a beneficial role in motor recovery because they have not controlled for the CST impairment.

Although both the FA of the affected CST and the M1-M1 rsFC have been reported to correlate with motor recovery in subcortical patients with stroke, the intrinsic association between the anatomic connection impairments and functional connectivity reorganization remains largely unknown. Within 4 weeks after subcortical stroke, the M1-M1 rsFC reduction has been found to be positively correlated with CST impairment.\textsuperscript{16} However, this study cannot answer whether the restoration or enhancement of the rsFC plays a beneficial role in motor recovery because both measures have not evolved into their final stable stages and they may experience largely different dynamic changes after stroke. The possible role of the rsFC in motor recovery could be clarified by investigating correlation between the M1-M1 rsFC and the FA of the affected CST in stable chronic (>6 months) stroke patients with excellent motor outcomes. Patients with stroke in the stable chronic stage were selected because both structural impairment and functional reorganization reached nearly unchanged levels at this stage. Only in stroke patients with excellent motor outcomes, we can infer a beneficial role of the rsFC increase in motor recovery from a negative correlation and a detrimental role from a positive correlation because the CST impairment prevents motor recovery, and these patients really have good motor outcomes. Because a negative correlation could better explain why stroke patients with different degrees of CST impairment could obtain similar good motor outcomes and why some patients with stroke could have a much higher M1-M1 rsFC than healthy controls despite always having lower FA of the affected CST, we hypothesize that the restoration or enhancement of the rsFC plays a beneficial role in motor recovery and predict a negative correlation between the M1-M1 rsFC and the FA of the affected CST in these patients with stroke. If the hypothesis is correct, rehabilitative strategies aimed to enhance the M1-M1 rsFC may benefit motor recovery in stroke patients with relatively severe CST impairment.

Another unanswered question is that of the association between the integrity of the affected CST and the M1-M1 anatomic connection. It has been shown that the impaired CST may affect indirectly connected fiber tracts in patients with stroke, such as the M1-M1 anatomic connection,\textsuperscript{17} through a mechanism of trans-synaptic axonal degeneration.\textsuperscript{18} So if we predict a positive correlation of FA values between the affected CST and the M1-M1 anatomic connection in patients with subcortical stroke.

In this study, we recruited 16 healthy controls and 20 chronic ischemic stroke patients with infarctions restricted to the internal capsule and neighboring regions that exhibited a good outcome in global motor function. We first compared differences in the FA values of the affected CST and the M1-M1 anatomic connection and in the M1-M1 rsFC strengths between the 2 groups. Then, we tested the correlations between every 2 of these 3 measures in patients with stroke. Through these hypothesis-driven analyses, we aimed to clarify the role of the restoration or enhancement of the rsFC in! motor recovery in patients with subcortical stroke.

Methods

Subjects

The experimental protocol was approved by the Medical Research Ethics Committee of Tianjin Medical University General Hospital, and written informed consent was obtained from all participants. The inclusion criteria are the following: (1) the subjects had experienced a first-onset ischemic stroke; (2) a single lesion was restricted to the internal capsule and neighboring regions; (3) subjects were right-handed before the stroke; (4) the amount of time poststroke onset was >6 months to ensure that the patients were at a stable chronic stage; (5) the subjects exhibited a good outcome in global motor function as assessed by a whole extremity Fugl-Meyer assessment of >90/100. The exclusion criteria were (1) recurrent stroke which was defined on the basis of both clinical history and MRI evaluation; (2) any other brain disorders or abnormalities that could be identified by medical history or imaging examinations; (3) subjects exhibiting lacunes and microbleeds were excluded based on T1-, T2-, and diffusion-weighted images; (4) subjects with severe white matter hyperintensity manifesting as a Fazekas et al\textsuperscript{19} score of > 1; (5) subjects with a history of drug dependency or psychiatric disorders. According to these criteria, twenty patients (13 men; mean age, 56.8±8.4 years) were included in this study. Sixteen healthy subjects (7 men; mean age, 58.8±7.3 years) were also recruited as controls.

Magnetic Resonance Data Acquisition

Magnetic resonance images were acquired using a 3.0 Tesla MR scanner (Signa Excite HDx; GE Healthcare, Milwaukee, WI). Tight but comfortable foam padding was used to minimize head movement, and earplugs were used to reduce scanner noise. Resting-state fMRI 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; gap=1 mm; 38 interleaved transversal slices; and 180 volumes. During the resting-state fMRI scans, all subjects were instructed to keep their eyes closed, stay as still as possible, think of nothing in particular, and not to fall asleep. DTI data were obtained using a spin-echo single-shot echo-planar imaging sequence. Diffusion-sensitized gradients were applied along 30 noncollinear directions with a b-value of 1000 s/mm\textsuperscript{2}. In addition, 3 sets of b=0 images were obtained. Using an integrated parallel acquisition technique with an acceleration factor of 2 allowed us to obtain images with less distortion from susceptibility artifacts. We collected 50 slices from each participant. The scan parameters were repetition time/echo time=11000/77.6 ms, field of view=256 mm×256 mm, matrix=128×128, flip angle=90°, slice thickness=3 mm, and no gap. Sagittal 3-dimensional T1-weighted images were acquired by a brain volume sequence (repetition time/echo time=7.8/3.0 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).

Preprocessing for Resting-State fMRI Data

The resting-state fMRI data were preprocessed using the Statistical Parametric Mapping software (SPM8, http://www.fil.ion.ucl.ac.uk/ spam). The first 10 volumes from 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 corrected for acquisition time delay between the slices. Then, head motion parameters
were estimated; none of the 36 subjects had a maximum displacement of >2 mm or a maximum rotation of >2°. The MRI data set was spatially normalized to the Montreal Neurological Institute (MNI) echo-planar imaging template and resampled into 3×3×3 mm³ voxels. Thereafter, several nuisance variables were regressed out from the fMRI data, including the averaged signals of the ventricular, white matter, and the whole brain, and the Friston 24 regressors (including 6 head motion parameters, 6 head motion parameters 1 time point before, and the 12 corresponding squared items). Next, a band-pass frequency filter (0.01–0.08 Hz) was applied to reduce low-frequency drift and high-frequency noise. Finally, the filtered blood oxygenation level dependent images were spatially smoothed using an isotropic Gaussian kernel of 8-mm full-width at half maximum.

Preprocessing for DTI Data

The DTI data were preprocessed using FMRIB’s free software FSL (Oxford Center for Functional MRI of the Brain, Oxford, United Kingdom). All diffusion-weighted images were visually inspected by 2 radiologists for apparent artifacts because of subject motion and instrument malfunction. For each examination of each subject, the diffusion-weighted images were registered to the corresponding b=0 images with an affine transformation to correct for eddy-current distortion and motion displacement. Then, the skulls in the images were removed using the brain extract toolbox. The diffusion tensor was reconstructed using the linear least-square fitting algorithm, which was used for calculating diffusion indices, including the fractional anisotropy (FA), 3 eigenvectors, and the mean diffusivity. Deterministic fiber tracking was applied using a fiber assignment by means of continuous tracking algorithm with an angle threshold of 50°. To reconstruct the tracts of interest, we used a multiple-ROI approach based on existing anatomic knowledge about tract trajectories. Specifically, for the CST, 3 ROIs were placed at the levels of the precentral gyrus, the posterior limb of the internal capsule, and the cerebral peduncle on the individual axial FA map (Figure 1A). These ROIs were selected based on several previous studies. Fibers that passed through all 3 ROIs were considered as the CST. Furthermore, nuisance fibers, such as those going to the contralateral hemisphere, cerebellum, or thalamus, were removed using specific ROIs. For the M1-M1 anatomic connection, 3 ROIs were placed at the bilateral precentral gyrus and the midsagittal slice of the corpus callosum on the FA map (Figure 1A). Unrelated fibers were removed using specific exclusion masks. The streamlines were seeded from the left M1 and passed through the corpus callosum and ended at the right M1 and vice versa. These procedures were repeated 3 months later by the same researcher.

Tractography was performed in all healthy controls (Figure 1B). Then, we coregistered the individual diffusion indices and tracts into MNI space using a 2-step method. First, we coregistered the brain-extracted b=0 images of each subject with his/her T1 images using an affine method (12 parameters); then the T1 images were finely coregistered into the T1 template of MNI space. Finally, the diffusion indices and the reconstructed tracts were written into MNI space using the affine parameters generated from the above steps and were resliced into 2×2×2 mm³. The resulting tracts of all healthy subjects were used to calculate the probability map of each tract of interest. The final probability maps were calculated as the mean probability of the 2× of tractography by the same researcher. Then, 25% probability was used to generate the bilateral CSTs and the M1-M1 anatomic connection masks (Figure 1C). These probability masks encompassed the centers of the CST and the M1-M1 connection common to all healthy controls. FA values were then extracted from these masks for the patients with stroke and the healthy controls. For the affected CST, we only extracted FA values from the cerebral peduncle, where the CST fibers have a relatively coherent arrangement. FA values can accurately estimate white matter integrity only in white matter regions with coherent fiber arrangement. For the same reason, only the FA values of the midsagittal slice (or the middle 10 mm section) of the M1-M1 connection were extracted. We used the mean FA value of all voxels within each ROI to represent the FA of the ROI.

Seed Masks

The left and right M1s were separately extracted from Brodmann areas 4 in the Brodmann atlas. Then, we separately extracted the overlapping regions of the left and right M1s with the 25% probability map of the M1-M1 anatomic connection to define the left and right seed masks for the rsFC analysis (left: MNI coordinates, −12, −30, and 54; cluster size, 61 voxels; right: MNI coordinates, 12, −30, and 54; cluster size, 62 voxels).

rsFC Analysis

Using the defined seed masks as the ROIs, the ROI-based rsFC analysis was performed. For each individual data set, the Pearson correlation coefficient between the mean time series of the left and right ROIs was computed and converted to z value using Fisher r-to-z transformation to improve the normality. Then, the general linear model was applied to quantitatively compare group differences in the rsFC between patients with stroke and healthy controls. In this process, age and sex were treated as covariates of no interest. Differences between 2 groups were considered significant if P<0.05. The value of Cohen d was used to describe the effect size (ES).

DTI Analysis

The general linear model was applied to quantitatively compare FA differences in the affected CST and the M1-M1 anatomic connection between patients with stroke and healthy controls. In this process,
Correlation Analysis

First, we explored the correlation between FA values of the affected CST and those of the M1-M1 anatomic connection in the patient group. Second, we assessed the relationship between the FA values of the affected CST and the M1-M1 rsFC. Finally, we examined the correlation of FA values of the M1-M1 anatomic connection with the M1-M1 rsFC. For all correlation analyses, we used partial correlations to factor out age and sex, and $P<0.05$ was considered to be statistically significant.

Subgroup Analyses

Patients with stroke were further divided into the left-brain damage (LBD) and right-brain damage (RBD) subgroups. Then, we repeated our analyses for the 2 subgroups, respectively. The same statistical methods and thresholds as those used for the total patient group were used in the subgroup analyses.

Voxel-Based Connectivity Analyses

To validate and extend our findings of the ROI-based analyses, we also performed whole-brain voxel-based analyses on the FA and the M1 rsFC. The methods of these voxel-based analyses are provided in the online-only Data Supplement.

Results

Demographic and Clinical Information

The clinical and demographic data of patients with stroke and controls are listed in Table. Compared with healthy controls, patients with stroke did not show any significant differences in age ($P=0.44$) and sex ($P=0.20$). The duration from stroke onset to the MRI scan ranged from 11 to 64 months (mean value, 29.8±16.9 months). The stroke lesions involved the internal capsule and the surrounding structures, including the internal capsule, thalamus, basal ganglia, and corona radiate (Figure 2); 9 of 20 patients had infarct lesions in the right hemisphere and 11 in the left hemisphere. The motor function of the patients was significantly recovered with an Fugl-Meyer assessment of >94/100 for whole extremities.

rsFC Analysis

Compared with healthy controls, stroke patients with a good outcome in global motor function showed increased ($P=0.024$; ES=0.57) M1-M1 rsFC (Figure 3A).

DTI Analysis

The general linear model analysis showed that stroke patients with subcortical infarction had a significantly decreased FA in the cerebral peduncle of the affected CST ($P=0.004$; ES=0.72; Figure 3B). Patients with stroke also had a trend toward decreased FA ($P=0.098$; ES = 0.41) in the whole affected CST. Compared with healthy controls, patients with stroke had decreased FA ($P=0.021$) in the midsagittal slice of the M1-M1 anatomic connection with an ES of 0.48 (Figure 3C). Similarly, patients with stroke also had decreased mean FA in the middle 10-mm section of the M1-M1 connection ($P=0.026$; ES=0.57).

Correlation Analyses

The FA values of the M1-M1 anatomic connection were positively correlated with the FA values of the affected CST in patients with stroke ($r=0.759$; $P<0.001$; Figure 4A). However, a significant negative correlation was found between the FA values of the affected CST and the M1-M1 rsFC ($r=-0.569$; $P=0.022$; Figure 4B). Similarly, a negative correlation was observed between the FA values in the M1-M1 anatomic connection and the M1-M1 rsFC in either the midsagittal slice ($r=-0.571$; $P=0.013$; Figure 4C) or the middle 10-mm section ($r=-0.5639$; $P=0.015$).
Subgroup Analyses
The results of the subgroup analyses are shown in the online-only Data Supplement. The RBD subgroup displayed similar results to those of the total patient group. Although the trend of the changes remained, the LBD subgroup displayed much weaker results. The LBD subgroup displayed a trend toward a smaller degree of damage of the affected CST relative to the RBD subgroup ($P=0.209; \text{ES}=0.403$). The FA of the affected CST in the RBD was significantly lower ($P<0.001; \text{ES}=1.52$) than that in LBD.

Voxel-Based Connectivity Analyses
The results of the voxel-based analyses of the FA and the M1 rsFC are provided in the online-only Data Supplement. Compared with healthy controls, patients with stroke exhibited significantly reduced FA in the ipsilesional CST and corpus callosum and significantly increased interhemispheric rsFC between the ipsilesional M1 and the contralesional M1 (MNI coordinates, $−9, −18, \text{and} 69$; peak $t$ value, $6.03$; cluster size, $34$ voxels).

Discussion
In this study, we found a negative correlation between the M1-M1 rsFC and the FA of the affected CST in subcortical stroke patients with good outcomes. In contrast to the detrimental role of the CST impairment in motor recovery, this finding suggests that the restored or enhanced M1-M1 connection fibers may play a beneficial role. We also found a positive correlation between the FA values of the affected CST and those of the M1-M1 anatomic connection, indicating that the M1-M1 anatomic connection impairment is secondary to the CST impairment.

In patients with chronic subcortical stroke, we repeated the previous finding of decreased FA in the affected CST. The decreased FA in the affected CST has been considered to reflect the pathological process of Wallerian degeneration that is secondary to the direct damage of the CST by stroke lesions. The impairment of the M1-M1 connection fibers has also been reported in patients with subcortical stroke. However, the direct association between the impairments of the CST and the M1-M1 connection has not been established in patients with stroke. Our positive correlation between the FA values of the M1-M1 connection fibers and those of the affected CST provides evidence for the hypothesis that the M1-M1 connection impairment may result from the transsynaptic axonal degeneration that is secondary to the CST impairment because both fibers are connected with neurons in the M1.

In patients with subcortical stroke, the M1-M1 rsFC is initially decreased\(^{11,12}\) and is gradually restored to a level near or greater than normal.\(^{12,14}\) Consistent with these previous findings, we also found increased M1-M1 rsFC in patients with chronic subcortical stroke. Local injury of the motor network may cause transient attenuation of activity in intact, connected motor regions in the ipsilesional and contralesional hemispheres, which is known as diaschisis.\(^{28,29}\) The unparallel activity changes in the bilateral M1 may be related to the initial decrease in the M1-M1 rsFC. In line with this inference, the interhemispheric activation imbalance has been found in the first week in rats with stroke.\(^{30}\) The restoration
of the M1-M1 rsFC may be related to the disappearance of the temporary transhemispheric diaschisis, axons sprouting to establish new connections and novel projection patterns, and functional reorganization within the motor network. However, the mechanisms of motor recovery after stroke are rather complex and need to be further studied.

A previous study on subcortical stroke (<4 weeks post stroke) has shown that the M1-M1 rsFC reduction is positively correlated with the CST impairment, which is inconsistent with our finding of a negative correlation between these 2 measures in chronic subcortical stroke. This discrepancy may be explained by the difference in the stages of stroke between the 2 studies. Within 4 weeks post stroke, the FA of the affected CST exhibits monotonous decrease. Although the rsFC also decreases initially (the first days to weeks), the following changes (increase or unchange) in the rsFC are largely different across subjects. Thus, the positive correlation cannot provide information on the role of the restoration of the rsFC in motor recovery because neither of the 2 measures reaches a final stable level. However, our finding of a negative correlation may represent an association at a stable chronic stage of stroke. Because our patients exhibited similar good outcomes in global motor function, the M1-M1 rsFC changes could explain why patients with various degrees of CST impairments exhibit similar good functional recovery. That is, the motor deficit resulting from the CST impairment could be compensated by the enhanced M1-M1 rsFC, which may reflect a form of reactive neural plasticity. It is clinically important to confirm a beneficial role of the restoration or enhancement of the M1-M1 rsFC in motor recovery in patients with stroke. Logically, rehabilitation targeting strengthening M1-M1 rsFC may benefit motor recovery in stroke patients with CST impairment. However, it is not clear how rehabilitation would influence rsFC. Further studies are needed to understand the mechanism quantified by rsFC and to clarify the effect of rehabilitation on rsFC.

We also found that the FA in the M1-M1 anatomical connection was negatively correlated with the M1-M1 rsFC. That is to say, patients with more severe impairment in M1-M1anatomical connection had a higher M1-M1 rsFC. As we know, there is a close relationship between anatomic and functional connectivity measures in healthy subjects. Specifically, the FA of the anatomic connection was positively correlated with the strength of the rsFC. Therefore, the negative correlation between the M1-M1 anatomic and functional connectivity in chronic stroke patients with CST impairment further confirms that the restoration or enhancement of the M1-M1 rsFC may play a beneficial role in motor recovery.

We found that the LBD subgroup had much weaker results than the RBD subgroup. Further analyses showed that the LBD subgroup had significantly less CST impairment than the RBD subgroup. These findings suggest that the degrees of the CST impairment may account for different statistical significances between the 2 subgroups. The similar trends of significant differences and correlations in the RBD and LBD subgroups suggest that lesion sides may not have a significant effect on connectivity changes in patients with stroke. The whole-brain voxel-wise analyses demonstrated that patients with stroke also exhibited significantly reduced FA in the affected CST and the M1-M1 anatomic connection and a significantly increased M1-M1 rsFC. These findings indicate that the connectivity changes examined in this study are the main characteristic changes in patients with chronic subcortical stroke.

**Limitations**

The study sample was a narrow range of highly functioning individuals post stroke with whole-extremity Fugl-Meyer assessment values ranging from 94 to 100. This may be acceptable from the perspective of improving the homogeneity of patients with stroke and may be reasonable for determining the role of the restoration or enhancement of the rsFC in motor recovery by investigating correlation between the M1-M1 rsFC and the FA of the affected CST. However, our conclusion should be validated in a more general stroke population. Another limitation of this study was that we did not collect enough behavioral data to investigate the links between connectivity changes and behavioral improvements in patients with stroke. In addition, most of correlations reported in this study did not reach statistical significance when multiple comparisons were corrected using the Bonferroni method. Further studies should recruit a large sample of stroke patients with a large range of motor outcomes and collect more behavioral parameters to validate our findings and to explore their clinical implications on rehabilitation.

**Conclusions**

Our findings suggest that the M1-M1 anatomic connectivity impairment may be secondary to CST damage. The enhanced M1-M1 rsFC may reflect a compensatory mechanism for motor deficits resulting from the impairment of the CST and the M1-M1anatomical connection, which may account for similar motor recovery in subcortical stroke patients with varying degrees of CST impairments.

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**Disclosures**

None.

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