Original Contribution

Interactions Between the Corticospinal Tract and Premotor–Motor Pathways for Residual Motor Output After Stroke

Robert Schulz, MD; Eunhee Park, MD, PhD; Jungsoo Lee, PhD; Won Hyuk Chang, MD, PhD; Ahee Lee, MS; Yun-Hee Kim, MD, PhD*; Friedhelm C. Hummel, MD*

Background and Purpose—Brain imaging has continuously enhanced our understanding how different brain networks contribute to motor recovery after stroke. However, the present models are still incomplete and do not fit for every patient. The interaction between the degree of damage of the corticospinal tract (CST) and of corticocortical motor connections, that is, the influence of the microstructural state of one connection on the importance of another has been largely neglected.

Methods—Applying diffusion–weighted imaging and probabilistic tractography, we investigated cross-network interactions between the integrity of ipsilesional CST and ipsilesional corticocortical motor pathways for variance in residual motor outcome in 53 patients with subacute stroke.

Results—The main finding was a significant interaction between the CST and corticocortical connections between the primary motor and ventral premotor cortex in relation to residual motor output. More specifically, the data indicate that the microstructural state of the connection primary motor–ventral premotor cortex plays only a role in patients with significant damage to the CST. In patients with slightly affected CST, this connection did not explain a relevant amount of variance in motor outcome.

Conclusions—The present data show that patients with stroke with different degree of CST disruption differ in their dependency on structural premotor–motor connections for residual motor output. This finding might have important implications for future research on recovery prediction models and on responses to treatment strategies. **(*Stroke*. 2017;48:00-00. DOI: 10.1161/STROKEAHA.117.016834.)**

Key Words: brain ■ diffusion ■ parietal lobe ■ stroke ■ white matter

In the past decade, functional[1] and structural connectivity analyses[2] have dramatically enhanced our understanding of network alterations after ischemic stroke. Noninvasive brain stimulation protocols have been developed and applied variously to use this knowledge to enhance motor recovery. Despite that our present models can explain more and more variance in residual motor output and recovery on the one hand,[3] and brain stimulation has evidenced its potential in neurorehabilitation[4] on the other hand, we are far from models and protocols that fit for every patient. The question arises if important information has been left unnoticed when looking at stroke-related network alterations: How can we further advance our network concepts? When and how do patients have to be recruited for specific brain stimulation protocols? Do we have to adjust our models and treatment assumptions and develop them toward individualization, for example, on specific clinical or imaging characteristics?

Structural analyses have predominantly investigated the influence of the integrity of the corticospinal tract (CST).[2] Functional analyses have extensively investigated the importance of changes of local brain activity and inter-regional corticocortical connectivity between key areas of the human motor network that are the primary motor cortex (M1) and secondary motor areas in the frontal and parietal lobe.

Received January 27, 2017; final revision received June 7, 2017; accepted July 6, 2017.

From the Department of Neurology, University Medical Center Hamburg-Eppendorf, Germany (R.S.); Department of Physical and Rehabilitation Medicine, Center for Prevention and Rehabilitation, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea (J.L., W.H.C., Y.-H.K.); Department of Physical and Rehabilitation Medicine, Kyungpook National University Medical Center, Daegu, Republic of Korea (E.P.); Department of Health Sciences and Technology, Department of Medical Device Management & Research, SAIHST, Sungkyunkwan University, Seoul, Republic of Korea (J.L., A.L., Y.-H.K.); Defitech Chair of Clinical Neuroengineering, Brain Mind Institute and Centre of Neuroprosthetics (CNP), Swiss Federal Institute of Technology (EPFL), Geneva, Switzerland (F.C.H.); Defitech Chair of Clinical Neuroengineering, Brain Mind Institute and Centre of Neuroprosthetics (CNP), Swiss Federal Institute of Technology (EPFL Valais), CRR (Clinique Romande de Réadaptation), Sion, Switzerland (F.C.H.); and Department of Clinical Neurosciences, Geneva University Hospital, Switzerland (F.C.H.).

*Drs Kim and Hummel contributed equally.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.117.016834/-/DC1.

Correspondence to Friedhelm C. Hummel, MD, Defitech Chair of Clinical Neuroengineering, Brain Mind Institute, SV, Centre of Neuroprosthetics (CNP), Swiss Federal Institute of Technology (EPFL), Campus Biotech, Rm H4.3.132.084, 9, Chemin des Mines, 1202 Geneva, Switzerland, E-mail friedhelm.hummel@epfl.ch or Yun-Hee Kim, MD, PhD, Department of Physical and Rehabilitation Medicine, Center for Prevention and Rehabilitation, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro Gangnam-gu, Seoul 06351, Republic of Korea, E-mail yunkim@skku.edu

Particularly, the premotor areas of the frontal lobe, which are the dorsal and ventral premotor cortex (PMv), the supplementary motor area, and cingulate motor areas, might render one potential substrate for brain reorganization after stroke as they have direct access to M1, as well as to the spinal cord.[5] For instance, studies have suggested reduced neuronal coupling between ipsilesional premotor cortices and M1 after stroke and that the reinstatement of normal connectivity over time is related to the amount of recovery.[6] Likewise, the importance of the microstructural state of single corticocortical networks between premotor areas and M1 has also been demonstrated by structural imaging.[7] Only few studies have investigated to what degree specific corticocortical structural[8] or functional[9–12] networks relate to motor output when taking the integrity of the CST as the main outflow tract of the human motor network into account: It has been shown that—independent from the influence of the CST—microstructural properties of specific premotor–motor connections, especially between M1 and the PMv, might be relevant for motor output in chronic stroke.[8]

Strikingly though, it has been largely neglected whether the influence of the corticocortical motor network on motor function might be a generalizable finding or whether it might depend from the state of the CST? The detection of such cross-network interactions might help to better understand brain reorganization patterns. Additionally, it might provide novel insights toward patient stratification, for example, brain stimulation protocols in the context of high intersubject variability of behavioral responses and responders and nonresponders.[4,13–15] One previous study has already reported that interhemispheric coupling strengths between both M1 correlated with motor function only in patients with intact CST, whereas patients with disrupted CST did not show a similar association.[16] Until now, structural cross-network interactions between corticocortical premotor–motor connections and the CST have not been investigated systematically in detail.

Continuing previous structural work on parietofrontal motor pathways as an important network for human motor control in healthy participants[17] and patients with stroke,[8,18–20] this study sought to investigate structural cross-network interactions between the CST and corticocortical motor pathways between M1, PMv, and the intraparietal sulcus (IPS).[8] Specifically, the question was addressed whether the importance of specific tracts of the parietofrontal ipsilesional corticocortical network after stroke might depend on the microstructural integrity of the CST or whether these factors are independent. A large group of patients with subacute stroke was analyzed applying diffusion tensor imaging, probabilistic tractography, and template-based tract analysis to assess tract-related biophysical properties of white matter microstructure[21] of the CST and 3 predefined intrahemispheric parietofrontal tracts between M1, PMv, and IPS. We hypothesized that the microstructural state of the different corticocortical connections would be associated with residual motor output depending on the level of damage to the CST.

Materials and Methods

Participants and Clinical Testing

Fifty-three patients (aged 60±11.2 years, 30 men; 4 left handed) with first-ever ischemic strokes (dominant hemisphere, 23) were recruited and tested 3 months after onset (mean, 99±12.4 days). Figure 1 gives an overview of the distribution of stroke locations with cortical, subcortical, and pontine lesions. Patients were evaluated by means of hand grip force (calculated as the ratio between the affected and unaffected hand) and the upper limb score of the Fugl-Meyer assessment. See Table I in the online-only Data Supplement for an overview of the demographic and clinical characteristics. Both measures were combined to one composite motor output score (MO) by principal component analysis (first principal component).[6,8,22] For the reconstruction of the motor tracts, we used structural brain imaging data of 26 healthy older participants (66±10.1 years, 15 men, all right handed) from a previous study.[22] The present study was approved by the local ethics committees (Hamburg, PV3777; Seoul, Institutional Review Board No. 2015-07-02). All participants gave written informed consent according to the Declaration of Helsinki.

Brain Imaging Sequences

In the patients with stroke, diffusion–weighted images were acquired using a 3T Phillips ACHIEVA magnetic resonance imaging (MRI) scanner (Philips Medical Systems, Best, the Netherlands). For the controls, diffusion–weighted and high-resolution T1-weighted anatomic images were available from a previous study and were acquired using a 3T Siemens Skyra MRI scanner (Siemens, Erlangen, Germany). Details of the sequence parameters are given in Text I in the online-only Data Supplement.

Image Processing and Probabilistic Tractography

The FSL software package 5.1 (http://www.fmrib.ox.ac.uk/fsl) was used to analyze the imaging data of the patients with stroke. In brief, after correcting for eddy currents and head motion, brains were skull stripped and fractional anisotropy (FA) maps were calculated for each participant fitting the diffusion tensor model at each voxel. The FA maps were then registered nonlinearly to the Montreal Neurological Institute standard space applying FSLs flirt and fnirt commands. Herein, stroke lesions were masked out and were not considered during the registration process.

The characterization of the CST and the 3 intrahemispheric connections between M1, PMv, and IPS bilaterally in the patients with stroke was conducted using structural and diffusion–weighted imaging data of 26 healthy controls taken from a previous study.[22] Herein, for the CST originating from M1, normalized and binarized group average tract masks of varying thresholds of the left and right average CST at the level from the mesencephalon to the cerebral peduncle (Montreal Neurological Institute coordinates, z=−25 to −20) were already available. These masks were used to calculate the mean FA for the ipsilesional and contralesional CST in the patients with stroke. CST integrity was finally reported as proportional FA values (ipsilesional/contralesional tract; see Texts II and III in the online-only Data Supplement for details on the mask creation of M1 and the CST tractography).

In addition to the CST, the available data set of the controls was also used to reconstruct 3 intrahemispheric corticocortical connections between M1, PMv, and IPS. The cortical seed mask for M1—biased to hand function and standardized in size and relation to the cortical gray matter/white matter boundary[8]—was taken from the previous study.[22] The same algorithm was now used to calculate additional PMv

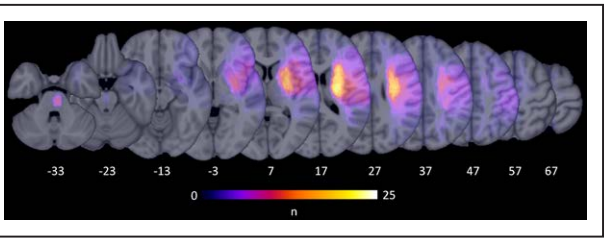

Figure 1. Stroke lesions. All masks of stroke lesions were brought to the right side and overlaid on a T1 template in Montreal Neurological Institute standard space. The color bar indicates the number of subjects (n) in which voxels lay within a stroke lesion.

and IPS masks for both hemispheres (Text II in the online-only Data Supplement). Probabilistic tractography was conducted in each of the 26 controls to reconstruct connections between M1 and PMv, PMv and IPS and between M1 and IPS within each hemisphere. An established pipeline, whose details are given in the Text IV in the online-only Data Supplement, was used to calculate common group average trajectories for each of the connections of 4 different spatial extents, that is, thresholds (Figure 2). These trajectories were used to calculate mean tract-related FA values across the whole tract in each patient and finally reported as proportional FA values (ipsilesional/contralesional hemisphere). When overlapping, the stroke lesion was not excluded in congruence with previous reports.[8] Consequently, mean proportional FA values reflected a combination of direct injury, as well as postinjury distant changes in fiber integrity. Because group average template tracts were used to read out individual tract FA values, proper congruence of the location and topography of the average tracts and the individual anatomy in the FA map was carefully checked before statistical analysis. In some participants, gyral anatomy particularly around the hand knob, the inferior precentral gyrus, and the postcentral sulcus was different from the Montreal Neurological Institute template; hence, some average tracts occasionally resided in sulci in single patients. Consequently, focal brain morphology was considered out of the normal range despite good overall registration to standard Montreal Neurological Institute and the proportional FA values of such tracts were omitted from the final analysis. Table I in the online-only Data Supplement summarizes which tract-related proportional FA values of which patients with stroke could be finally used in the analyses.

Statistical Analysis

The data were analyzed using R statistical package 3.1.3. In a first analysis, linear mixed-effects modeling with repeated measures was used for the analysis of the proportional FA values of the different tracts of interest in the patients with stroke. Relevant white matter disintegrity was assumed when the proportional FA values were significantly lower than 1 (Text V in the online-only Data Supplement). In a second analysis, 3 separate multiple linear regression models were fitted using R's lm for the proportional FA value of each of the 3 corticocortical tracts (TRACT), with MO (log-transformed) as the dependent variable and CST integrity, as well as the interaction CST*TRACT as independent variables. Nonsignificant interactions or main effects were kept given the primary focus of this study to investigate cross-network interactions after stroke. Cross-correlations between CST and corticocortical tract FA values were moderate (Pearson's R, all ≤0.35). For both analyses, age, hemisphere (dominant or nondominant hemisphere lesioned), and time after stroke were included as additional independent variables to adjust the target effects.[8,22] Estimated coefficients are given with 95% confidence interval. For visualization, mean correlations between TRACT and MO were estimated for 4 arbitrary CST integrities, that is, proportional FA values between 0.6 and 0.9 with higher values indicating better CST tract integrity. Behavioral scores are given as mean±SD. Statistical significance was assumed at $P<0.05$.

Results

Probabilistic Tractography and Tract-Related FA

Probable trajectories between M1 and PMv, PMv and IPS and between M1 and IPS were successfully obtained in the healthy participants. Figure 2 shows the group averages of the tracts with reasonable spatial reproducibility across the participants. Highest spatial homogeneity of the tracts was found

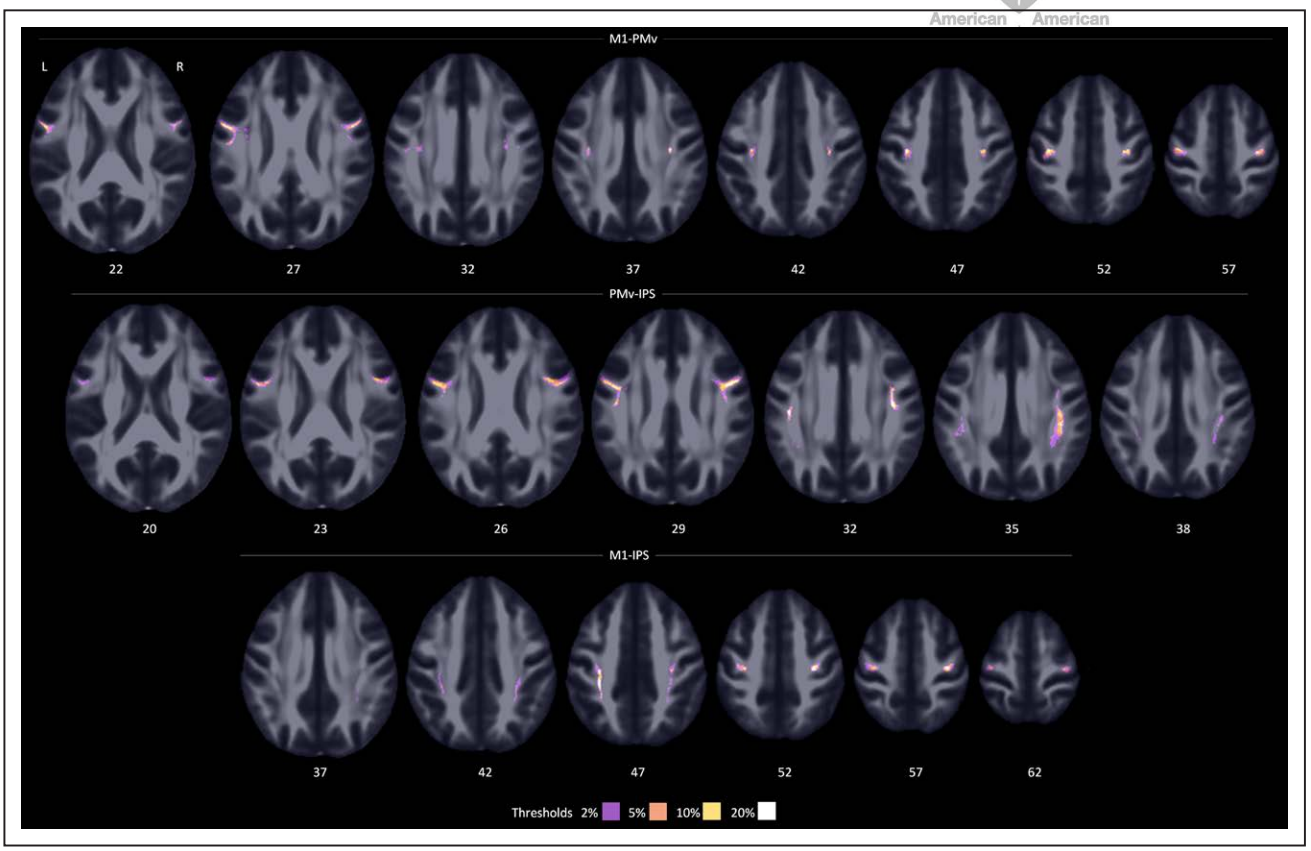

Figure 2. Group averaged trajectory map for intrahemispheric corticocortical motor connections. Probable courses of the intrahemispheric fiber tracts between primary motor (M1) and ventral premotor cortex (PMv; M1–PMv), PMv and intraparietal sulcus (IPS; PMv–IPS) and M1 and IPS (M1–IPS) as derived from probabilistic tractography in 26 healthy controls. Group average trajectories based on 4 different thresholds (see Methods section of this article) are overlaid on a fractional anisotropy template in Montreal Neurological Institute standard space. L denotes left hemisphere; and R, right hemisphere.

for M1–PMv with significant convergence of the trajectories toward M1 hand knob area and the anterior bank of the precentral gyrus for PMv. For PMv–IPS and M1–IPS, the variability in the trajectories toward IPS was higher, particularly for the left hemisphere. The trajectories for the CST have been already reported in a previous study.[22] Tract-related white matter integrity was significantly affected by stroke in all 4 tracts (<1), estimated mean proportional FA were 0.82 (95% confidence interval, 0.78–0.86) for CST, 0.88 (0.84–0.93) for M1–PMv, 0.85 (0.80–0.90) for PMv–IPS, and 0.92 (0.87–0.97) for M1–IPS. M1–IPS was significantly less affected than CST (P=0.02; corrected by false discovery rate). The other proportional FA values did not show significant differences. Tract-related FA values for both hemispheres are given in Table II in the online-only Data Supplement.

Interactions Between Corticospinal and Corticocortical Networks and Their Impact on Residual Motor Output

The main finding was a significant cross-network interaction between CST integrity and the microstructural state of the connection between M1 and PMv for residual motor output in patients with stroke (P=0.014; Table). Figure 3 visualizes this interaction showing that M1–PMv integrity specifically contributes to MO in patients in which the CST is more affected by the stroke. In contrast, in patients with more preserved CST integrity, M1–PMv integrity does not explain a relevant amount of variance in MO. A similar strong trend was also observed for PMv–IPS (P=0.064). The model M1–IPS did not show significance in regard to this interaction between the CST and the corticocortical pathway.

Table. Linear Regression Models

Model (n)	Predictor	Coefficient	95% Confidence Intervals		*P* Values
			Lower	Upper	
M1–PMv (n=42)	Age	–0.005	–0.014	0.004	0.295
	Dom	–0.231	–0.466	0.004	0.054
	Time	–0.005	–0.016	0.007	0.394
	CST	11.995	3.920	20.071	0.005*
	M1–PMv	9.292	2.304	16.280	0.011*
	CST*M1–PMv	–11.142	–19.858	–2.426	0.014*
	Intercept	–8.151	–14.596	–1.706	0.015*
PMv–IPS (n=42)	Age	–0.004	–0.014	0.006	0.469
	Dom	–0.170	–0.423	0.082	0.179
	Time	–0.005	–0.016	0.007	0.429
	CST	7.993	1.407	14.579	0.019*
	PMv–IPS	5.361	–0.310	11.032	0.063
	CST*PMv–IPS	–6.694	–13.803	0.415	0.064
	Intercept	–4.749	–9.979	0.482	0.074
M1–IPS (n=31)	Age	0.001	–0.014	0.016	0.903
	Dom	–0.153	–0.526	0.219	0.404
	Time	–0.004	–0.022	0.013	0.625
	CST	10.928	–0.892	22.748	0.068
	M1–IPS	7.084	–2.880	17.048	0.155
	CST*M1–IPS	–9.859	–22.320	2.602	0.116
	Intercept	–6.719	–16.231	2.794	0.158

Individual models (with n sample size) were fitted for each corticocortical tract to investigate corticospinal-corticocortical network interactions after stroke. The composite motor output score (MO, log-transformed, see Statistics) was the dependent variable. Corticospinal tract (CST), M1–PMv, PMv–IPS and M1–IPS tract-related white matter integrity (included as proportional fractional anisotropy values), Dom-information whether the dominant or nondominant hemisphere was lesioned (reference is nondominant). Time indicates time after stroke in days. Coefficients are given with upper and lower border of 95% confidence intervals. Adjusted R^2 and P values for the individual models were 0.43 (P<0.001) for M1–PMv, 0.31 (P=0.003) for PMv–IPS, and 0.09 (P=0.23) for M1–IPS. IPS indicates intraparietal sulcus; M1, primary motor; and PMv, ventral premotor cortex.

Discussion

The main finding of this study was a significant cross-network interaction between the CST and the corticocortical connection between M1 and PMv in relation to residual motor output after stroke. The data indicate that the microstructural state of M1–PMv seems to play a relevant role for residual motor output in patients with stroke with a significant damage to the CST. In contrast, in patients with slightly affected CST, the microstructural state of M1–PMv did not seem to explain a relevant amount of variance in motor outcome.

This finding extends the present knowledge about the interrelationships between the microstructural states of the motor networks at the corticospinal and corticocortical level and motor output after stroke and might have also implications for our understanding of recovery processes and ultimately treatment protocols by means of noninvasive brain stimulation.

Network Alterations After Stroke

Previous structural imaging studies have mainly investigated the influence of the CST for motor output after stroke showing that better CST integrity relates to better outcome. More recently, structural alterations of corticocortical connections between primary and secondary motor areas, such as M1, the dorsal and ventral premotor areas, and also posterior parietal brain regions, have been assessed in more detail as well.[2,8]

Importantly though, these analyses have largely neglected potential interdependencies between the different networks at the corticospinal and corticocortical level in relation to motor outcome. Thus, the isolated consideration of single networks will lead to findings whose interpretation might be limited. Therefore, the simultaneous consideration of corticocortical connections, especially together with the CST as the major output pathway might provide novel insights into brain reorganization after stroke. Relevant questions arising are (1) to what extent the microstructural state of premotor–motor connections would add to explain variance to the known involvement of the CST and (2) whether the relevance of these premotor–motor connections would depend on the magnitude of the CST damage or whether it would be a rather general phenomenon. To date, only the first question has been experimentally addressed in few trials by investigating

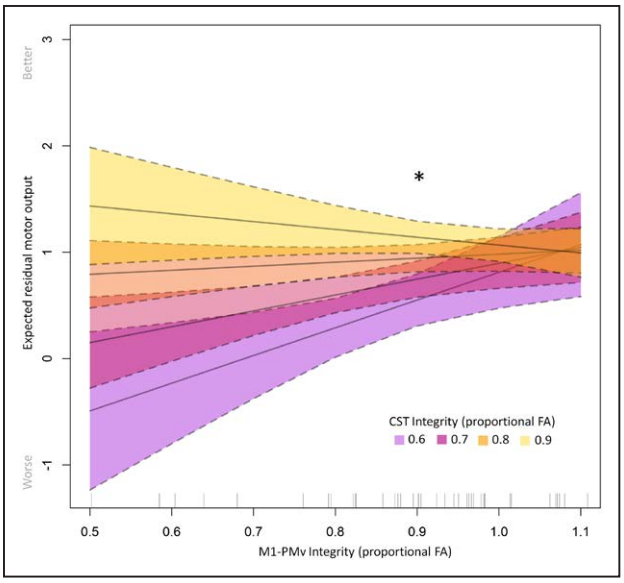

Figure 3. Cross-network interactions for the connection (M1–PMv) and corticospinal tract (CST) after stroke. Effect plot for the significant cross-network interaction between the microstructural integrity of the CST and the microstructural state of the cortico-cortical connection between PMv and M1 (estimated correlations for 4 different proportional fractional anisotropy [FA] values of the CST between 0.6 and 0.9) significantly related to expected composite motor output after stroke (dimensionless, log-transformed, see Statistics). It shows that the integrity of M1–PMv is positively associated with motor output in patients with disrupted CST (low proportional FA values). In contrast, patients with more preserved CST integrity (higher proportional FA values) do not show this positive influence of M1–PMv integrity. Plot shows the estimated means (solid lines) with 95% confidence interval (dotted lines). *Significant interaction CST*M1–PMv as the basis for this visualization.

corticocortical networks under simultaneous consideration of the CST. For instance, a positive contribution to residual motor output in patients with chronic stroke has been reported for the microstructural state of ipsilesional parietofrontal pathways between the anterior IPS and PMv and particularly between PMv and M1, in fact in addition to that of the CST.[8] In line, it has been also shown that the combination of CST injury and functional connectivity between ipsilesional M1 and the premotor cortex was a better marker of the residual motor status than either measure alone.[12] A similar additive effect has been obtained for the combined analysis of CST integrity and interhemispheric connectivity.[9] However, to date, the question whether the magnitude of damage to the CST would determine the relevance of corticocortical structural connections for motor output has not yet been addressed in detail.

Cross-Network Interactions Between Corticospinal and Corticocortical Networks After Stroke

The goal of the present study was to tackle this open question. We hypothesized that the contribution of corticocortical connections to motor output would depend on the state of the CST, which would go beyond a simple additive effect in correlating brain structure of different motor tracts with behavior. Indeed, such cross-network interaction was present for M1–PMv and CST, providing a novel insight into the relevance of premotor–motor connections for motor output after stroke. This effect seemed to be specific to M1–PMv and not a general phenomenon for any parietofrontal connections which supports the view of M1–PMv as an important pathway for recovered hand function after stroke.[8] Previous studies have consistently reported that ipsilesional premotor areas such as PMv and their interplay with M1 are contributing to motor function, spontaneous recovery,[6] and also motor learning after stroke.[23] It has been commonly assumed that this interplay was related to the reorganization of cortical motor networks in patients with stroke with varying degrees of motor impairment.[6,8] However, the present data now suggest that such relationships between premotor–motor connectivity and motor output might not hold true for all patients independent of the impairment of their CST and the level of motor deficit, respectively. In contrast, the interplay between premotor areas and M1 might be rather relevant in patients with significant damage to the CST. Notably, this would further strengthen our concept that secondary motor areas in the frontal lobe undergo plastic changes after stroke to support motor output particularly in those patients with the biggest demand for supportive cortical motor areas. Prospective future studies will have to validate our model arguing that patients with high lesion load to the CST but preserved microstructural state of M1–PMv would show superior residual motor function compared with patients with disrupted M1–PMv connectivity. In patients with well-preserved CST integrity, most likely also less affected in average, the state of M1–PMv fiber tracts would not influence residual motor output. The present evidence of significant structural cross-network interactions is also supported by a functional imaging study, which has found that interhemispheric coupling strength correlated with motor function only in patients with intact CST, whereas patients with disrupted CST did not show a similar association.[16]

Potential Implications of Cross-Network Interactions

The present findings might have interesting implications for the refinement of prediction models for stroke recovery, on the one hand, and, on the other hand, for research focusing on patient-tailored treatment protocols by means of noninvasive brain stimulation. First, there is already stimulating data for prediction models of motor recovery based on clinical scores and electrophysiological and structural measures of CST integrity.[3,24,25] These models are designed to be workable in the clinical setting, as analyses of the CST as a potential biomarker can be easily conducted in automated frameworks. However, by focusing only on the CST, the prediction might be limited,[25] and the inclusion of information on corticocortical networks and their interactions with the CST as additional biomarkers could improve these models, a matter that has to be tested prospectively. Second, noninvasive brain stimulation techniques have been increasingly developed to enhance motor recovery after stroke.[4] To date, these approaches have been generally applied independently of specific patient characteristics. Neglecting them, which could have an impact on the selection of the stimulation site or the protocol, might be one possible explanation for the heterogeneous responses, even in the view of

responders and nonresponders in brain stimulation trials in patients with stroke. Data based on the present analytic approach might provide a rational for patient stratification to predict individual responses to stimulation or to determine the best cortical site for an intervention. In fact, in terms of M1 stimulation—the most widely used approach to enhance motor recovery—responses are particularly weak in patients with serious impairment and high lesion load to the CST.[26] Consequently, where should we stimulate severely affected patients with heavily disrupted CST? In line with the present results, it has been already discussed that premotor areas such as PMv might be alternative targets as these areas normally have a higher probability of survival, they might act via parallel corticospinal pathways bypassing the disrupted CST originating from M1 as defined at the beginning, and, for example, PMv might have a high potential for plastic reorganization.[27] Indeed, first proof-of-concept studies have indicated that this type of stimulation might be a safe and reasonable approach to enhance motor recovery in patients with rather severe deficits.[28] Based on the present data, we would hypothesize that facilitating stimulation of ipsilesional PMv, for example, by means of anodal transcranial direct current stimulation would result in significant behavioral effects particularly in patients with disrupted CST on the one hand and high tract-related FA of M1–PMv on the other hand. In these patients, only PMv stimulation might drive adaptive plastic alterations both at the level of preserved corticospinal fiber tracts originating from PMv and also at the level of residual premotor–motor fibers targeting intact M1 neurons. In contrast, in patients with either well-preserved CST or high lesion load to the CST and concurrently disconnected M1 and PMv, we hypothesize that PMv stimulation might only show marginal effects. Accordingly, facilitating ipsilesional M1 stimulation might be preferentially efficient in patients with well-preserved CST, independent from the microstructural state of M1–PMv. Prospectively tested, such models might pave the way of precision medicine by tailoring interventional strategies for motor recovery after stroke.[29]

Limitations

Some important limitations of the present study are worth to consider. First, the present study was conducted in the subacute stage after stroke. Whether the present findings of relevant cross-network interactions also hold true for earlier stages or the chronic phase of recovery are open questions for future research. Second, group average templates of cortico-cortical and CSTs from healthy controls were used to infer tract-related white matter properties of different motor pathways in a large group of patients with stroke. Probabilistic and averaged in nature, these tracts might differ to individual brain anatomy on the one hand. On the other, such approaches will allow us to perform the necessary more complex analyses on larger samples. Third, based on clear a priori hypotheses,[8] we focused on the CST and defined parietofrontal motor connections at the cortical level. The present findings are not exhaustive, and the state of other parts of the motor network, such as frontal or prefrontal parts, and other cortico-subcortical or cortico-cerebellar circuits might also influence comparable analyses.

Sources of Funding

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government, Ministry of Science, ICT and Future Planning (MSIP, NRF-2017R1A2A1A05000730 to Dr Kim).

Disclosures

None.

References

1. Rehme AK, Grefkes C. Cerebral network disorders after stroke: evidence from imaging-based connectivity analyses of active and resting brain states in humans. *J Physiol*. 2013;591:17–31. doi: 10.1113/jphysiol.2012.243469.
2. Koch P, Schulz R, Hummel FC. Structural connectivity analyses in motor recovery research after stroke. *Ann Clin Transl Neurol*. 2016;3:233–244. doi: 10.1002/acn3.278.
3. Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. *Brain*. 2012;135(pt 8):2527–2535. doi: 10.1093/brain/aws146.
4. Schulz R, Gerloff C, Hummel FC. Non-invasive brain stimulation in neurological diseases. *Neuropharmacology*. 2013;64:579–587. doi: 10.1016/j.neuropharm.2012.05.016.
5. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. *Physiol Behav*. 2002;77:677–682. doi: 10.1016/S0031-9384(02)00929-0.
6. Rehme AK, Eickhoff SB, Wang LE, Fink GR, Grefkes C. Dynamic causal modeling of cortical activity from the acute to the chronic stage after stroke. *Neuroimage*. 2011;55:1147–1158. doi: 10.1016/j.neuroimage.2011.01.014.
7. Schulz R, Braass H, Liuzzi G, Hoerniss V, Lechner P, Gerloff C, et al. White matter integrity of premotor-motor connections is associated with motor output in chronic stroke patients. *Neuroimage Clin*. 2015;7:82–86. doi: 10.1016/j.nicl.2014.11.006.
8. Schulz R, Koch P, Zimerman M, Wessel M, Bönstrup M, Thomalla G, et al. Parietofrontal motor pathways and their association with motor function after stroke. *Brain*. 2015;138(pt 7):1949–1960. doi: 10.1093/brain/awv100.
9. Volz LJ, Sarfeld AS, Diekhoff S, Rehme AK, Pool EM, Eickhoff SB, et al. Motor cortex excitability and connectivity in chronic stroke: a multimodal model of functional reorganization. *Brain Struct Funct*. 2015;220:1093–1107. doi: 10.1007/s00429-013-0702-8.
10. Carter AR, Astafiev SV, Lang CE, Connor LT, Rengachary J, Strube MJ, et al. Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke. *Ann Neurol*. 2010;67:365–375. doi: 10.1002/ana.21905.
11. Rosso C, Valabregue R, Attal Y, Vargas P, Gaudron M, Baronnet F, et al. Contribution of corticospinal tract and functional connectivity in hand motor impairment after stroke. *PLoS One*. 2013;8:e73164. doi: 10.1371/journal.pone.0073164.
12. Wu J, Quinlan EB, Dodakian L, McKenzie A, Kathuria N, Zhou RJ, et al. Connectivity measures are robust biomarkers of cortical function and plasticity after stroke. *Brain*. 2015;138(pt 8):2359–2369. doi: 10.1093/brain/awv156.
13. Kang N, Summers JJ, Cauraugh JH. Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2016;87:345–355. doi: 10.1136/jnnp-2015-311242.
14. Nicolo P, Ptak R, Guggisberg AG. Variability of behavioural responses to transcranial magnetic stimulation: origins and predictors. *Neuropsychologia*. 2015;74:137–144. doi: 10.1016/j.neuropsychologia.2015.01.033.
15. Li LM, Uehara K, Hanakawa T. The contribution of interindividual factors to variability of response in transcranial direct current stimulation studies. *Front Cell Neurosci*. 2015;9:181. doi: 10.3389/fncel.2015.00181.
16. Carter AR, Patel KR, Astafiev SV, Snyder AZ, Rengachary J, Strube MJ, et al. Upstream dysfunction of somatomotor functional connectivity after corticospinal damage in stroke. *Neurorehabil Neural Repair*. 2012;26:7–19. doi: 10.1177/1545968311411054.
17. Vingerhoets G. Contribution of the posterior parietal cortex in reaching, grasping, and using objects and tools. *Front Psychol*. 2014;5:151. doi: 10.3389/fpsyg.2014.00151.

18. Inman CS, James GA, Hamann S, Rajendra JK, Pagnoni G, Butler AJ. Altered resting-state effective connectivity of fronto-parietal motor control systems on the primary motor network following stroke. *Neuroimage*. 2012;59:227–237. doi: 10.1016/j.neuroimage.2011.07.083.
19. Wang L, Yu C, Chen H, Qin W, He Y, Fan F, et al. Dynamic functional reorganization of the motor execution network after stroke. *Brain*. 2010;133(pt 4):1224–1238. doi: 10.1093/brain/awq043.
20. Rehme AK, Eickhoff SB, Rottschy C, Fink GR, Grefkes C. Activation likelihood estimation meta-analysis of motor-related neural activity after stroke. *Neuroimage*. 2012;59:2771–2782. doi: 10.1016/j.neuroimage.2011.10.023.
21. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed*. 2002;15:435–455. doi: 10.1002/nbm.782.
22. Schulz R, Frey BM, Koch P, Zimerman M, Bönstrup M, Feldheim J, et al. Cortico-cerebellar structural connectivity is related to residual motor output in chronic stroke. *Cereb Cortex*. 2017;27:635–645. doi: 10.1093/cercor/bhv251.
23. Johansen-Berg H, Dawes H, Guy C, Smith SM, Wade DT, Matthews PM. Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. *Brain*. 2002;125(pt 12):2731–2742. doi: 10.1093/brain/awf282.
24. Byblow WD, Stinear CM, Barber PA, Petoe MA, Ackerley SJ. Proportional recovery after stroke depends on corticomotor integrity. *Ann Neurol*. 2015;78:848–859. doi: 10.1002/ana.24472.
25. Buch ER, Rizk S, Nicolo P, Cohen LG, Schnider A, Guggisberg AG. Predicting motor improvement after stroke with clinical assessment and diffusion tensor imaging. *Neurology*. 2016;86:1924–1925. doi: 10.1212/WNL.0000000000002675.
26. Lindenberg R, Zhu LL, Rüber T, Schlaug G. Predicting functional motor potential in chronic stroke patients using diffusion tensor imaging. *Hum Brain Mapp*. 2012;33:1040–1051. doi: 10.1002/hbm.21266.
27. Plow EB, Cunningham DA, Varnerin N, Machado A. Rethinking stimulation of the brain in stroke rehabilitation: why higher motor areas might be better alternatives for patients with greater impairments. *Neuroscientist*. 2015;21:225–240. doi: 10.1177/1073858414537381.
28. Cunningham DA, Varnerin N, Machado A, Bonnett C, Janini D, Roelle S, et al. Stimulation targeting higher motor areas in stroke rehabilitation: a proof-of-concept, randomized, double-blinded placebo-controlled study of effectiveness and underlying mechanisms. *Restor Neurol Neurosci*. 2015;33:911–926. doi: 10.3233/RNN-150574.
29. Koch PJ, Hummel FC. Toward precision medicine: tailoring interventional strategies based on noninvasive brain stimulation for motor recovery after stroke. *Curr Opin Neurol*. 2017;30:388–397. doi: 10.1097/WCO.0000000000000462.

Stroke
Journal of the American Heart Association

Interactions Between the Corticospinal Tract and Premotor–Motor Pathways for Residual Motor Output After Stroke

Robert Schulz, Eunhee Park, Jungsoo Lee, Won Hyuk Chang, Ahee Lee, Yun-Hee Kim and Friedhelm C. Hummel

Stroke. published online September 13, 2017;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

Data Supplement

Interactions Between the Corticospinal Tract and Premotor-Motor Pathways for Residual Motor Output After Stroke

Robert Schulz, MD; Eunhee Park, MD, PhD; Jungsoo Lee, PhD; Won Hyuk Chang, MD, PhD; Ahee Lee, MS; Yun-Hee Kim, MD, PhD*; Friedhelm C. Hummel, MD*

* contributed equally

Table I | Clinical Data & Model Contribution

Clinical characteristics are given for each patient. M male, F female. Stroke locations are given: BG Basal ganglia, CR Corona radiata, F Frontal lobe, IC Internal capsule, P Parietal lobe, SC Striatocapsular, T Temporal cortex, TH Thalamus, PO Pons, PI Peri-insula, CI Cingulate cortex, S1 Primary sensory cortex. Time as days after stroke. Dom. Hem. indicates whether the dominant hemisphere was lesioned (1) or not lesioned (0). Grip summarizes whole-hand grip force as proportional values of the affected and unaffected hand. UEFM Fugl-Meyer score of the upper extremity (range 0-66). The information "Model Inclusion" summarizes which tract-related FA values could be considered in the final analyses. CST corticospinal tract, M1 primary motor cortex, PMv ventral premotor cortex, IPS intraparietal sulcus. Mean values are given ±SD.

ID	**Clinical characteristics**							*Model Inclusion*			
	Age	**Sex**	**Location**	**Time**	**Dom. Hem.**	**Grip**	**UEFM**	*CST*	*M1-PMv*	*PMv-IPS*	*M1-IPS*
1	33	M	PO	93	1	0.14	62	x	x	x	x
2	35	M	CR	94	0	0.13	42	x	x	x	x
3	54	M	CR,SC	112	0	0.00	30	x	x	x	
4	63	M	CR	88	1	0.04	21	x	x	x	
5	60	M	F,P,T	100	0	0.00	46	x	x	x	x
6	60	M	SC	113	0	0.00	14	x	x	x	
7	79	F	CR,SC	103	0	0.05	37	x	x	x	
8	48	F	CR,SC	98	0	0.01	41	x	x	x	
9	71	F	PO	93	0	0.07	58	x	x	x	x
10	53	F	TH	87	0	1.16	60	x	x	x	x
11	75	M	SC	90	0	0.11	58	x	x	x	
12	66	M	CR	88	0	0.10	55	x	x	x	x
13	65	F	SC	80	0	0.89	66	x	x	x	x
14	66	M	F,P,CR,SC	115	1	0.34	66	x		x	
15	55	M	SC	98	1	0.69	62	x			
16	60	F	PO	93	1	0.31	59	x	x	x	x
17	70	M	PO	84	1	0.67	66	x			
18	67	F	T,PI,CR,SC	91	1	0.00	4	x	x		
19	55	F	CR	99	0	0.09	45	x	x	x	
20	54	M	SC	94	0	0.68	57	x			x
21	66	M	PO	77	1	0.21	50	x	x	x	x
22	47	F	SC	115	0	0.11	59	x			
23	55	M	F,CI	105	1	0.71	43	x			x
24	37	F	SC	112	1	0.60	60	x	x	x	x
25	62	M	PI	92	1	0.00	20	x	x	x	x
26	52	M	SC	97	1	0.20	55	x			x
27	52	F	CR	96	0	0.10	34	x	x	x	
28	67	M	PO	94	0	0.00	20	x	x	x	x
29	35	F	PI	101	1	0.00	14	x	x	x	x
30	68	M	SC	94	0	0.17	58	x	x	x	
31	42	F	PI	110	1	0.00	20	x	x	x	x
32	45	F	CR	104	0	0.08	47	x	x		
33	69	F	PO	92	0	0.00	11	x			
34	69	F	SC	109	1	0.00	40	x		x	
35	49	M	PO	88	0	0.27	36	x	x	x	x
36	67	F	CR	98	0	0.00	18	x	x	x	x
37	52	F	CR	85	0	0.00	16	x	x	x	x
38	40	M	PI	89	0	0.57	50	x	x	x	x
39	58	F	CR	104	1	0.00	4	x	x	x	
40	64	M	CR	92	1	0.14	24	x	x	x	x
41	68	M	PO	93	0	0.00	20	x	x	x	x
42	69	M	PO	93	1	0.00	30	x	x	x	x
43	40	M	F,CI	87	0	0.66	57	x	x	x	x
44	67	M	CR	105	1	0.00	17	x	x	x	x
45	69	M	PO	106	0	0.26	66	x	x	x	
46	79	F	PO	124	0	0.00	24	x			
47	62	M	PO	137	0	0.05	44	x			
48	77	M	CR	117	0	0.75	55	x	x	x	x
49	70	F	S1	129	0	0.23	55	x	x	x	x
50	80	F	CR	99	1	0.06	54	x	x	x	x
51	67	M	CR,SC	112	1	0.00	4	x	x	x	
52	73	F	SC	81	1	0.21	55	x	x	x	x
53	54	M	PO	85	1	0.01	53	x	x	x	x
Mean±SD	**60±11**	**30 M**		**99±12**	**23 D**	**0.2±0.3**	**40.8±19.1**				

Text I | Brain Imaging – Sequence Parameters

In the patients with stroke, diffusion-weighted images were acquired using a 3T Phillips ACHIEVA MRI scanner (Philips Medical Systems, Best, The Netherlands). Seventy-five axial slices were obtained covering the whole brain with gradients (b = 1000 s/mm^2) applied along 45 non-collinear directions with the following sequence parameters; repetition time (TR) = 8770 ms, echo time (TE) = 60 ms, field of view (FOV) = 220 x 220 mm, slice thickness (ST) = 2.25 mm, in-plane resolution (IPR) = 1.96 x 1.96 mm. For the controls, diffusion-weighted and high-resolution T1-weighted anatomic images were available from a previous study and were acquired using a 3T Siemens Skyra MRI scanner (Siemens, Erlangen, Germany). For the former, 75 axial slices were obtained covering the whole brain with gradients (b = 1500 s/mm^2) applied along 64 non-collinear directions with the following parameters: TR = 10000 ms, TE = 82 ms, FOV = 256 x 204 mm, ST = 2.0 mm, IPR = 2 x 2 mm. For the latter, a three-dimensional magnetization-prepared, rapid acquisition gradient-echo sequence (MPRAGE) was used with the following parameters: TR = 2500 ms, TE = 2.12 ms, FOV = 256 x 208 mm, 256 axial slices, ST = 0.94 mm, IPR = 0.83 x 0.83 mm [1].

Text II | Creation of cortical seed masks

To calculate cortical seed regions for tractography in the controls, the T1 structural image was used for brain segmentation into white and gray matter. The Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/) was used for subsequent automatic cortical parcellation. Selected surface-based cortical gray matter masks were then transferred to FSL's MNI-T1 1mm standard space and used to calculate individual masks related to the white matter and gray matter boundary including the whole extent of M1, PMv and IPS: For M1 and PMv, a mask covering the precentral gyrus was used. The creation of the masks were guided by anatomic and connectivity based suggestions for parcellation for M1 [2] and PMv [3]. For IPS, the labels *11157* and *12157*, respectively, from *aparc2009* parcellation was considered to calculate a mask covering the whole extent of IPS. Based on this information, each cortical mask was produced for the individual control subject in a standardized semi-automated fashion. The multiplication of the masks with the individual binarized FA map, thresholded at values of >0.1, was conducted to include only seed voxels with a reasonable connection to major white matter trajectories. For M1 and PMv, the tract reconstruction was additionally biased toward hand representations. Therefore, functional imaging [4] and electrophysiological [5–8] data were incorporated into the mask creation. [9,10]: 500 voxels adjacent to peak coordinates of interest (Supplementary Tab. III) within M1 and PMv were automatically selected using an in-house Matlab script (Matlab R2010b, Mathworks, US) providing smaller motor masks for M1 and PMv related to hand function and standardized in size and relation to the cortical white matter / gray matter boundary. This information is adapted from our previous report [1].

Text III | Probabilistic Tractography of the Corticospinal Trajectories

The CST reconstruction had been already conducted in the 26 healthy controls [1]: In brief, seeding from the hand representation in M1 (see Text 1), 50000 streamline were sent downwards applying masks of the posterior limb of the internal capsule, the pontomedullar junction and the ventral medulla oblongata as waypoint masks. Together with interhemispheric and basal ganglia exclusion masks this approach has been found to allow distinguish corticospinal and interfering corticofugal fibers. After thresholding each output distribution (0.1%, 0.5%, 1% and 2%, respectively, minimum 5) of the overall streamlines (*waytotal*) and binarization, the common group average was calculated – as for the corticocortical connections - for each of the four thresholds by taking the sum of all individual threshold- and subject-specific trajectories. Only those voxels were considered to belong to the CST which were found in at least 65% of the number of subjects contributing to the tract, in agreement with previous studies [9]. For the stroke patients the binarized CSTs were finally used to estimate threshold-specific individual FA values at the level from the mesencephalon to the cerebral peduncle (MNI coordinates z=-25 to z=-20). Finally tract-related FA values were averaged across the four thresholds and reported as proportional values (ratio ipsilesional / contralesional hemisphere). This information is adapted from a previous report [1].

Text IV | Probabilistic Tractography of the Corticocortical Trajectories

Probabilistic tractography was used to reconstruct probable connections between (i) M1 and PMv, (ii) PMv and IPS and (iii) M1 and IPS in the healthy controls. For tractography, a large amount of streamlines were sent bi-directionally from the cortical seed and target regions (50000 samples from M1 and PMv, 5000 samples from IPS due to its large extent). Interhemispheric and subcortical exclusion masks (basal

ganglia, brainstem) were used to guide the tractography. After normalization by *waytotal*, the connectivity distributions of both directions were combined via (A+B)-(A*B) and thresholded by four different levels (T1-T4, 2%, 5%, 10% and 20% [10] of the maximum value [11] as objective guidelines lack how to threshold probabilistic tractography distributions. After that, each tract (T1-T4) was summed across the 26 controls and finally constrained by 65% [10] of the number of participants contributing to that tract and binarized. Based on each of the resulting standard tracts, the tract-related fractional anisotropy (FA) – a measure of white matter integrity - was estimated in each stroke patient for each threshold (T1-T4) and averaged across the four thresholds for both the ipsilesional and contralesional hemispheres. In agreement with previous studies, proportional FA values (ratio the ipsilesional/contralesional hemisphere) were calculated to assess FA asymmetry and used for the correlative analyses [9]. This information is adapted from a previous report [1].

Text V | Statistics: Tract-related FA Values in the Stroke Patients

To analyze the proportional FA values for the CST (n=53) and the three corticocortical connections M1-PMv (n=42), PMv-IPS (n=42) and M1-IPS (n=31), R's *lmer* was used to estimate one combined linear mixed-effects model with repeated measures. Estimated means are given for all tracts with 95% confidence intervals (CI), corrected for age, the information whether the dominant or non-dominant hemisphere was lesioned and time after stroke. Post-hoc multiple pairwise comparisons of the means were conducted using *lsmeans* and false-discovery rate (FDR) correction.

Table II | Tract-Related FA Values in the Stroke Patients

Estimated means of absolute FA values are given for each of the tracts with 95% CI based on one combined linear mixed-effect analysis with repeated measures. The model revealed a significant hemisphere*tract interaction ($p<0.001$). The control variables age, dominance and time were not significant but kept in the model to adjust the target effects. Tract-specific differences between FA-values of the ipsilesional and contralesional hemispheres were compared pairwise, *P*-values are given after FDR correction for four tests.

Tract	Hemisphere	Est. Mean	95% CI	
			Lower	Upper
CST (n=53)	Ipsilesional	0.487	0.471	0.503
	Contralesional	0.593	0.578	0.609
	P-value	<0.001		
M1-PMv (n=42)	Ipsilesional	0.366	0.349	0.384
	Contralesional	0.417	0.400	0.434
	P-value	<0.001		
PMv-IPS (n=42)	Ipsilesional	0.357	0.340	0.374
	Contralesional	0.419	0.401	0.436
	P-value	<0.001		
M1-IPS (n=31)	Ipsilesional	0.386	0.367	0.406
	Contralesional	0.422	0.402	0.441
	P-value	0.040		

Table III | MNI-Coordinates Used for Calculating the Motor Masks

Functional and electrophysiological data were included in seed and target mask creation to bias the tract reconstruction toward the hand representation in the M1 and PMv motor masks. Therefore 500 voxels adjacent to peak coordinates were selected automatically. Notably, for the PMv coordinate, MNI coordinates from four studies were averaged. MNI coordinates (x,y,z) are given for right and left hemisphere.

Mask	Coordinates (x,y,z)
M1	(37, -25, 62); (-37, -25, 62) [4]
PMv	(57, 14, 21); (-57, 14, 21) [5–8]

References:

1. Schulz R, Frey BM, Koch P, Zimerman M, Bönstrup M, Feldheim J, et al. Cortico-Cerebellar Structural Connectivity Is Related to Residual Motor Output in Chronic Stroke. *Cereb. Cortex*. 2017;27:635–645.
2. Geyer S, Ledberg A, Schleicher A, Kinomura S, Schormann T, Bürgel U, et al. Two different areas within the primary motor cortex of man. *Nature*. 1996;382:805–7.
3. Tomassini V, Jbabdi S, Klein JC, Behrens TEJ, Pozzilli C, Matthews PM, et al. Diffusion-weighted imaging tractography-based parcellation of the human lateral premotor cortex identifies dorsal and ventral subregions with anatomical and functional specializations. *J. Neurosci.* 2007;27:10259–69.
4. Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE. Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage*. 2006;31:1453–74.
5. Davare M, Andres M, Cosnard G, Thonnard J-L, Olivier E. Dissociating the role of ventral and dorsal premotor cortex in precision grasping. *J. Neurosci.* 2006;26:2260–8.
6. Davare M, Lemon R, Olivier E. Selective modulation of interactions between ventral premotor cortex and primary motor cortex during precision grasping in humans. *J. Physiol.* 2008;586:2735–42.
7. Davare M, Montague K, Olivier E, Rothwell JC, Lemon RN. Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. *Cortex.* 2009;45:1050–7.
8. Davare M, Rothwell JC, Lemon RN. Causal connectivity between the human anterior intraparietal area and premotor cortex during grasp. *Curr. Biol.* 2010;20:176–81.
9. Schulz R, Park C-H, Boudrias M-H, Gerloff C, Hummel FC, Ward NS. Assessing the Integrity of Corticospinal Pathways From Primary and Secondary Cortical Motor Areas After Stroke. *Stroke*. 2012;43:2248–2251.
10. Schulz R, Koch P, Zimerman M, Wessel M, Bönstrup M, Thomalla G, et al. Parietofrontal motor pathways and their association with motor function after stroke. *Brain*. 2015;138:1949–1960.
11. Hughes EJ, Bond J, Svrckova P, Makropoulos A, Ball G, Sharp DJ, et al. Regional changes in thalamic shape and volume with increasing age. *Neuroimage*. 2012;63:1134–42.