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Relationship Between Cortical Excitability Changes and Cortical Thickness in Subcortical Chronic Stroke

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Relationship Between Cortical Excitability Changes and Cortical Thickness in Subcortical Chronic Stroke

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Ischemic stroke leads to excitability changes of the motor network as probed by means of transcranial magnetic stimulation (TMS). There is still limited data that shows to what extent structural alterations of the motor network might be linked to excitability changes. Previous results argue that the microstructural state of specific corticofugal motor tracts such as the corticospinal tract associate with cortical excitability in chronic stroke patients. The relationship between changes of cortical anatomy after stroke, as operationalized by means of decreases or increases in local cortical thickness (CT), has scarcely been addressed. In the present study, we re-analyzed TMS data and recruitment curve properties of motor evoked potentials and CT data in a group of 14 well-recovered chronic stroke patients with isolated supratentorial subcortical lesions. CT data of the stroke patients were compared to CT data of 17 healthy controls. Whole-brain and region-of-interest based analyses were conducted to relate CT data to measures of motor cortical excitability and clinical data. We found that stroke patients exhibited significantly reduced CT not only in the ipsilesional primary motor cortex but also in numerous secondary motor and non-motor brain regions, particularly in the ipsilesional hemisphere including areas along the central sulcus, the inferior frontal sulcus, the intraparietal sulcus, and cingulate cortices. We could not detect any significant relationship between the extent of CT reduction and stroke-related excitability changes of the motor network or clinical scores.

Keywords: gray matter, cortex, recovery, motor, MRI, cortical excitability

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INTRODUCTION

Ischemic stroke leads to time- and recovery-dependent changes of motor cortical excitability which can be probed by means of transcranial magnetic stimulation (TMS). Motor evoked potentials (MEP) and recruitment curve properties have been considered as surrogates for the functional state of the motor network, including important corticofugal motor pathways such as the corticospinal tract (CST). Studies have related excitability measures to motor deficits and recovery processes after stroke (1–7). Over time, the field has moved from unimodal approaches to multimodal analyses of brain structure and function to better understand intersubject variability in stroke recovery (8). For instance, one study combined TMS and MRI to assess cortical excitability, interregional connectivity and damage to the CST and found that these factors accounted for more than 80% of

the variance in functional impairment in chronic stroke patients (9). Other studies have recently evidenced significant relationships between CST microstructure and cortical excitability (10, 11).

Apart from important motor pathways, such as the CST, stroke-related alterations of brain structure also affect local cortical anatomy: For instance, cortical thinning has been observed in primary and secondary motor and non-motor brain regions of the ipsi- and contralesional hemispheres (5, 12–18), predominantly in cortices directly connected to the stroke lesion (14, 19) or in the deepest layers of the motor cortices (13). By showing gradual cortical thickening in frontal and temporal cortices (20) or increases of cortical gray matter volume (21, 22), other studies have argued for the existence of neuroplastic brain alterations after stroke to promote recovery processes. However, the precise relationship between cortical thickness (CT) alterations—either loss or gain in CT—and motor recovery remains under debate. There are studies which have found significant associations between cortical anatomy and clinical scores (13, 16, 21, 22) and others which have not (5, 14, 15, 19, 20).

Given these data, the question arises whether CT alterations, particularly located in key motor areas, might explain intersubject variability in excitability of the motor network. Specifically, these areas might comprise the primary motor cortex and also frontal and parietal cortices of the ipsilesional hemisphere since studies have repeatedly shown that the parietofrontal motor network might be critically involved in motor functioning and recovery processes after stroke (23–25). The regional analysis of CT could add information at the cortex level to answer this question, thus supporting available data from previous functional imaging studies or structural imaging studies which focused on corticofugal or interregional corticocortical motor tracts (8). In fact, only a few studies have explored potential associations between CT and TMS-based measures of cortical excitability in stroke patients. For hand dexterity, Borich et al. found that the CT of the precentral gyrus together with cortical excitability were informative predictors. However, the authors did not address any potential interrelationship between both variables (26). Another study compared CT data of the ipsilesional primary motor cortex with its excitability but did not detect any association. As potential limitations, this analysis was constrained to the primary motor cortex and included patients with subcortical strokes but also patients with direct cortex involvement. CT was thinner in patients with cortical strokes (5). Notably, inter-study variability in the composition of the patient groups with lesion locations with (5, 16, 27, 28) or without direct damage to the cortex (12–15, 17–19, 29, 30) might be a relevant influential factor for variability in structure-function relationships. A comparison of different methods to estimate CT has indicated that directly damaged cortical areas exhibit an increased risk for invalid CT values due to local effects of the stroke lesion (31).

The present study was designed to shed new light on the potential relationship between regional CT alterations, changes of cortical excitability, and residual motor function after stroke. For that, we reanalyzed available TMS, MRI, and clinical data of a group of supratentorial subcortical chronic stroke patients. Specifically, high-resolution structural MRI data was used to quantify CT of the ipsilesional and contralesional hemispheres. Data of healthy participants of similar age and gender were used to determine brain regions with significant loss or gain in CT in the stroke cohort. Whole-brain and region-of-interest (ROI) based analyses were conducted to relate CT data to measures of cortical excitability and clinical data.

METHODS

Participants and Clinical Testing

This study is based on the cohort of chronic stroke patients of our previous report on the influence of cortico-cerebellar structural connectivity on excitability of the motor network (10). The original inclusion criteria were: first-ever supratentorial ischemic stroke with persistent hand motor impairment in the chronic stage of recovery ($\geq$ 6 months), 18 years of age or older, and no contraindication for TMS or MRI. In total, 18 patients were included in that study. In the present work, four patients were excluded due to cortical lesions that could have affected the interpretation of the results. Seventeen healthy participants of similar age and gender served as the control group. Distribution of stroke hemispheres regarding the dominant and non-dominant hemispheres were taken into account. Fourteen control participants were treated as having their dominant hemisphere being pseudo-affected by the stroke. All participants gave written informed consent according to the Declaration of Helsinki to participate in the study which was approved by the local ethics committee (PV5357). Patients underwent clinical testing including grip and pinch forces, Fugl-Meyer assessment of the upper extremity (UEFM), and the nine-hole peg test (NHP). Grip and pinch forces and NHP performances were obtained from both the affected and unaffected hands. These data have been already introduced by our previous report and are taken 1:1 (10).

TMS Data Acquisition and Analysis

Details of the TMS methodology can be found in our original publication (10). In brief, a Magstim 200 magnetic stimulator with a 70-mm figure-eight coil and electromyographic electrodes placed over the first dorsal interosseous muscle on both hands in a belly-tendon montage was used to collect MEP. The resting motor threshold (RMT) at the hotspot was determined to the nearest 1% of the maximum stimulator output (32). To calculate the properties of the MEP's recruitment curve (RC), blocks of 11 stimuli, in a pseudorandomized order to avoid hysteresis effects and with intensities ranging from 90 to 160% of RMT, were administered. RC data were acquired in the affected (AH) and unaffected hemispheres (UH). Signal software 4.05 (Cambridge, Electronic Design, Cambridge, UK) was used to analyze the data. The first trial of each block was discarded due to the possibility that the initial MEP showed higher amplitudes than subsequent responses. At least 5 trials of every intensity level (90–160% RMT) were used to fit the RC, except for one patient and one control in which a reduced range (90–150%) was used due to a high stimulation intensity at 160%. The RC data were plotted as intensity vs. MEP size (peak-to-peak amplitude). Each individual

TABLE 1 | Demographic and clinical data.

ID	Age	Gender	Tas (months)	DoHe	AH	Stroke location	LAC	Grip (Kg)		Pinch (Kg)		NHP (pegs/s)		UEFM
								AH	UH	AH	UH	AH	UH	
1	78	F	20	L	L	BG, IC	No	19.33	24.00	7.00	7.50	0.77	0.78	66
2	74	M	20	L	L	CR	Yes	42.00	34.67	10.67	8.83	0.57	0.54	66
3	55	M	66	L	L	BG, IC	No	46.33	45.33	12.17	11.33	0.65	0.89	66
4	75	M	58	L	R	PLIC	No	26.67	40.67	7.50	12.00	0.33	0.84	39
5	61	M	75	L	L	BG, IC	No	31.00	42.67	8.00	9.83	0.51	0.85	47
6	76	M	80	L	L	BG, IC	No	24.33	31.67	8.17	8.50	0.44	0.78	50
7	61	M	88	L	L	PLIC	No	31.00	34.67	8.33	9.67	0.65	0.67	64
8	73	M	62	R	R	BG, IC	No	31.33	35.33	9.83	10.67	0.60	0.72	63
9	60	M	9	L	L	TC	Yes	35.33	43.33	9.00	8.17	0.59	0.75	55
10	58	M	31	L	L	TC	Yes	24.00	22.33	7.17	6.17	0.91	0.86	66
11	64	M	7	L	R	BG, IC	Yes	4.33	21.33	4.00	6.67	0.50	0.79	52
12	63	M	11	L	L	BG, CR	No	20.33	46.00	3.00	8.00	0.53	1.00	51
13	84	F	23	L	L	BG, CR	Yes	9.33	15.00	4.00	6.33	0.43	0.73	39
14	54	M	29	L	L	CR, PLIC	Yes	17.33	42.00	6.33	8.83	0.57	0.73	59
Mean Stroke	66.86	M:86%	41.36	L:13	L:11	—	LAC:6	25.90*	34.21	7.51	8.75	0.58**	0.78	55.93
SD Stroke	±9.53	—	±28.78	—	—	—	—	±11.57	±10.03	±2.59	±1.80	±0.15	±0.11	±9.84
Mean Control	66.76	M:82%	—	L:16	L:13	—	—	34.80	33.22	7.33	7.18	0.77	0.73	66.00†
SD Control	±8.04	—	—	—	—	—	—	±12.17	±10.79	±2.42	±2.57	±0.18	±0.13	±0.00

Age in years, gender (M, male; F, female), time after stroke (tas, in months), dominant (DoHe) and affected (AH) hemisphere, stroke location (BG, basal ganglia; CR, Corona radiate; IC, internal capsule; TC, thalamocapsular; PLIC, posterior limb of the internal capsile), lacunar infarct (LAC), absolute grip and pinch force values for the affected (AH) and the unaffected (UH) hemisphere, nine-hole peg test (NHP), and the Fugl-Meyer assessment of the upper extremity. Significant group differences are indicated by asterisks ($p \leq 0.05$, **$p \leq 0.01$).*
† UEFM score of 66 was assumed for controls.

intensity was then fitted into a sigmoid Boltzmann function which allowed to estimate MEP_{max} (plateau of RC) and $Slope_{max}$ (maximum slope of RC), separately for AH and UH as stroke-related alterations has been evidenced for these two measures. Importantly, these data are taken 1:1 from our previous report which should be consulted by the interest reader for further details (10).

Brain Imaging and Cortical Thickness Analyses

A 3T Siemens Skyra MRI scanner (Siemens, Erlangen, Germany) and a 32-channel head coil were used to acquire high-resolution T1-weighted anatomical images by means of a three-dimensional magnetization-prepared, rapid acquisition gradient-echo sequence with the following parameters: TR = 2,500 ms, TE = 2.12 ms, FOV = 240 mm, 256 coronal slices with a voxel size of 0.8 x 0.8 x 0.9 mm. Datasets were processed with Freesurfer version 6.0.0 (http://surfer.nmr.mgh.harvard.edu/) using the default options to measure CT (33, 34). The reconstructions were visually inspected and, if required, manually corrected following established recommendations from the Freesurfer's documentation. To compare affected (AH) and unaffected hemispheres (UH), all images were registered to the Freesurfer's common space symmetrical template, *fsaverage_sym*, that allowed to flip all lesions to the left hemisphere (35). Therefore, in this study we treated the left hemisphere as the AH and the right hemisphere as the UH. Surface data were smoothed with a full-width-half-maximum Gaussian kernel of 10 mm.

Statistics

For group comparisons and correlative analyses with clinical and TMS data, we first followed a whole-brain approach. Using the Freesurfer's *mri_glmfit* utility, general linear models are specified for the whole brain's surface, which comprises CT estimates for every vertex of every subject. To evaluate CT differences between patients and controls, a first model with CT as the dependent variable was fit treating group as the factor of interest. Color-coded statistical parametric maps were displayed using *freeview*. To assess the relationship between CT and cortical excitability or motor function, similar separate models were fit with TMS measures of AH or clinical data of the affected hand treated as the independent variables. All models were adjusted for the effect of age. *P*-values in every model were corrected for multiple comparisons using a Z Monte Carlo simulation over 10,000 iterations with a cluster-forming threshold of $P < 0.0001$ (36). Statistical significance was assumed at cluster-wise corrected *P*-values < 0.05. Size of clusters and number of significant vertices are given in the results section.

In addition to these surface-based whole-brain analyses, a ROI analysis of primary and secondary motor areas was also conducted using R statistical package (version 4.0.3, https://www.r-project.org/). Mean CT values were extracted from clusters of the parietofrontal motor network and cingulate cortices exhibiting a significant loss in CT in the stroke patients compared to healthy controls. These data (now as independent explanatory variable of interest) were fit into separate multiple linear regression models to explain variability in cortical excitability

($Slope_{max}$, MEP_{max}) and clinical scores (maximum grip and pinch force, NHP of the affected hands, UEFM). The respective data of the unaffected hemisphere or hand and age were treated as covariates in line with our previous report (10). $Slope_{max}$ and MEP_{max} values were log-transformed to improve data distribution. Models were checked for normal distribution of residuals and relevant multi-collinearity was excluded by estimating the variation inflation factor. Cook's distance was used to check for outliers. Statistical significance was determined as $P < 0.05$. *P*-values are presented as uncorrected values.

RESULTS

Demographic and Clinical Data

Analyses were conducted in 14 patients (12 males, mean age 66.86 years, mean 41 months after stroke, one left-handed, 12 patients with lesions on their dominant hemisphere, 6 lacunar strokes). Demographic and clinical data of the patients and controls are given in **Table 1**. The distribution of the stroke lesions is shown in **Figure 1**.

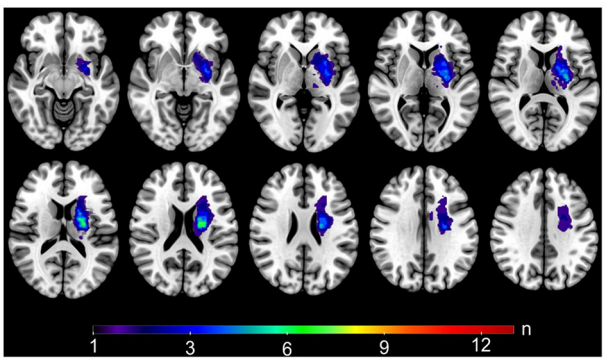

FIGURE 1 | Stroke lesions. Lesion mask overlap showing the distribution of the stroke lesions on a standard MNI template. For visualization purposes, all the masks were registered to the MNI space and flipped to the left hemisphere. Warm colors show areas of bigger overlap.

Cortical Thickness in Stroke Patients

Whole-brain analysis revealed a significantly reduced CT in patients when compared to controls, after accounting for age, in multiple cortical areas with predominance of the ipsilesional hemisphere (**Figure 2**). The largest clusters of significant CT reductions were found in the ipsilesional pre-, post-, and paracentral gyri, in the central and paracentral sulci, the cuneal gyrus and the calcarine and parieto-occipital sulci as well as in the superior parietal gyrus and postcentral sulcus. In the contralesional hemisphere, the extent of CT reduction was rather moderate and did only reach statistical significance in the central sulcus, the cuneal gyrus, and in the calcarine sulcus. **Tables 2**, **3** summarize cluster locations and sizes, cluster-related averaged CT values for patients and controls, and absolute mean group differences. There were no significant CT increases in patients compared to controls in the present cohort.

Based on a priori hypotheses for functional and structural domains within the motor network with primary and secondary motor areas in frontal, parietal (8, 24) and also cingulate cortices (37–39), significant clusters were extracted for an additional ROI analysis. **Figure 3** displays the 9 clusters with significant loss in CT that were chosen: Frontal 1, Central 1–3, Superiorparietal 1–3, Cingulate 1–2. The clusters Central 3 and Superiorparietal 3 were located on the unaffected hemisphere, all others on the ipsilesional hemisphere. For ROI analysis, the large ipsilesional cluster 1 has been divided into clusters Central 1 and Central 2.

Relationship Between CT, Cortical Excitability, and Clinical Scores

Whole-brain analyses did not detect any significant associations, neither between CT estimates and the TMS data ($Slope_{max}$ and MEP_{max}) of AH nor between CT and the clinical scores in the stroke patients. In line, the ROI analysis of the frontal, central, superiorparietal and cingulate motor areas did not uncover any significant relationships with these parameters (**Tables 4**, **5**).

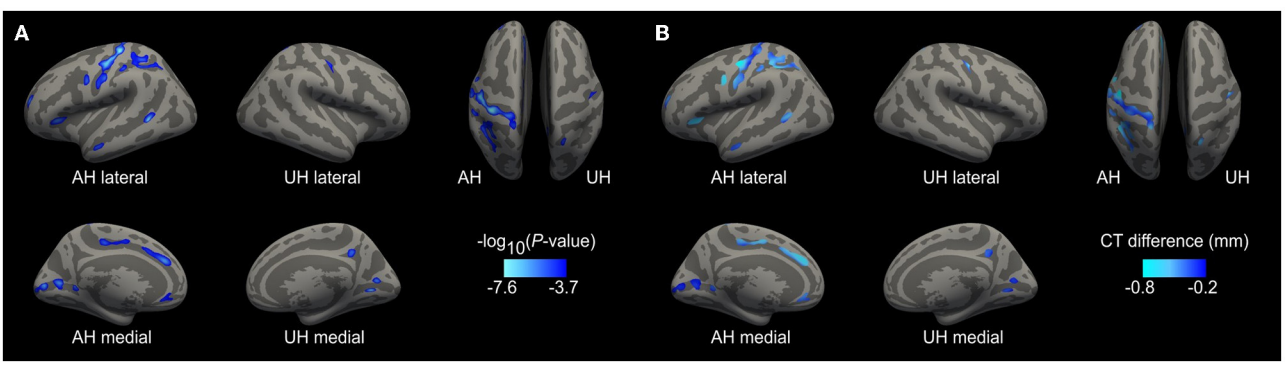

FIGURE 2 | Cortical thickness reductions after stroke. Whole-brain analysis results from comparison of patients with controls. For visualization purposes, the left hemisphere is modeled as the affected hemisphere (AH) and the right hemisphere as the unaffected hemisphere (UH). **(A)** Show clusters of significant CT thinning in the patients. The scale displays values of $-\log_{10}$ (*P*-value). Lighter blue tones mean higher significance. **(B)** Display the vertex-wise difference of CT values in mm (patients-controls) using the significant clusters as masks. Lighter blue shows areas of increased cortical thinning.

TABLE 2 | Location of the significant CT clusters in the AH of patients when compared to controls.

Number	Location	Size (mm^2)	no. of vertices	Mean CT Patients (SD)	Mean CT Controls (SD)	Mean CT Difference
1*#	Paracentral gyrus Central and paracentral sulci	1370.89	3,469	1.55 (0.21)	1.99 (0.31)	−0.44
2*	Superior parietal gyrus Postcentral, and intraparietal sulci	825.83	1,868	1.91 (0.19)	2.39 (0.24)	−0.48
3*	Superior frontal, anterior cingulate, and middle anterior cingulate gyri Anterior cingulate and middle anterior cingulate sulci	355.55	558	2.47 (0.16)	3.01 (0.18)	−0.54
4	Cuneal gyrus Calcarine sulcus	323.06	504	1.52 (0.10)	1.81 (0.11)	−0.29
5	Calcarine sulcus	277.62	372	1.53 (0.08)	1.81 (0.09)	−0.28
6*	Middle posterior cingulate gyrus Middle posterior cingulate and marginal cingulate sulci	228.57	541	2.22 (0.17)	2.71 (0.22)	−0.49
7	Lateral fissure and superior circular sulcus	185.07	407	2.07 (0.14)	2.64 (0.13)	−0.57
8	Middle temporal gyrus Superior temporal sulcus	162.32	253	2.51 (0.13)	2.98 (0.16)	−0.47
9	Middle frontal sulcus	156.42	221	2.01 (0.08)	2.43 (0.11)	−0.42
10	Calcarine sulcus	112.66	242	2.09 (0.25)	2.46 (0.27)	−0.37
11	Anterior cingulate gyrus and sulcus	112.56	160	2.32 (0.13)	2.74 (0.17)	−0.42
12*	Precentral gyrus Inferior part of the precentral sulcus	109.58	231	2.33 (0.16)	2.93 (0.18)	−0.6
13*	Postcentral sulcus	102.7	271	2 (0.10)	2.45 (0.14)	−0.45
14	Middle temporal gyrus Superior temporal sulcus	98.59	152	2.68 (0.14)	3.06 (0.14)	−0.38

All clusters are corrected for multiple comparisons (cluster-wise corrected P-values < 0.05).
**Clusters were selected for ROI analysis.*
#This large cluster in the surface analysis has been divided into Clusters 1 and 2 for the ROI analysis.

TABLE 3 | Location of the significant CT clusters in the UH of patients when compared to controls.

Number	Location	Size (mm^2)	no. of vertices	Mean CT Patients (SD)	Mean CT Controls (SD)	Mean CT Difference
1	Cuneal gyrus Calcarine sulcus	148.75	180	1.49 (0.08)	1.8 (0.09)	−0.31
2	Subparietal sulcus	126.12	267	2.31 (0.11)	2.69 (0.1)	−0.38
3	Medial lingual gyrus Calcarine gyrus	125.8	188	1.45 (0.07)	1.79 (0.09)	−0.34
4*	Superior parietal gyrus	85.01	151	1.98 (0.11)	2.52 (0.15)	−0.54
5*	Central sulcus	84.71	221	1.75 (0.15)	2.21 (0.2)	−0.46

All clusters are corrected for multiple comparisons (cluster-wise corrected P-values < 0.05).
**Clusters were selected for ROI analysis.*

DISCUSSION

The present work shows that chronic stroke patients with isolated subcortical supratentorial stroke lesions exhibit significantly reduced CT in numerous primary and secondary motor and non-motor brain areas, particularly in the ipsilesional hemisphere including areas along the central sulcus, the inferior frontal sulcus, the intraparietal sulcus, and cingulate cortices. However, we could not detect any significant relationship between the extent of these CT reductions and stroke-related changes in motor cortical excitability or clinical scores.

Cortical thinning, especially in brain regions connected to the stroke lesion, has been previously reported by imaging studies. For instance, a longitudinal study comparing stroke patients in the acute phase within three months after stroke found a significant loss in CT in a small area at the superior frontal gyrus at the lateral border of the supplementary motor area (14). However, these CT changes did not correlate with changes in clinical scores. Another work investigated chronic stroke

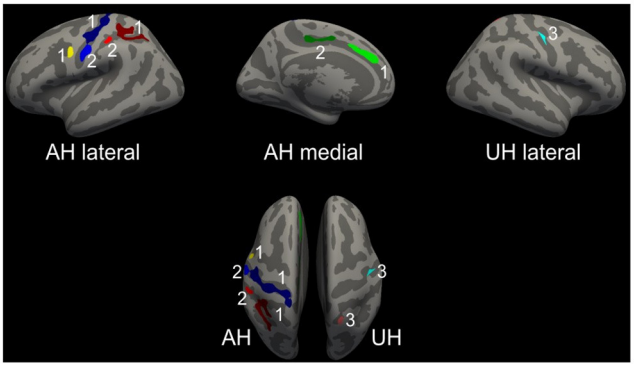

FIGURE 3 | Regions-of-interest for cluster-wise analysis. Selected clusters for ROI analyses. For visualization purposes, the left hemisphere is modeled as the affected hemisphere (AH) and the right hemisphere as the unaffected hemisphere (UH). Central ROIs are displayed in blue tones, the frontal one in yellow, parietal ROIs in red tones and the cingulate ROIs in green. Upper row with lateral and medial views on AH and UH. Lower row with superior view of AH and UH.

TABLE 4 | Patients' linear regression models with the CST's integrity measurements as the response variable.

Outcome	Region	Coefficient	Conf. Interval		*P*-value	Adj. R^2
			Lower	Upper		
$Slope_{maxAH}$	Central 1	0.35	−1.14	1.84	0.61	−0.08
	Central 2	0.30	−0.91	1.52	0.59	−0.08
	Central 3*	0.44	−0.5	1.39	0.32	0
	Cingulate 1	0.15	−1.02	1.31	0.79	−0.10
	Cingulate 2	−0.07	−1.23	1.09	0.89	−0.11
	Superiorparietal 1	0.40	−0.95	1.75	0.53	−0.06
	Superiorparietal 2	0.67	−0.34	1.67	0.17	0.09
	Superiorparietal 3*	−0.10	−1.28	1.08	0.85	−0.11
	Frontal 1	0.36	−0.48	1.19	0.36	−0.02
MEP_{maxAH}	Central 1	0.66	−0.66	1.97	0.29	−0.10
	Central 2	0.57	−0.59	1.73	0.30	−0.11
	Central 3*	0.54	−0.36	1.43	0.21	−0.05
	Cingulate 1	0.47	−0.81	1.76	0.43	−0.16
	Cingulate 2	0.19	−0.89	1.28	0.70	−0.22
	Superiorparietal 1	0.72	−0.51	1.96	0.22	−0.06
	Superiorparietal 2	0.78	−0.03	1.59	0.06	0.15
	Superiorparietal 3*	0.19	−1.08	1.45	0.75	−0.23
	Frontal 1	0.52	−0.20	1.24	0.14	0.01

**Central 3 and Superiorparietal 3 are UH clusters. The rest are AH clusters. P-values are uncorrected.*

patients and found significant loss in CT in the ipsilesional primary motor cortex. However, this study did not conduct a whole-brain analysis to look for CT alterations in other brain regions (12). Similarly, other studies have limited their CT analyses to pre-specified regions such as the primary motor cortex (5, 17).

In the present cross-sectional whole-brain analysis we show that CT alterations are not limited to the primary motor cortex but are also evident in multiple cortical areas of non-primary motor-related brain regions of the ipsilesional hemisphere. Specifically, we found significant loss in CT in the ventral premotor cortex and in cortices along the intraparietal sulcus. These data are well in line with multiple reports, which have evidenced that the parietofrontal motor network shows relevant functional (23, 40, 41) and structural changes (22, 24) which also relate to recovery after stroke. Moreover, cortices of the cingulate motor areas also showed a significant loss in CT. As cingulate motor areas are structurally and functionally connected with motor, premotor and also somatosensory areas (37, 38, 42), we speculate that network disconnection effects by the stroke lesion are likely to drive these CT reductions as well. In the literature, one study in acute ischemic brainstem strokes also found thinning of the cingulate cortices (18). Previous functional imaging studies have already shown that cingulate motor areas exhibit recovery-dependent increases in brain activation (43, 44), potentially reflecting enhanced processing of somatosensory feedback after stroke (45). Since we have not detected any increases in CT in these areas, but only significant decreases, one might hypothesize that such adaptive processes might be time-specific phenomena and more likely to be relevant in acute or subacute stages than in chronic stages of recovery.

Loss of CT was predominant in, but not limited to, the ipsilesional hemisphere. On the contralesional hemisphere we only detected CT reductions in a few regions in the central sulcus, the cuneal gyrus and calcarine sulcus. This predominance of CT alterations in the ipsilesional hemisphere is in line with a number of previous studies (19, 46). For instance, Cheng et al. estimated an average loss in CT of 0.15 mm after one year in the ipsilesional and 0.13 mm in the contralesional hemisphere in cortices that are structurally connected to the stroke lesions (19).

Some studies have shown that not only loss, but also gain in CT or cortical gray matter volume can be detected after stroke (16, 20, 22). For instance, Liu et al. observed gradual CT increases after basal ganglia stroke in the temporal and frontal lobes (20). The present cohort did not exhibit such increases in CT when compared to the healthy controls. In our study, absolute CT values, and not the relative change of CT over time, were used for group comparison (20). Thus, sensitivity of our cross-sectional approach might be inferior compared to longitudinal statistics to detect subtle gain in CT. Another study found increases of gray matter volume in bilateral supplementary motor areas after right-hemispheric stroke only. These changes also correlated with preserved motor functions (21). A systematic analysis of the influence of the side of the stroke lesion for loss or gain in CT of the affected or unaffected hemispheres would remain a topic for upcoming studies. In line with this idea, precise information regarding subcortical stroke locations, such as striatal vs. non-striatal lesions, might be further influential factors for stroke-related CT alterations in both hemispheres (20). To what extent such information might also influence cross-sectional group comparisons for CT analyses should be addressed by future research.

Earlier reports have been increasingly using multimodal approaches to better understand stroke recovery. For instance,

TABLE 5 | Patients' linear regression models with the motor output measurements as the response variable.

Outcome	Region	Coefficient	Conf. Interval		*P*-value	Adj. R^2
			Lower	Upper		
$Gripa_{AH}$	Central 1	−2.77	−35.61	30.06	0.85	0.21
	Central 2	−0.47	−28.68	27.74	0.97	0.21
	Central 3*	3.06	−19.44	25.55	0.77	0.21
	Cingulate 1	−1.77	−27.30	23.76	0.88	0.21
	Cingulate 2	−13.49	−37.39	10.41	0.24	0.31
	Superiorparietal 1	−5.06	−36.01	25.88	0.72	0.22
	Superiorparietal 2	3.96	−21.34	29.27	0.73	0.22
	Superiorparietal 3*	−4.66	−31.76	22.43	0.71	0.22
	Frontal 1	−2.57	−23.13	17.98	0.79	0.21
$Pinch_{AH}$	Central 1	−0.07	−7.61	7.46	0.98	0.21
	Central 2	0.21	−6.07	6.50	0.94	0.21
	Central 3*	0.36	−4.7	5.42	0.88	0.21
	Cingulate 1	−2.18	−7.81	3.46	0.41	0.26
	Cingulate 2	−2.94	−8.45	2.57	0.26	0.30
	Superiorparietal 1	−0.39	−7.48	6.71	0.91	0.21
	Superiorparietal 2	1.68	−3.89	7.25	0.52	0.24
	Superiorparietal 3*	−1.18	−7.14	4.79	0.67	0.22
	Frontal 1	−0.13	−4.53	4.28	0.95	0.21
NHP_{AH}	Central 1	0.31	−0.12	0.74	0.14	0.13
	Central 2	0.13	−0.27	0.53	0.48	−0.03
	Central 3*	0.22	−0.07	0.52	0.12	0.16
	Cingulate 1	0.09	−0.29	0.47	0.60	−0.05
	Cingulate 2	0.17	−0.20	0.54	0.33	0.02
	Superiorparietal 1	0.22	−0.21	0.65	0.28	0.04
	Superiorparietal 2	0.24	−0.05	0.54	0.10	0.19
	Superiorparietal 3*	−0.07	−0.46	0.32	0.70	−0.07
	Frontal 1	0.13	−0.14	0.40	0.31	0.03
UEFM	Central 1	4.63	−26.12	35.38	0.75	0.00
	Central 2	−1.10	−27.67	25.48	0.93	−0.01
	Central 3*	6.41	−14.58	27.4	0.52	0.03
	Cingulate 1	−7.71	−31.41	15.99	0.49	0.03
	Cingulate 2	−5.09	−29.18	19.00	0.65	0.01
	Superiorparietal 1	0.40	−28.97	29.77	0.98	−0.01
	Superiorparietal 2	3.64	−18.32	25.60	0.72	0.00
	Superiorparietal 3*	−12.22	−36.32	11.88	0.29	0.09
	Frontal 1	−1.68	−19.86	16.49	0.84	−0.01

**Central 3 and Superiorparietal 3 are UH clusters. The rest are AH clusters. P-values are uncorrected.*

one study investigated the relationship between CT of the primary motor cortex, motor cortical excitability, and clinical measures. While TMS measures could be related to motor functions, CT values could not (5). In the present study, we aimed at addressing this relationship with respect to other cortical brain regions in an exploratory manner. Against our hypothesis, and in accordance with the previously mentioned study, we did not find any significant association, neither between CT and TMS measures of cortical excitability, nor between CT and motor functions after stroke. Concerning the association between CT and clinical scores, there are other studies which could not detect significant correlations (5, 14, 15, 19, 20, 47).

One potential explanation for these negative results is that, although we only included supratentorial subcortical strokes, our patients still exhibited variable lesion locations. Among the affected regions were the corona radiata, internal capsule, basal ganglia, and thalamus. This heterogeneity might still translate into a relevant variability also in the extent of cortical degeneration and atrophy via degeneration of connecting fiber tracts (14, 46). This might complicate the detection of significant relationships spatially converging to a distinct brain region. On the other hand though, even in cohorts with very homogenous stroke locations such as isolated basal ganglia strokes, CT-outcome associations have not been detected (20). This might indicate that CT alone is unlikely to explain a relevant amount of intersubject variability in clinical scores. Hence, other established surrogate markers of the integrity of the motor network, such as fractional anisotropy of the CST, seem to be more informative for residual motor functioning and recovery after stroke (8). Given more recent results for layer-specific CT changes after stroke (13), these negative results could suggest that a higher resolution and the precise CT estimation related to layer V of the primary motor cortex or secondary motor cortices contributing to the CST (48, 49) might be capable to capture such associations between cortical anatomy, electrophysiology, and motor function. For clinical scores, such layer-specific CT estimates have been found to show significant correlations (13).

There are critical limitations to note. First, stroke patients from the initial cohort that had a cortical stroke had to be excluded from the sample. Therefore, only 14 patients could be finally included in the present analyses. On the one hand, this allowed us to interpret CT alterations as secondary degeneration remote from the lesion, because direct lesion effects of the cortical anatomy could be excluded. On the other hand, the reduced sample size influences the power of the statistical analyses. Our results should be verified or falsified in independent datasets. Second, the study was cross-sectional in nature. Longitudinal analyses with repeated sessions of MRI and TMS—throughout the recovery phase—might help to uncover time-dependent associations between cortical thickness and excitability, which undergo changes after stroke. Third, the present cohort included patients with rather mild deficits with a mean NIHSS score of 2 and a mean UEFM score of 56. Thus, whether our findings might be different in stroke patients with more severe deficits, remains a topic for upcoming prospective studies.

DATA AVAILABILITY STATEMENT

Although there are data sharing restrictions imposed by the ethical review board, data will be made available upon reasonable request, which includes submitting an analysis plan for a secondary project.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Chamber of Physicians,

Hamburg (No. PV5357). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JGP conducted the CT analyses and prepared the manuscript. SG conducted the TMS and imaging experiments and supervised the CT analyses. CC contributed to the statistical analyses. CG contributed to the design of the study. RS contributed to the design of the study, the statistical analyses, and prepared the manuscript. All authors revised the manuscript for important intellectual content.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2 Exposition of the published work

2.1 Introduction

2.1.1 Epidemiological context

Apoplexy (from ancient Greek ἀποπληξία, apoplexia, “struck down with violence”) is the Greek term coined by Hippocrates[1] and used in the Antiquity to refer to what we know today as stroke.[1,2] Old depictions in the literature, like the death of the Elamite king Humban-Nimena III in the 7th century BC,[3] show that this pathology has been present since ancient times.

In modern times, stroke remains extremely prevalent in our society. According to recent epidemiological data, it represented the second-leading cause of mortality and disability in adults worldwide in 2019.[4] In the European Union (EU) in 2017 there were 1.12 million incident strokes, almost 10 million prevalent stroke cases, 7 million disability-adjusted life years, and almost 0.5 million deaths.[5] In addition, the number of people living with stroke in the EU was estimated to increase by 27% between 2017 and 2047.[5] In Germany 1.7% of all women and 1.5% of men over 18 years old reported either having suffered a stroke in the previous 12 months or living with chronic stroke-related disabilities.[6] Furthermore, it was shown that the 12-month prevalence of strokes in persons over 55 years old increased from less than 1% to 6.4% for women and to 6.1% for men.[6] Prominent reasons for these increases are the aging of the population; and lifestyle choices associated with risk factors like high body mass index and high systolic blood pressure.[4]

According to the World Health Organization, stroke can be divided into ischemic and hemorrhagic events. The latter includes both, intracerebral and subarachnoid hemorrhage. Ischemic stroke can be defined as “brain, spinal cord, or retinal cell death attributable to ischemia, based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury”[7]. In 2019, ischemic strokes represented 62.4% of total stroke cases worldwide.[4] It is usually caused by the occlusion of a cerebral blood vessel.[8] Common causes of occlusion are atherosclerosis with superimposed thrombosis and cerebral embolisms, e.g. as the consequence of atrial fibrillation.[8,9] Rare causes of ischemic stroke include arterial dissection or vasculitis.[8,9] Cerebral ischemia triggers a complex cascade of pathophysiological processes ultimately leading to neuronal apoptosis.[9,10]

Upper motor limb deficits represent 40% to 80% of the disabilities following a stroke[11,12] Multiple motor rehabilitation strategies, including physical training interventions and the use of pharmacological agents or virtual reality,[13,14] have been developed. Nonetheless, a review of 15 randomized controlled trials of motor rehabilitation after stroke showed a significant effect of the intervention in only one study.[13] Thus, further research is needed to better understand inter-subject variability in spontaneous recovery after stroke, residual motor function, and treatment response.

2.1.2 Biomarkers as tools for motor recovery inference after stroke

As pointed out by Boyd et al.,[15] a possible reason for negative results in rehabilitation trials might be the heterogeneity of stroke. A “one size fits all” approach in study designs might not correctly capture associations between intervention and outcome.[15] A possible solution is the use of biomarkers, defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”,[16] to guide the design and intervention type in clinical trials.

Searching for biomarkers, the field has moved from unimodal to multimodal approaches to analyze brain structure and function, and to relate these measures to clinical scores. The structural integrity of the corticospinal tract (CST), the principal motor pathway in mammals,[17] has been widely studied and used to infer motor outcome after stroke.[15,18–23] The functional integrity of the motor cortex and the CST can be studied using transcranial magnetic stimulation (TMS). TMS-derived motor evoked potentials (MEP) can be recorded in peripheral hand muscles, typically the first dorsal interosseous muscle (FDI), using electromyography (EMG). The relationship between the TMS stimulus intensity and the MEP response is described by a sigmoid curve, named the stimulus response or recruitment curve (RC).[24,25] Different outcome parameters can be derived: a) slope, which indicates the strength or gain of descending CST;[24,26] b) the area under the curve as a measure of overall CST output;[26] c) the maximum MEP amplitude, as the maximum synchronized CST output;[27,28] d) the inflection point or the slope’s steepest point, which signifies the intensity at which the maximum gain is observed.[26,27] Maximum amplitude of the MEP and RC properties have been related to motor output after stroke.[27–35] More recently, studies have also evidenced correlations between structural and functional integrity of the CST.[27,36]

Stroke-related alterations are also reflected in the cortical anatomy. Neuronal death following retrograde Wallerian degeneration[37–39] has been reported to lead to cortical thinning in primary and secondary motor and non-motor brain regions bilaterally.[28,37,40–45] It has been shown that cortical thinning is most pronounced in cortices directly connected to the primary stroke lesion.[37,41] Reporting an increase in cortical thicknesses (CoT) in frontal and temporal cortices, predominantly contralesional,[46,47] or increased cortical gray matter volume [48,49] other studies have argued for the existence of cortical adaptive neuroplastic processes to promote recovery. However, the precise association between CoT alterations and motor function remains elusive. Some studies have found associations between CoT or gray matter volume and clinical scores,[48–50] but most have not.[28,37,41,42,46] It is an open question whether CoT may provide information that improves current models that combine clinical scores with structural and functional CST measurements to predict the recovery of motor function after stroke.[29,34] For example, previous studies have indicated that CoT alterations might occur in cortices of the frontoparietal network whose structural and functional characteristics have been repeatedly related to motor function and recovery processes.[51–53] Other regions of interest include the cingulate cortices. Cingulate motor areas have been identified by direct cortical stimulation using

electrodes[54] and connections to the frontoparietal network have been observed in structural and functional connectivity studies.[55,56]

So far, only one previous study has investigated the relationships between CoT and functional integrity of the CST.[28] Although CoT alterations and changes in the functional integrity of the CST were observed, there were no associations between these measurements.[28] One potential limitation was that this study restricted the analyses to the primary motor cortex (M1). The potential influence of CoT of other regions of the frontoparietal network was not accounted for. Another limitation was the inclusion of patients with subcortical and cortical stroke locations. Recent studies suggested that lesions directly affecting the cortex complicate CoT estimations and structure-outcome relationships.[57] Thus, while addressing these limitations, in the present study we set out to shed new light on the potential relationships between CoT and functional integrity of the CST in chronic subcortical stroke patients.

2.2 Methods

2.2.1 Study cohort

A cohort of 18 chronic stroke patients and 17 healthy controls of similar age and gender was taken from a previous study and included in the present analysis.[27] All patients had experienced a first, unilateral, supratentorial ischemic stroke with hand motor deficits, and they were in the chronic phase of the disease (>6 months after the event). All patients were at least 18 years old and had no contraindications for TMS or MRI. Due to cortical lesions, four patients were excluded from the CoT analysis. The Fugl-Meyer assessment of the upper extremity (UEFM), the nine-hole peg test (NHP), and whole hand grip and pinch force values served as clinical motor scores.

2.2.2 Estimation of the cortical thickness

The cerebral cortex is the outermost layer of neural tissue in the brain of humans and other mammals. It has the topology of a highly folded and curved 2-D sheet.[58,59] This anatomical characteristic makes its manual study time-consuming and difficult. Therefore, automated tools that allow the estimation of its structural properties, such as CoT, have been developed.

A frequently used pipeline for CoT estimation is the surface-based method implemented in the FreeSurfer image analysis suite.[60] The high resolution T1-weighted MRI images of the participants are first registered with the MNI305 atlas, using an affine registration, for computing seed points in later pipeline's steps. Image intensity variations are then corrected and normalized using the white matter's intensity as a reference. Then, a skull stripping algorithm is applied to remove extra cerebral voxels. Next, the remaining voxels are labeled as white matter or as something different to white matter. Cutting planes that separate the cerebral hemispheres from the cerebellum and brain stem are then computed. Using a connected components algorithm, a preliminary segmentation is created, where interior holes representing white matter are automatically filled creating a filled volume for each hemisphere. An initial surface is generated by tessellating these volumes. This surface is refined by making it follow the intensity gradients between the white and gray matter. The refined surface, named the white surface, is then nudged

outwards to follow the intensity gradients between the gray matter and the cerebrospinal fluid, generating the pial surface. Manual interventions are possible at different steps of the pipeline as quality check measures. A detailed explanation of these steps, including the algorithms, is available[58,59] Visual steps of the pipeline's outputs are shown in Figure 1.

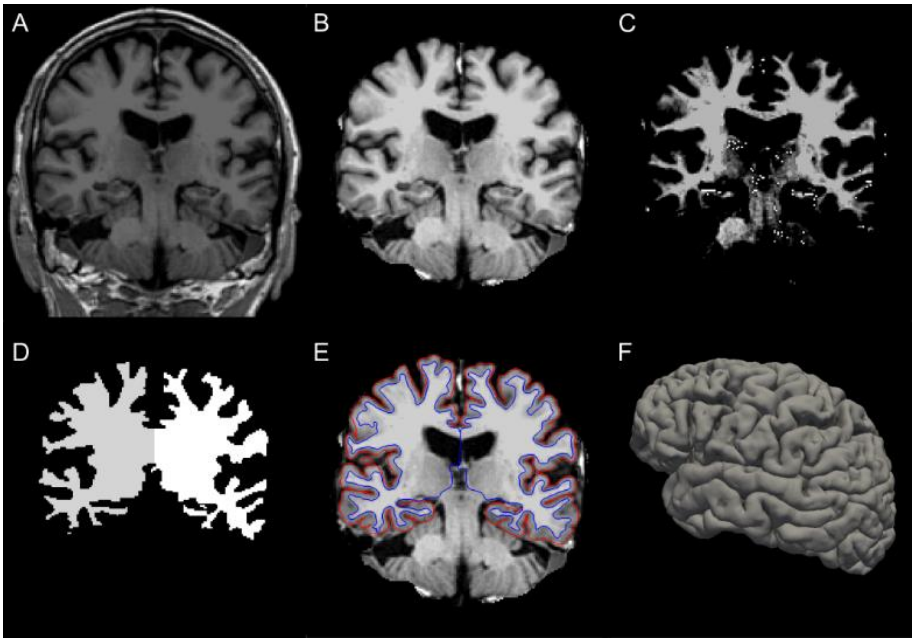

Figure 1. Visual steps of the FreeSurfer's surface-based pipeline for CoT estimation in a participant of the study. (**A**) Initial T1-weighted anatomical MRI image. (**B**) Intensity normalized and skull stripped volume. (**C**) White matter volume. (**D**) Result of the calculation connected components of the white matter. Gray indicates results for the right hemisphere and white for the left hemisphere. (**E**) The white surface (in blue) and the pial surface (in red) for both hemispheres projected on a coronal slide. (**F**) 3-D representation of the pial surface of the left hemisphere.

CoT is finally measured as the average distance between the white and pial surfaces.[61] Regional CoT values vary between 1 and 4.5 mm , with overall average of 2.5 mm.[61]

Due to most patients having a left-sided lesion (11 out of 14), the left hemisphere was considered as the affected hemisphere (AH) and the right hemisphere as the unaffected hemisphere (UH). To allow the comparison of CoT values from different hemispheres, participants were registered with the FreeSurfer's symmetrical template, *fsaverage_sym*, which accounts for interhemispheric differences.[62]

2.2.3 Transcranial magnetic stimulation data

For functional TMS-derived CST properties, maximum MEP (MEP_{max}) and maximum slope of the RC values ($Slope_{max}$) were used from the original report.[27] For a detailed description of the data acquisition, we refer the reader to the included publication.

2.2.4 Statistics

An initial whole-brain approach was used to compare CoT between patients and healthy participants and to explore correlations between CoT and clinical scores (grip, pinch, NHP, UEFM) or TMS measures ($Slope_{max}$ and MEP_{max}). FreeSurfer's utility *mri_glmfit* was used to fit general linear models with CoT as the response variable. All models were corrected for the nuisance variable age. The problem of multiple

comparisons was addressed by applying a Z Monte Carlo simulation over 10,000 iterations with a cluster-forming threshold of $P<0.0001$.[63]

Clusters located in the frontoparietal motor network or in the cingulate cortices obtained after comparing both groups were selected for an additional region of interest (ROI) analysis. The mean CoT value of each ROI was extracted and fit as the explanatory variable in multiple linear regression models using R (version 4.0.3).[64] Linear models were corrected for age and for the outcome variable of the unaffected hand (for grip, pinch, and NHP models).

2.3 Results

2.3.1 Demographics

Fourteen stroke patients were included in the final analyses (12 males, mean age 67 years old (SD ± 9.53), mean time after stroke 41 months ± 28.78, one was left-handed, 12 patients were affected on their dominant hemisphere). A summary of demographic and clinical data is included in the publication.

2.3.2 Whole-brain CoT analysis

The group comparison between patients and controls reveled cortical thinning in multiple brain areas as depicted in Figure 2.

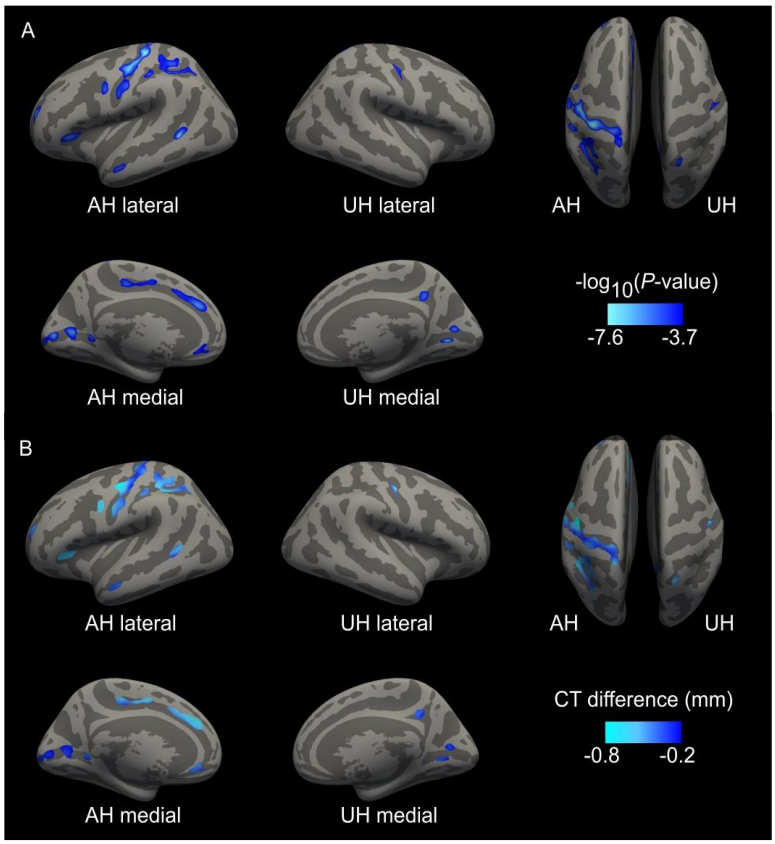

Figure 2. Cortical thickness reductions after stroke. A whole-brain analysis was used to compare the CoT of patients and controls. For visualization purposes, the left hemisphere is modeled as the affected hemisphere (AH) and the right hemisphere as the unaffected hemisphere (UH). (**A**) Clusters of significant lesser CoT in the patients. The scale displays values of -log10 (P-value). Lighter blue tones mean higher significance. (**B**) Vertex-wise group differences of CoT values in mm (patients-controls) using the significant clusters as masks. Lighter blue shows areas of greater cortical thinning in patients.

Bigger clusters exhibiting cortical thinning in patients were found in the ipsilesional hemisphere. Specifically, lesser CoT was detected in the pre-, post- and paracentral gyri, the postcentral sulcus, the central and paracentral sulci, the cuneal gyrus, the calcarine and parieto-occipital sulci, and the superior parietal gyrus. Although CoT alterations were also observed in the unaffected hemisphere, these were less prominent and the only significant clusters were located in the central sulcus, the cuneal gyrus, and the calcarine sulcus. Statistical details of these group differences are included in the publication. General linear models did not reveal any correlations, neither between CoT and TMS measurements, nor between CoT and clinical scores.

2.3.3 Region of interest analysis

Nine clusters located in the frontoparietal network or cingulate cortices were selected for ROI analysis. These were Frontal 1, Central 1-2, Superiorparietal 1-2, Cingulate 1-2 on the ipsilesional hemisphere; and Central 3 and Superiorparietal 3 on the contralesional hemisphere (Figure 3).

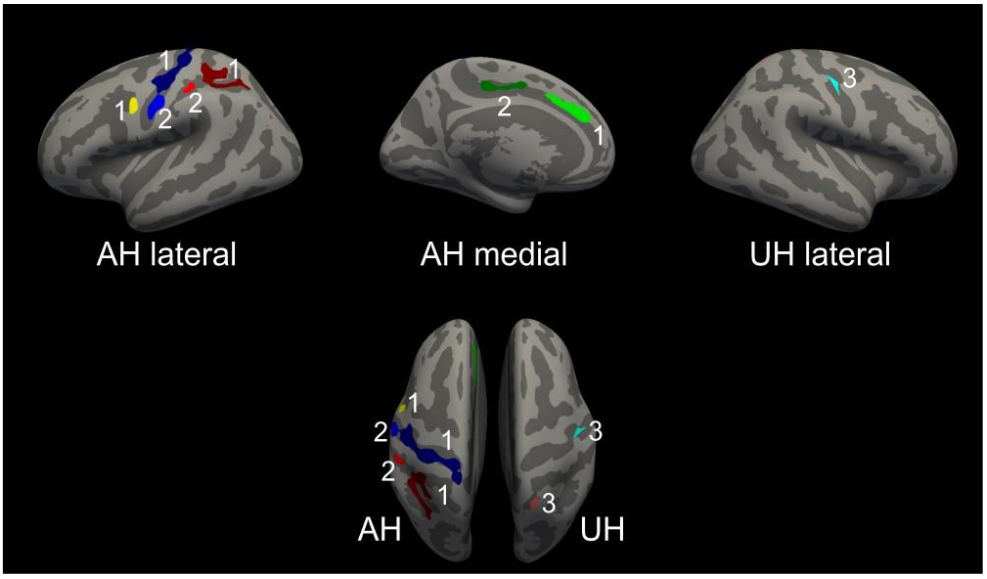

Figure 3. Selected clusters for ROI analysis. For visualization purposes, the left hemisphere is modeled as the affected hemisphere (AH) and the right hemisphere as the unaffected hemisphere (UH). Central ROIs are displayed in blue tones, the frontal one in yellow, parietal ROIs in red tones and the cingulate ROIs in green.

Linear regression models with CoT as the explanatory variable did not show any significant associations, neither between CoT and TMS measurements, nor between CoT and clinical scores.

2.4 Discussion

The present study aimed at investigating CoT alteration in chronic stroke patients with isolated subcortical lesion locations and possible associations of CoT with clinical scores of upper limb function and TMS-derived measurements of excitability of the CST. It shows that a thinner cortex is observed in patients in numerous primary and secondary motor and non-motor brain regions. These changes were more marked in the ipsilesional hemisphere. However, they were not correlated with neither clinical motor measurements nor with TMS measurements.

Cortical thinning is a known phenomenon after stroke. In a recent review that evaluated the effects of stroke on CoT beyond the primary lesion, Cortese et al.[65] showed that the most common finding is a thinner cortex. The present lesser CoT in stroke patients supports this observation. Although the current findings were more marked in the ipsilesional hemisphere, we also observed contralesional clusters of thinner cortical. Thus, we hypothesize that the present focal CoT changes are a direct consequence of the stroke and not due to generalized processes, like aging, which have a more global profile of CoT alterations.[66] In fact, Cheng et al.,[37] using probabilistic fiber tractography showed that cortical thinning occurs bilaterally, particularly in regions connected to the primary stroke lesion. This might explain the distribution of the present clusters, predominantly located in the frontoparietal network and cingulate cortices. Multiple studies have shown relevant functional[51,67,68] and structural[49,52] changes in the frontoparietal network after stroke. Likewise, cingulate areas show dense structural and functional connections with motor, premotor and somatosensory areas.[54–56,69]

Post-stroke Wallerian degeneration has been proposed as a possible mechanism of cortical thinning after stroke.[37,38] After an axotomy, degeneration of the axonal structures distal to the damage, and later retrograde neuronal death likely due to loss of retrograde trophic support, occurs.[70] Werring et al.[71] studied the microstructural integrity of the CST of five stroke patients using diffusion tensor imaging and observed patterns of changes similar to those found in animal models of Wallerian degeneration. They argued that changes in diffusion parameters were caused by the breakdown of the myelin sheath and disintegration of the axonal microfilaments, and thus, alterations in the directional structures.[71] Regarding the contralesional changes, Cheng et al.[37] observed that the CoT changes in the contralesional hemisphere were associated with the structural integrity of the corpus callosum. They proposed a mechanism of contralesional cortical degeneration via loss of ipsilesional intermediary interneurons, which they termed “transcallosal diaschisis”.[37]

In contrast to the present findings, there are studies that have found cortical thickening after stroke. Liu et al.[46] examined thirty five basal ganglia stroke patients over five time points. Using resting-state functional MRI they divided the participants into two groups: those predominantly overlapping with the motor network region, the striatal motor network-dominant group (SMD); and the remaining as the non-striatal motor network-dominant (N-SMD) group. They found a steady cortical thickening in both groups. However, the patterns of CoT thickening were different between the groups. The SMD showed bilateral cortical thickening, mainly in the contralesional ventrolateral prefrontal cortex, prefrontal pole, the middle temporal gyrus, and the ipsilesional frontal pole, and superior frontal gyrus. The N-SMD showed only contralesional CoT increases, predominantly in the frontal pole, the medial prefrontal cortex, and the anterior cingulate cortex. They argued that the observed cortical thickening is the product of motor recovery and compensation, especially because contralesional changes were more prominent.[46]

Another study that supports the idea of cortical thickening as an expression of cortical reorganization after stroke is the one by Sterr et al.[47] They evaluated the effects of constraint-induced movement therapy (CIMT, forced-use therapy), a rehabilitation

therapy in which patients are forced to use their paretic hand. After the therapy, cortical thickening was observed in both the active and placebo group in the contralesional postcentral gyrus and middle frontal gyrus. Hence, the cross-sectional design of the present study might explain why we did not find cortical thickening.

Associations between CoT changes after stroke and motor function, evaluated with clinical scores, have been inconsistent. Liu et al.[46] and Sterr et al.[47] did not find associations between the extent of cortical thickening and the degree of improved motor function after stroke. Likewise, studies reporting cortical thinning did not show correlations with clinical scores.[28,37,41,42] More recently, Rojas et al.[72] found that initial contralesional CoT values of acute stroke patients in the precentral gyrus, superior frontal sulcus, and temporal and cingulate cortices were positively associated with motor function after 3 to 6 months after the event. This study suggested that CoT estimates might serve as a marker for structural brain reserve of motor-related brain regions to support motor recovery after stroke. Lotan et al.[50] were able to calculate a distribution of five cortical layers using MRI. The deepest layer (L5) was a compound of anatomical cortical layers V and VI. They observed, that after stroke, the cortical thinning occurs almost exclusively in the deepest layer. They also found a correlation with these CoT alterations in L5 and motor scores, but not so between the whole thickness and motor outcome. Thus, one might hypothesize that the current approach to CoT, usually taken as a whole, might lack the necessary resolution to capture real associations between changes in cortical structure and motor function after stroke. Another explanation for the inconsistent results might be the heterogeneity in stroke cohorts. However, the lack of correlations observed by Liu et al.[46] after subdividing their stroke sample to reduce the heterogeneity speak against this.

In the present study no association between cortical brain structure and functional integrity of the CST was found. In a previous report, Buetefisch et al.[28] explored how the motor function of the hand related to the ipsilesional CoT of M1, and the structural and functional integrity of M1 and the CST after stroke. The ipsilesional hemisphere showed a thinner cortex when compared to the contralesional hemisphere. The functional integrity of the CST was associated with the motor function. In the case of CoT or structural integrity of the CST, neither showed a correlation with hand function. Ipsilesional CoT and the function of the ipsilesional CST were also not associated. As an explanation for this result, they proposed that by recruiting only MEP positive patients, they biased their results towards participants with relatively preserved corticomotor neurons in M1. This possible explanation also applies to the present results, for only MEP positive patients were included in the present study.

The present study has limitations to notice. First, cortical stroke patients from the original cohort had to be excluded, because CoT values around the stroke lesion have been found to be non-reliable.[57] Thus, only 14 patients were included in the final analysis. On the one hand, this allowed us to confidently interpret the CoT alterations as remote changes secondary to the stroke lesion. On the other hand, the reduced sample size also affected the statistical power of our analysis. Furthermore, the inclusion of only subcortical stroke patients limits the generalizability of the present results to patients with cortical lesions. Caution is advised when evaluating the literature because most of the studies exclude cortical strokes (Table 1). This might

be an expression of the limitation of the current CoT estimation methods. Another limitation is the cross-sectional design of the study. Therefore, within-group differences or time-sensitive CoT changes could not be evaluated. Including only MEP positive patients, as already mentioned, represents another limitation that might bias the sample towards well-recovered patients.

As future directions for the study of CoT, Lotan et al.[50] already showed that the MRI study of the cortical lamination patters might provide helpful information in stroke, and similar results have been observed in cortical dysplasia.[73] Tomer et al.[74] have proposed a novel method for calculating cortical layers *in vivo* with typical MRI sequences. Future studies might employ this technique to investigate if a layer-based analysis of the CoT will better capture associations between motor function or functional measurements of CST integrity after stroke to further improve available models in motor recovery research after stroke.

Concluding, our findings support the idea that, although CoT alterations are observed after stroke, no simple correlation can be found between this and TMS-derived measurements of CST integrity, even while only studying subcortical stroke patients.

Study	Participants (% subcort.)	Design & Time After Stroke	Measures	Main Finding
Buetefisch et al. (2018)[28]	18 (55.55%)	C-S >6 m	CS, CoT, TMS, DTI	TMS associated with CS. Lesser CoT in ipsilesional M1 not associated with CS or TMS.
Brodtmann et al. (2012)[43]	12 (83.33%)	L <2 h, 3 h, 24 h, 1 w, 6 w, 12 w	CS, CoT	Greater contralesional CoT over time. CoT changes positively associated with NIHSS.
Chen et al. (2021)[44]	48 (100% Brainstem)	C-S <3 d	CoT	Lesser bilateral CoT compared to controls.
Cheng et al. (2015)[41]	12 (100%)	L 3-5 d, 3 m	CS, CoT, DTI	Lesser ipsilesional CoT in areas connected to the primary stroke lesion. No associations between CoT and CS.
Cheng et al. (2020)[37]	18 (100%)	L 3-5 d, 1 y	CS, CoT, DTI	Lesser bilateral CoT in areas connected to the primary stroke lesion. No associations between CoT or DTI and CS.
Hong et al. (2021)[45]	37 (100% Lacunar)	R	CoT, DTI	Lesser ipsilesional CoT and reduced ipsilesional ODI. CoT was associated with ODI from the corresponding hemisphere.
Jones et al. (2016)[42]	17 (100%)	C-S >6 m	CS, CoT, MRS	Lower bilateral CoT, tNAA, Glx in the patients compared to controls. tNAA and Glx were associated with CS, but CoT was not.
Liu et al. (2020)[46]	33 (100% Basal ganglia)	L <7 d, 14, 30, 90	CS, CoT	Greater bilateral CoT not associated with CS.
Lotan et al. (2019)[50]	20 (100%)	C-S >3 m	CS, CoT, DTI	Lesser ipsilesional CoT in deepest M1 layer. Negative association between CoT changes and NIHSS.
Sterr et al. (2013)[47]	31 (58.06%)	L >6 m, 2 w after CI therapy	CS, CoT, DTI	CS improved after intervention. Greater contralesional CoT after intervention, but not associated with CS after intervention.
Zhang et al. (2014)[40]	26 (100%)	C-S >6 m	CS, CoT, rs-fMRI, reach-to-grasp Task	Lesser ipsilesional CoT in M1 compared to controls. Region of CoT changes showed increased task-evoked activation, ALFF, and rsFC.

Table 1 | Survey of CoT studies. Abbreviations: ALFF, amplitude of low-frequency fluctuation; CI, constraint induced; CS, clinical scores; C-S, cross-sectional; CoT, cortical thickness; DTI, diffusion tensor imaging; Glx, glutamine; L, longitudinal; M1, primary motor cortex; NIHSS, National Institutes of Health Stroke Scale; ODI, orientation dispersion index; R, retrospective; rsFC, resting-state functional connectivity; rs-fMRI, resting-state functional magnetic resonance imaging; subcort., subcortical.

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4 Summary

4.1 English

Structural and functional changes of the corticospinal tract (CST) after ischemic stroke have been related to motor recovery processes. Cortical thickness (CoT), a measure of brain cortical structure, might serve as a biomarker to improve current prediction models. Different studies have observed cortical thinning or, less common, thickening after stroke. However, the associations between CoT and clinical motor function or the functional integrity of the CST remain unclear. In the present study, whole-brain and region of interest analyses were conducted in fourteen chronic subcortical stroke patients to explore how CoT is related to motor function or to transcranial magnetic stimulation (TMS)-derived measurements of CST function, namely properties of the recruitment curve of the maximum motor evoked potentials. As a control group, 17 healthy participants were used. Bilateral, predominantly ipsilesional, cortical thinning was observed in the patients when compared with controls in primary and secondary motor and non-motor brain regions. Nonetheless, these CoT alterations were neither associated with clinical motor function nor with CST excitability. Future studies including layer-based CoT analyses are warranted to shed further light on the interrelationships between brain structure, CST function, and motor function after stroke.

4.2 Deutsch

Strukturelle und funktionelle Veränderungen des kortikospinalen Trakts (CST) nach einem ischämischen Schlaganfall können zur Vorhersage der Erholung der motorischen Funktion herangezogen werden. Die kortikale Dicke (CoT), ein Maß für die kortikale Struktur des Gehirns, könnte ein Biomarker sein, mit dem derzeitige Vorhersagemodelle weiter verbessert werden könnten. Verschiedenen Studien haben eine Ausdünnung oder, seltener, eine Verdickung des Kortex nach einem Schlaganfall beobachtet. Die Zusammenhänge zwischen CoT und der klinischen motorischen Funktion oder der funktionellen Integrität der CST sind jedoch weiterhin nicht gut verstanden. In der vorliegenden Studie wurden bei 14 chronischen Schlaganfallpatienten mit subkortikalen Läsionen Ganzhirnanalysen und regionsspezifische Analysen durchgeführt, um zu untersuchen, wie die CoT mit der motorischen Funktion und mit Maßen der kortikospinalen Erregbarkeit, quantifiziert durch Analyse von Rekrutierungskurven von magnetisch evozierten motorischen Potenzialen, zusammenhängt. Als Kontrollgruppe wurden Daten von 17 gesunden Probanden herangezogen. Bei den Patienten wurde im Vergleich zu den Kontrollpersonen eine bilaterale, vorwiegend ipsiläsionale kortikale Ausdünnung in primär und sekundär motorischen und nicht-motorischen Hirnregionen beobachtet. Diese CoT-Veränderungen waren jedoch weder mit der motorischen Funktion noch mit der Erregbarkeit des CST verbunden. Weitere Studien, z.B. auch unter Anwendung schichtbasierter CoT-Analysen, sind gerechtfertigt, um die die Zusammenhänge zwischen der Hirnstruktur und der CST-Funktion und der motorischen Funktion nach einem Schlaganfall weiter besser zu erfassen.

5 Author contribution

The work presented here was carried out in the Department of Neurology of the University Medical Center Hamburg-Eppendorf (UKE) under the supervision of Prof. Dr. med. Christian Gerloff (Doktorvater) and PD Dr. med. Robert Schulz (Mentor).

This study was a secondary analysis building on the original work of Dr. Stephanie Guder. MRI raw data were re-analyzed, recruitment curve properties were available and integrated in the novel statistical analyses. Dr. Schulz conceptualized the present investigations. Prof. Gerloff contributed to the study design.

The present work included the image pre- and post-processing including cortical thickness analyses and statistical modeling. The first draft of the publication was written by me, including the preparation of all figures and tables. Revisions during preparation of the manuscript and during peer-review were primarily done by Dr. Schulz and me.

Results of the present analysis were published as a full article entitled “Relationship Between Cortical Excitability Changes and Cortical Thickness in Subcortical Chronic Stroke” in Frontiers in Neurology on March 2022.

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7 Curriculum vitae

Der Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

8 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....