Research Article

Structural remodeling in related brain regions in patients with facial synkinesis

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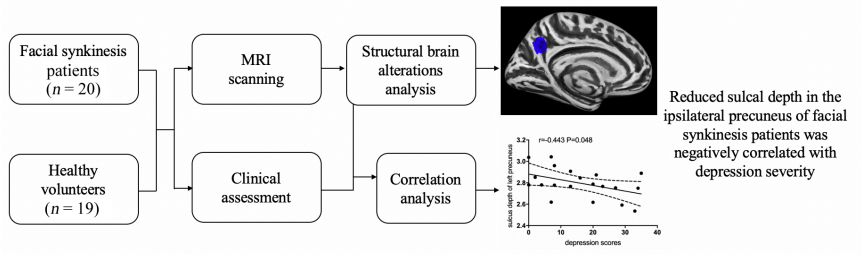
Graphical Abstract *Searching the cortex with surface-based morphometry: a small step towards the fundamental of stubborn facial synkinesis*



Abstract

Facial synkinesis is a troublesome sequelae of facial nerve malfunction. It is difficult to recover from synkinesis, despite improved surgical techniques for isolating the peripheral facial nerve branches. Furthermore, it remains unclear whether long-term dysfunction of motor control can lead to irreversible plasticity-induced structural brain changes. This case-control study thus investigated the structural brain alterations associated with facial synkinesis. The study was conducted at Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China. Twenty patients with facial synkinesis (2 male and 18 female, aged 33.35 ± 6.97 years) and 19 healthy volunteers (2 male and 17 female, aged 33.21 ± 6.75 years) underwent magnetic resonance imaging, and voxel-based and surface-based morphometry techniques were used to analyze data. There was no significant difference in brain volume between patients with facial synkinesis and healthy volunteers. Patients with facial synkinesis exhibited a significantly reduced cortical thickness in the contralateral superior and inferior temporal gyri and a reduced sulcal depth of the ipsilateral precuneus compared with healthy volunteers. In addition, sulcal depth of the ipsilateral precuneus was negatively correlated with the severity of depression. These findings suggest that there is a structural remodeling of gray matter in patients with facial synkinesis after facial nerve malfunction. This study was approved by the Ethics Review Committee of the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China (approval No. 2017-365-T267) on September 13, 2017, and was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR1800014630) on January 25, 2018.

Key Words: brain plasticity; cortical thickness; depression; facial nerve paralysis; facial synkinesis; peripheral nerve injury; sulcal depth; structural remodeling; surface-based morphometry; voxel-based morphometry

Chinese Library Classification No. R445.2; R745.1+2; R363

Introduction

Facial synkinesis is a troublesome sequela of idiopathic facial nerve paralysis, characterized by involuntarily synchronous contractions of neighboring muscles that accompany voluntary facial movement. It is difficult to recover from synkinesis (Husseman and Mehta, 2008; Pourmomeny et al., 2014; Xie et al., 2020), and the underlying pathogenesis of facial synkinesis has not yet been elucidated. The most widely acknowledged mechanism is the paradoxical co-contraction of related muscles that is caused by a misdirected regeneration of nerve fibers following damage (Baker et al., 1994; Takeda et al., 2015). Despite progress in the surgical techniques of selective neurectomy and the use of botulinum toxin-A injection to isolate peripheral facial nerve branches, in one study, half of the patients with post-facial paralysis synkinesis were left with persistent synkinesis (Azizzadeh et al., 2019). Therefore, it is possible that long-term rigid synkinesis may involve dysfunction of motor control in the central nervous system, even after a collateral neurectomy.

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Several recent reports have reported that peripheral nerve injury can induce profound alterations in the brain, and the functional outcome might be closely associated with both peripheral recovery and brain adaptation (Davis et al., 2011; Socolovsky et al., 2017). Using task-based and resting-state functional magnetic resonance imaging (MRI), our previous studies have also demonstrated that facial synkinesis is accompanied by brain reorganization (Wang et al., 2019; Wu et al., 2019). Our results confirmed the involvement of both motor and cognitive brain regions, which extends our understanding of the neural mechanism underlying facial synkinesis. However, facial synkinesis fails to respond to treatments that are based on the principles of neural plasticity, including those that have had clinical success in other neuropathies (Apkarian et al., 2004; Borckardt et al., 2009). According to our current understanding of neural plasticity, structural plasticity is the result of functional plasticity (Cohen et al., 2017). In the early stages following brain damage, brain plasticity first results in reversible functional changes. Structural plasticity, which is characterized by changes in the anatomical connectivity between neurons, can subsequently occur according to functional plasticity (Taylor et al., 2009; Goswami et al., 2016; Cohen et al., 2017). While some longitudinal studies have reported that brain structural changes and symptom improvement are reversible in the early stage, in the chronic stage following brain damage, it is difficult to reverse structural changes and normalize brain structure. Indeed, in a study with patients with chronic illness, brain changes became relatively irreversible if the problem was long standing and intractable (Goswami et al., 2016; Cohen et al., 2017). To summarize, a stable change in brain structure could be a critical factor leading to dysfunction in motor control and the intractable symptoms of facial synkinesis. Therefore, it is necessary to explore structural brain alterations that occur following long-term functional reorganization in patients with facial synkinesis. Moreover, once the mechanisms underlying maladaptive functional alterations have been identified, it could be possible to inhibit this process in the early stages to prevent the occurrence of synkinesis.

To this aim, the present study compared the brain structure between patients with facial synkinesis and healthy volunteers using voxel-based morphometry analysis of the whole brain and cortical surface-based morphometry analysis. Morphometric parameters included brain volume, cortical thickness, and surface complexity (the local gyrification index, fractal dimension, and sulcal depth). We also investigated the relationship between morphometric measures and clinical assessment results (including evaluations of facial nerve function and of depression) in patients with facial synkinesis. Our results shed more light on facial synkinesis-related neural plasticity, which could aid the development of early interventions for certain synkinesis after facial nerve dysfunction.

Participants and Methods

Participants

This observational case-control study was performed at the Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, China, from January 2017 to December 2019. Twenty patients with a clinical diagnosis of facial synkinesis and 19 healthy volunteers of a comparable age and sex were enrolled. Facial synkinesis was diagnosed by two experienced plastic surgeons and was qualitatively evaluated by clinical assessments of involuntary facial movements. Patients were asked to perform five trigger movements, including forehead raise, eye closure, smile, lower lip pulled down, lip pout, and whistling. Special attention was paid to detect co-contraction of the orbicularis oculi muscle and elevator mouth muscles, which have been reported to be affected in all patients with synkinesis (Chuang et al., 2015). The inclusion criteria for patients with facial synkinesis were as follows: (1) adult patients (18–50 years) with first-onset unilateral facial paralysis who had been left with facial synkinesis (without restriction of sex or disease course); (2) patients had undergone no nerve repair or transfer surgery; and (3) no other medical or psychological disorders. Healthy volunteers were eligible for inclusion if they were aged 18–50 years and had no medical or psychological disorders. The exclusion criteria for both groups were as follows: (1) the presence of concurrent peripheral neuropathy; and (2) contraindications for MRI scanning. Additionally, we excluded patients with central facial nerve injury, facial nerve injury caused by tumor, facial trauma, or facial surgery, and a history of epilepsy or a family history of epilepsy.

The Ethics Review Committee of the Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine approved this study (approval No. 2017-365-T267) on September 13, 2017 (**Additional file 1**), and written informed consent (**Additional file 2**) was obtained from all participants, in accordance with the *Declaration of Helsinki*. This study was registered with the Chinese Clinical Trial Registry (registration number: ChiCTR1800014630) on January 25, 2018. The study design is summarized in **Figure 1**. This study was reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement (**Additional file 3**).

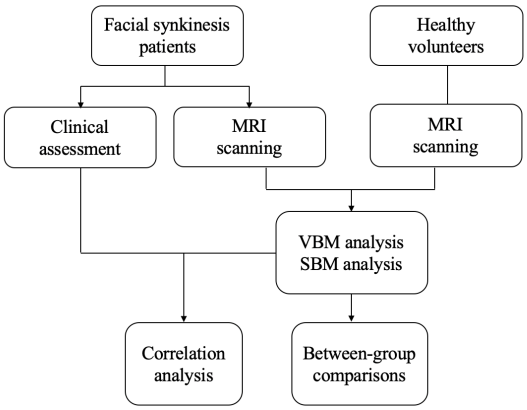

Figure 1 | **Study flow chart.**
MRI: Magnetic resonance imaging; SBM: surface-based morphometry; VBM: voxel-based morphometry.

Clinical assessments

Facial nerve function was assessed using the Sunnybrook Facial Grading System, which includes assessments of facial resting symmetry, symmetry of voluntary movement, and synkinesis (Chee and Nedzelski, 2000). The score ranges from 0 to 100, with higher scores indicating better facial nerve function. The severity of depression was evaluated using the self-report Beck Depression Inventory (BDI), since depression is commonly experienced by patients with facial paralysis (Beck et al., 1961; Nellis et al., 2017). The BDI score ranges from 0 to 63, with higher scores indicating more severe depressive symptoms.

MRI acquisition

MR images were acquired using a 3.0T scanner (MR750, GE Healthcare, Milwaukee, WI, USA). During the scanning, foam padding was used to restrict head movements, and participants were instructed to lie still and rest quietly. Structural images were acquired using a 3-dimensional T1-weighted fast spoiled gradient-recalled echo sequence with the following scan parameters: repetition time/echo time = 8.16/3.18 ms, inversion time = 450 ms, flip angle = 8°, field of view = 256 × 256 mm^2, acquisition matrix = 256 × 256, section thickness = 1.0 mm sagittal acquisition, number of averages = 1, interslice space = 0 mm.

Data analysis

MRI data were analyzed using the CAT12 toolbox (http://www.neuro.uni-jena.de/cat/) within the Statistical Parametric Mapping 12 toolbox (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) on the MATLAB 2014b platform for voxel-based morphometry (VBM) and surface-based morphometry (SBM) analysis. After converting Digital Imaging and Communication in Medicine (DICOM) files into Neuroimaging Informatics Technology Initiative (NIfTI) images, the brain images of patients with right-sided facial synkinesis were flipped along the y-axis prior to analysis so that the synkinetic side of all patients was on the left to facilitate comparability across subjects. Then, the anatomical images were reoriented so that the origin approximated the anterior commissure and the orientation approximated the MNI space for each subject. The acquired images were visually inspected for scanner artifacts and gross anatomical abnormalities.

VBM analysis

After an initial quality check, images were bias-corrected, segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) images, and spatially normalized to the MNI space using linear and non-linear transformations using a unified model that included high-dimensional DARTEL normalization. The normalized partial volume images were then modulated by dividing them by the fields. Then, the modulated gray matter images were smoothed by an 8-mm full-width at half-maximum isotropic Gaussian kernel for further statistical analysis. The total GM, WM, and CSF volumes were obtained separately using the segmented images. The total intracranial volume was calculated as the sum of GM, WM, and CSF volumes.

SBM analysis

The workflow for the SBM analysis, implemented in the CAT12 toolbox, included an established algorithm for creating a 3-dimensional cortical surface model (Dahnke et al., 2013). Then, morphometric parameters, including the cortical thickness, local gyrification index, fractal dimension, and sulcal depth, were calculated on the basis of the absolute mean curvature approach (Luders et al., 2006; Yotter et al., 2010).

The T1-weighted volumes were segmented to estimate WM distance, which was the distance from the WM boundary to each GM voxel, and local maxima (equal to the cortical thickness) were then projected to other GM voxels using a neighbor relationship that was determined by the WM distance. This projection-based method also included partial volume correction, and allowed us to handle sulcal blurring and sulcal asymmetries without sulcus reconstruction (Ziegler et al., 2012). For the inter-subject analysis, we used an algorithm that enables spherical mapping of the cortical surface (Yotter et al., 2011). The local complexity maps were re-parameterized into a common coordinate system. In addition to cortical thickness analysis, measures of structural complexity (the local gyrification index, fractal dimension, and sulcal depth) were extracted on the basis of the absolute mean curvature (Luders et al., 2006). Central cortical surfaces were created for both hemispheres separately. Finally, resampled surface data for cortical thickness and structural complexity were separately smoothed using 18-mm and 25-mm full-width at half-maximum Gaussian kernel, respectively, prior to further statistical analyses.

Quality control

While effective noise-reduction approaches are included in the CAT12 toolbox, the resulting volume and surface images can still be affected and were therefore re-checked by visual inspection; all the resulting volume and surface images showed satisfactory homogeneity. All segmentations were accurate and manual correction was not required. Therefore, none of the subjects were excluded from either group.

Statistical analysis

Between-group comparisons of age and sex were made using a two-sample *t*-test and a Chi-squared test, respectively. Statistical analyses were performed using the software package SPSS 22.0 (IBM, Armonk, NY, USA).

MRI data were analyzed using Statistical Parametric Mapping 12. Between-group comparisons in the structural MRI parameters were made using independent two-sample *t*-tests. Age, sex, and total intracranial volume were set as covariates. Significance for the SBM analysis was set at a vertex-level threshold of $P < 0.001$ without correction and a cluster-wise threshold of $P < 0.05$ with family-wise error (FWE) correction, while significance for the VBM analysis was set at $P < 0.05$ with FWE correction.

In addition to between-group differences in structural morphology, we also analyzed the relationship between structural parameters and clinical measures (the BDI and Sunnybrook Facial Grading System scores) using Spearman's correlation coefficient implemented in SPSS 22.0. We used a region of interest-based approach by extracting parameters from the significantly different brain regions that were identified in the between-group comparisons of structural MRI.

Results

Demographic and clinical characteristics of patients with facial synkinesis and healthy controls

No significant between-group difference was found in age or sex ($P > 0.05$). A total of 9 patients with right-sided and 11 patients with left-sided facial synkinesis were finally recruited. The duration from onset to examination was 26.05 ± 14.20 months. In patients with facial synkinesis, the mean Sunnybrook score was 42.45 ± 19.24 and the mean BDI score was 16.00 ± 11.54 (**Table 1**).

Table 1 | **Demographic and clinical characteristics of participants**

Characteristic	Facial synkinesis (*n* = 20)	Healthy control (*n* = 19)	*P*-value
Age* (yr)	33.35±6.97	33.21±6.75	0.950
Sex†			0.957
Male	2	2	
Female	18	17	
Duration (mon)*	26.05±14.20	–	–
Affected side (right/left)†	9/11	–	–
Sunnybrook scores*	42.45±19.24	–	–
Beck Depression Inventory score*	16.00±11.54	–	–

Data are expressed as the mean ± SD (*) or number (†). Age was analyzed using an independent two-sample *t*-test, and sex was analyzed using the Chi-squared test.

VBM analysis in patients with facial synkinesis and healthy controls

The VBM analysis revealed no significant between-group difference in the global volumes of GM, WM, CSF, or total intracranial volume ($P > 0.05$; **Table 2**).

SBM analysis in patients with facial synkinesis and healthy controls

Compared with healthy controls, patients with facial synkinesis exhibited a significantly smaller cortical thickness involving the right (contralateral to the synkinetic side) superior temporal and middle temporal regions (**Figure 2** and **Table 3**), and a smaller sulcal depth of the left (ipsilateral to the synkinetic side) precuneus (**Figure 3** and **Table 3**). There was no significant between-group difference in the fractal dimension or gyrification index ($P > 0.05$).

Table 2 | **Global tissue volumes (mL) of patients with facial synkinesis and healthy controls**

Global brain volumes	Facial synkinesis (*n* = 20)	Healthy control (*n* = 19)	*T*-value	*P*-value
Gray matter volume	642.70±47.26	650.08±9.78	–0.56	0.58
White matter volume	501.90±39.62	502.67±41.39	–0.06	0.95
Cerebrospinal fluid volume	265.00±44.70	283.88±69.15	–1.05	0.30
Total intracranial volume	1410.45±104.33	1437.33±113.36	–0.81	0.42

Data are expressed as the mean ± SD, and were analyzed using independent two-sample *t*-tests.

Table 3 | **Differences in cortical thickness and sulcus depth between patients with facial synkinesis and healthy controls in surface-based morphometry analysis**

	T-value	Size (vertices)	Overlap (%)	Region
Cortical thickness				
Facial synkinesis patients < healthy controls				
Right hemisphere	–4.5	989	60	Superior temporal
			39	Middle temporal
Sulcus depth				
Facial synkinesis patients < healthy controls				
Left hemisphere	–4.3	862	100	Precuneus

Associations between clinical assessments and structural parameters in patients with facial synkinesis

Within the facial synkinesis group, a negative correlation was found between BDI scores and sulcal depth of the left precuneus (r = –0.443, P < 0.05). However, sulcal depth of the left precuneus was not associated with Sunnybrook scores. Furthermore, there was no significant relationship between cortical thickness in the right superior temporal or middle temporal regions and clinical assessments (the right superior temporal region and depression scores: r = –0.152, P > 0.05; the right superior temporal region and Sunnybrook scores: r = 0.405, P > 0.05; the right middle temporal region and depression scores: r = –0.196, P > 0.05; the right middle temporal region and Sunnybrook scores: r = 0.331, P > 0.05; **Figure 4**).

Considering that there are large sex-related differences in the brain, we additionally carried out an analysis using only data from female patients (*n* = 18/20) and female healthy volunteers (*n* = 17/19). The results are shown in **Additional file 4**.

Discussion

In the present study, we investigated the morphological brain alterations in patients with facial synkinesis, as well as associations between clinical assessments and structural parameters. Overall, the structural analysis revealed that patients with facial synkinesis presented with more significant cortical atrophy. The SBM analysis demonstrated that patients with facial synkinesis exhibited smaller cortical thickness in the right (contralateral to the synkinetic side) superior temporal and middle temporal regions, and reduced sulcal depth of the left (ipsilateral to the synkinetic side) precuneus, compared with healthy volunteers. Furthermore, we found a negative association between sulcal depth of the ipsilateral precuneus and BDI scores in patients with facial synkinesis. In addition, there were no significant differences in VBM parameters between patients and healthy volunteers.

Neural plasticity, which includes both functional and structural plasticity, has been extensively researched in the context of peripheral neuropathy (Davis et al., 2011; Osborne et al., 2018). Functional plasticity can be viewed as alterations of synaptic connections or of the efficiency of neural circuits. Structural plasticity, in which functional circuits are established de novo, are thought to occur according to functional plasticity (Sweatt, 2016; Cohen et al., 2017). Our previous work has revealed that facial synkinesis-related functional reorganization decreases the distance between regions representing distinct facial movements, and an abnormal functional state of hypercompensation in the ipsilateral insular that is significantly associated with facial nerve function (Wang et al., 2019; Wu et al., 2019). These previous results confirmed the involvement of both motor and cognitive regions in facial synkinesis. In terms of structural changes, we found no significant difference in VBM parameters between two groups, while SBM parameters differed between patients with facial synkinesis and healthy volunteers. VBM is an advanced and powerful method for assessing the brain morphological/volumetric changes using voxel-by-voxel comparisons, and has previously been applied to various neurological conditions. SBM is a group of brain morphometric techniques to construct and analyze surfaces that represent structural boundaries within the brain. In addition to the VBM analysis, the use of SBM allows researchers to determine whether any GM volume differences are caused by differences in cortical thickness or in the size of cortical surface area. Moreover, it is possible to investigate any such differences with millimeter precision and examine the surface of the cortex in more detail, such that early structural changes can be detected more easily.

With respect to surface-based parameters, we found a reduction of cortical thickness in the right superior temporal and middle temporal regions in patients with facial synkinesis. This finding can be attributed to changes of the superior temporal sulcus (STS), since the STS separates the superior temporal gyrus from the middle temporal gyrus. Recent studies have revealed that the STS is involved in multisensory processing, especially for facial movement, eye gaze, and expression (Engell and Haxby, 2007; Hagan et al., 2009). The involvement of the STS in facial expression recognition has also been reported (Haxby et al., 2000). Facial expressions involve morphological changes, such as frowning, widening the eyes, pulling the corners of the lips up and backwards, lip stretching or tightening, nose wrinkling, and opening the mouth (Ekman and Rosenberg, 2005; Srinivasan et al., 2016). As well as the physical component of morphological changes, these expressions involve an affective component, whereby information about the expresser's internal feelings is conveyed. Conventional laboratory tasks have also revealed that facial expression recognition relies more heavily on the perception of morphological patterns than on affective information (Calvo and Nummenmaa, 2016). In the present study, brain regions involved in face processing were affected in patients with facial synkinesis, which indicates that these patients could have dysfunctions in perceiving morphological facial changes. Thus, it is possible that synkinesis after facial palsy is also associated with facial expression recognition, rather than isolated impairments in facial muscles.

Another key finding of this study is that sulcal depth of the ipsilateral precuneus was reduced in patients with facial synkinesis. Furthermore, we found a negative association between depression severity and sulcal depth of the ipsilateral precuneus. The precuneus is a part of the posteromedial parietal lobe, as well as the default mode network, and has been found to play an important role in visuospatial imagery, episodic memory retrieval, self-processing operations, and mood control (Cavanna and Trimble, 2006; Li et al., 2019). In addition, previous functional MRI studies have reported that the precuneus is part of the extended face processing

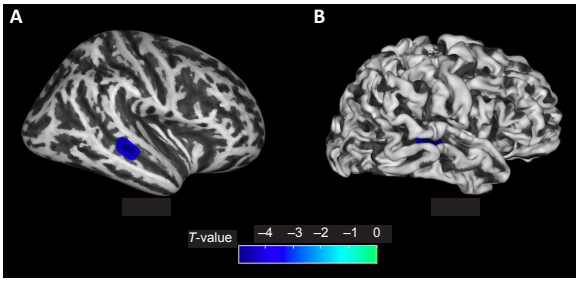

Figure 2 | The difference in cortical thickness between patients with facial synkinesis and healthy controls in the surface-based morphometry analysis.
Inflated (A) and pial (B) surface maps of the lateral surface of the right (contralateral to the synkinetic side) hemisphere. There was a smaller cortical thickness in patients with facial synkinesis compared with healthy volunteers (dark gray = sulci; light gray = gyri).

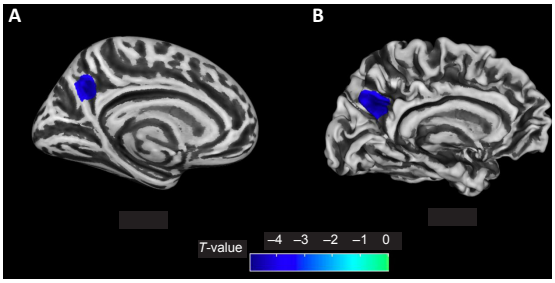

Figure 3 | The difference of sulcal depth between patients with facial synkinesis and healthy controls in the surface-based morphometry analysis.
Inflated (A) and pial (B) surface maps of the medial surface of the left (ipsilateral to the synkinetic side) hemisphere. There was a smaller sulcal depth in the patients with facial synkinesis compared with the healthy volunteers (dark gray = sulci; light gray = gyri).

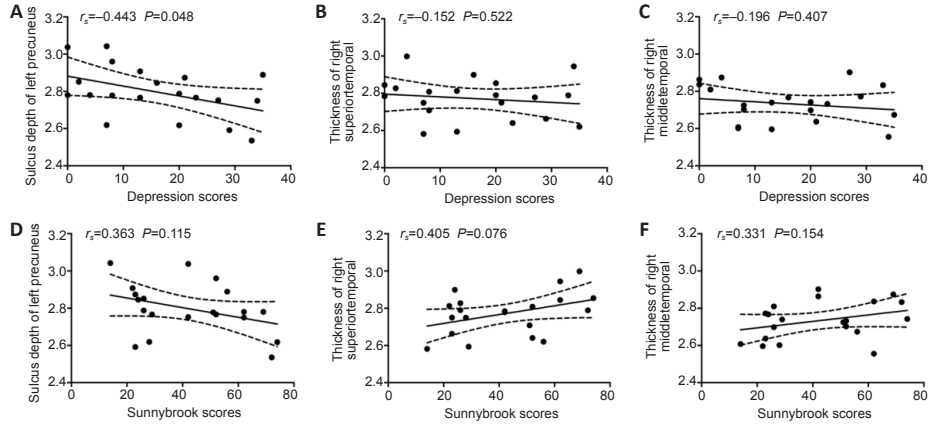

Figure 4 | Spearman's correlation coefficient analysis between clinical assessments and structural parameters in three regions of interest in the facial synkinesis group.
(A) Correlation between sulcal depth of the left precuneus and depression scores; (B) correlation between cortical thickness of the right superior temporal region and depression scores; (C) correlation between cortical thickness of the right middle temporal region and depression scores; (D) correlation between sulcal depth of the left precuneus and Sunnybrook scores; (E) correlation between cortical thickness of the right superior temporal region and Sunnybrook scores; (F) correlation between cortical thickness of the right middle temporal region and Sunnybrook scores.

network (Fox et al., 2009). In Sreenivas et al.'s (2012) task-based functional MRI study, the precuneus showed activation in response to emotional faces, across different emotions. In the present study, the depression-related reduction of sulcal depth in the ipsilateral precuneus might be indicative of an involvement of emotional and cognitive factors in facial synkinesis. We can therefore suggest that morphological alterations observed are secondary to long-term dysfunction of motor control/regulation, which are stable, such that facial synkinesis is extremely hard to recover from. Early intervention that focuses on synkinesis-related structural brain alterations could help to prevent certain synkinesis following facial nerve dysfunction. In view of the above, functional improvement in facial synkinesis could be promoted by targeting the modulation of maladaptive brain plasticity changes, such as separation of the representation sites of distinct facial movements, suppression of hypercompensation in the insular, and prevention of cortical atrophy in brain regions involving cognitive and emotional processing. Our preliminary results could therefore inform future work about the target of plasticity-based neuromodulation for motor functional improvement after facial nerve injury.

One limitation of this study is the relatively small sample size, which might have limited the statistical power of our results. The second limitation is the lack of a cognitive assessment of behavioral characteristics in patients with facial synkinesis, especially since brain areas involved in facial expression recognition were affected. Future work could therefore further investigate the association between changes in brain morphology and cognitive functions. Finally, to pinpoint the morphological changes caused specifically by facial synkinesis, a better comparison to make might be with patients who have recovered from facial paralysis rather than healthy controls; indeed, morphological abnormalities in related brain regions have been reported to recover to normal levels with the gradual recovery of facial nerve function (Bitter et al., 2011; Wu et al., 2015). Considering the scarcity of people who have recovered from facial paralysis, we recruited healthy controls. In future research, we will include more patients, define a more appropriate control group, enroll more homogeneous subjects, and conduct a long term follow-up study to more fully explore the morphological changes associated with facial synkinesis. Nonetheless, we believe that the findings from this preliminary study provide insights into the possibility of targeting plasticity-based neuromodulation for motor function improvement after facial nerve injury.

To conclude, we demonstrated that stable structural changes occur after facial synkinesis. Cortical atrophy was found in brain regions involved in cognitive and emotional processing. This provides new information about the morphological changes associated with facial synkinesis. Moreover, our data-driven analysis identified the ipsilateral precuneus as a potential target for intervention against depression, as well as several key regions that underlie persistent synkinesis symptoms. These insights could therefore aid the development of new strategies for the treatment of facial synkinesis.

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Institutional review board statement: *The study was approved by Ethics Review Committee of the Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (approval No. 2017-365-T267) on September 13, 2017, and registered with the Chinese Clinical Trial Registry (registration number: ChiCTR1800014630) on January 25, 2018.*

Declaration of participant consent: *The authors certify that they have obtained all appropriate participant consent forms. In the form the participants have given their consent for the images and other clinical information to be reported in the journal. The participants have understood that their names and initials will not be published and due efforts will be made to conceal their identity.*

Reporting statement: *This study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.*

Biostatistics statement: *The statistical methods of this study were reviewed by the biostatistician of School of Rehabilitation Medicine, Shanghai University of Traditional Chinese Medicine, China.*

Data sharing statement: *Individual participant data that underlie the results reported in this manuscript, after deidentification (text, tables, figures, and appendices) will be available indefinitely at ResMan Research Manager (http://www.medresman.org/) within 6 months after the completion of the trial without any charge. Other raw data can be achieved through contact with the corresponding author.*

Plagiarism check: *Checked twice by iThenticate.*

Peer review: *Externally peer reviewed.*

Open peer reviewer: *Lázaro Juliano Teixeira, Prefeitura Municipal de Balneário Camboriú, Brazil.*

Additional files:

Additional file 1: *Hospital Ethics Approval (Chinese).*

Additional file 2: *Informed consent form (Chinese).*

Additional file 3: *STROBE checklist.*

Additional file 4: *Results of only female participants.*

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P-Reviewer: Teixeira LJ; C-Editor: Zhao M; S-Editors: Yu J, Li CH; L-Editors: Cason N, Yu J, Song LP; T-Editor: Jia Y

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 2,3
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 3
Methods			
Study design	4	Present key elements of study design early in the paper	Page 4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5-7
Bias	9	Describe any efforts to address potential sources of bias	Page 6
Study size	10	Explain how the study size was arrived at	Page 4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 6-7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 4
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Page14
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 7
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*NA*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 7
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 8-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 10-11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.