Central functional reorganization and recovery following facial-hypoglossal neurorrhaphy for facial paralysis

Miao Ling a, Binbin Su b, Diya Su c, Dezhi Li d, Binbin Wang a, Hong Wan a, Michael Schumacher e, Lanxin Ji b,*, Song Liu a, d,*,

a Department of Injury and Repair, and Beijing Key Laboratory of Central Nervous System Injury, Beijing Neurosurgical Institute, Capital Medical University, Beijing 100070, China
b Tianjin Neuroimaging Center of Excellence, China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China
c Dalian University Affiliated Xinhua Hospital, Dalian 116000, China
d Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China
e U 1195, INSERM and Université Paris-Saclay, 94270 Le Kremlin-Bicêtre, France

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ABSTRACT

Objective: Functional deficits induced by nerve injuries can be restored by achieving effective reinnervation of the denervated targets and functional reorganization of the central nervous system after nerve reconstruction. In this study, we investigated the effect and extent of cortical functional reorganization related to the ability of transferred hypoglossal neurons to restore facial function in facial paralysis patients after a surgical bridge of neurorrhaphy ectopically between the ipsilateral hypoglossal nerve and injured facial nerve.

Methods: We treated 23 patients (35.4 ± 10.3 years, 10 males) and followed them up for 2.9 ± 0.61 years. We used motor-task-related functional magnetic resonance imaging to map activation change at multiple time points before and after neurorrhaphy; 20 normal subjects were included as control.

Results: All patients regained facial function to some extent after neurorrhaphy. Enhanced activation in motor-related cortices gradually returned to normal levels and was positively correlated with regained facial function. The related cortical functional areas included the left middle temporal gyrus, left inferior frontal gyrus, insula, bilateral motor cortex and the supplementary motor area extending to the paracingulate involved in intensive eye closing, as well as the left superior temporal gyrus, right putamen and the bilateral motor cortex involved in lip pursing. Intriguingly, significant correlations were found between the pre-surgery activation while intensive eye closing in bilateral motor cortex and recovery of facial nerve function induced by the neurorrhaphy treatment.

Conclusion: This is the first study mapping activation change in motor cortices at multiple time points before and after repair of the facial nerve. The cortical functional reorganization found may suggest potential treatment targets in the central nervous system for adjuvant therapies such as repetitive transcranial magnetic stimulation to further improve functional recovery.

1. Introduction

Facial nerve (FN) injury results in paralysis of the facial mimic muscles, which needs to be treated to restore lost function. Clinically, the House–Brackmann (HB) grading scale is often used to evaluate facial nerve function after injury, which is classed from grade I (normal function) to grade VI (total paralysis of facial mimic muscles) (House and Brackmann, 1985). For patients with facial paralysis whose HB grade exceeds grade IV, surgical treatment of the facial nerve is generally required. Regarding surgical treatment, the hypoglossal nerve is often chosen as an ideal axonal source for ectopic neurorrhaphy with the distal stump of the injured FN because its proximal stump is not usually available. Several factors determine the extent of regained facial function after neurorrhaphy, including the number and speed of motor axons regenerating toward the target muscles, the histological condition of the injured FN and its target muscles, appropriate target reinnervation, and
cortical functional reorganization to adapt to new tasks after reinnervation (Li et al., 2021; Navarro et al., 2007).

To date, no treatment in patients can ensure recovery of normal sensorimotor function after a severe nerve injury. This lack of treatment is considered a plateau that has been reached for the refinement of surgical repair techniques (Lundborg, 2003). Combination treatment to improve axonal regeneration, to promote target reinnervation and to enhance cortical reorganization is envisaged to overcome this limitation. In previous studies, we performed hypoglossal-facial neurorrhaphy to treat FN injury (Su et al., 2018; Zhang et al., 2015). We noticed that facial symmetry at rest could be restored in these patients, but evident asymmetry was still present during facial action. These results advise that despite effective reinnervation of the paralyzed facial muscles and restoration of the muscle tone and contraction after repair surgery, the hypoglossal motor neurons whose axons had been redirected towards the injured FN had not sufficiently adapted to their new functions. Thus, whether and how the hypoglossal motor neurons could change over time their original function to control facial mimic muscles remained to be investigated.

Peripheral nervous system and central nervous system (CNS) are functionally integrated together, and nerve injury unavoidably results in cortical modifications (Kaas, 1991; Kaas and Collins, 2003; Wall et al., 2002). It has been reported that neurons in CNS initially controlling the transferred nerve can be reeducated to assume new functions after ectopic neurorrhaphy (Molina-Rueda et al., 2019). In previous studies using blood oxygen level-dependent functional magnetic resonance imaging (fMRI), we found changes with time in the activation of facial movement related areas of patients after FN injury (Xiao et al., 2015). The study performed by Rottler et al. shows that hypoglossal-facial transfer resulted in an outcome-dependent cortical reorganization with activation of the cortical tongue area for restituted movement of the lip (Rottler et al., 2014). In this study, we further investigated the relationship between regained function and cortical reorganization in patients with FN injury followed by hypoglossal-facial neurorrhaphy. We combined clinical examination, electrophysiological evaluation, and fMRI to analyze changes in functional areas in the cortex during facial reinnervation. The discovery of the cortical functional areas with reorganized activity and the relationship between these areas and regained facial function may help to predict the prognosis of FN injuries and may particularly indicate potential treatment targets in the CNS for adjuvant therapies, such as repetitive transcranial magnetic stimulation (TMS).

2. Subjects and methods

Twenty-three patients (35.4 ± 10.3 years, 10 males) with unilateral facial paralysis (9 right-side affected) due to FN injury after the removal of an acoustic tumor in the cerebellopontine angle (CPA) area were treated with hypoglossal-facial neurorrhaphy and followed by clinical examination, electrophysiological evaluation and task- and resting-state fMRI in our neurosurgical department from September 2016 to June 2020. Twenty normal age and gender matched subjects (39.7 ± 10.3 years, 10 males) were included in this study as a normal control (NC) group. As shown in Fig. 2, fMRI scans were performed three times in the patients at the time prior to neurorrhaphy surgery (pre), at the first postoperative visit 3–8 months after neurorrhaphy (post-V1), and at the second postoperative visit 9–17 months after surgery (post-V2), and only once in NCs. The last follow-up consultation was performed by video calls 24–27 months after neurorrhaphy without fMRI examination.

This study was approved by the local ethics committee (KY2017-006–02) and conducted in accordance with the ethical principles of the “Administrative Measures for the Clinical Application of Medical Technology” (2009) of the Chinese Ministry of Health. Participants and their families were informed about various aspects of the examinations and/or treatment, including the advantages and trade-offs, and signed all relative consent forms.

2.1. Neurorrhaphy treatment

Hypoglossal-facial neurorrhaphy was performed identically to previous studies on patients under general anesthesia (Fig. 1) (Su et al., 2018; Zhang et al., 2015). Briefly, the injured FN and ipsilateral hypoglossal nerve were exposed under a neurosurgical microscope (Zeiss, Jena, Germany). One half of the hypoglossal nerve was cross-sectioned at a site closely distal to the descendens hypoglossi. One extremity of a predegenerated sural nerve graft (PNG) was surgically bridged end-to-side to the hypoglossal nerve at the partial cross-section site. The PNG was obtained from the ipsilateral sural nerve that was transected for predegeneration one week prior to neurorrhaphy. In response to nerve transection, Schwann cells dedifferentiate and proliferate in the distal segment of the nerve, providing a suitable nerve graft with favorable environment for axonal regeneration (Su et al., 2018; Zhang et al., 2015). The two distal ends of the PNG were created at another extremity and then bridged end-to-side to each of the two main FN branches, respectively.

2.2. Facial exercise program

Each subject performed daily facial exercise 2 weeks after neurorrhaphy, including massages and passive facial motions, such as passive eye closing, lip pursing, cheek drumming, and brow lifting. Passive paralyzed facial muscle exercise can delay the occurrence of muscle atrophy and promote nerve regeneration, reinnervation and subsequent functional recovery (Lindsay et al., 2010; Zhang et al., 2015). We advised patients to perform facial exercise more than three times daily for at least 30 min each time.
2.3. Evaluation of FN function and tongue movement

FN function was assessed regularly using the HB grading scale (House and Brackmann, 1985) before neurorrhaphy and at 3 months, 6 months, 9 months and beyond after neurorrhaphy. HB grading scale is a clinical widely used method to evaluate facial nerve function. It is classed from grade I (normal function) to grade VI (total paralysis of facial mimic muscles). A detail description of HB grading scale is shown in Supplementary s-Table 1.

Each patient was also photographed and videotaped to record any changes in facial symmetry and motion.

2.4. Electrophysiological examination

Electrophysiological examination was performed using Nicolet Viking Select (Nicolet; Middleton, Wisconsin, USA) to evaluate the spontaneous activity of the paralyzed facial muscles at rest and to record muscle action potentials during muscle movement under the examiner’s oral instruction. It is well known that the intact muscle tissue is non-electrically active when it is at rest, but the denervated muscle produces spontaneous activities, which are called fibrillation potentials and positive sharp waves. In contrast, an innervated muscle produces a disorderly group of action potentials with varying rates and amplitudes while voluntarily contracted, which is referred to as recruitment muscle

| Case | Sex(F/M) | Age(y) | FP side | Pre FP duration (mo.) | Pre HB grade | Post-V1 HB grade | Post-V2 HB grade | Last follow up HB grade |
|------|-----------|--------|---------|-----------------------|--------------|------------------|------------------|------------------------|
| 1    | F         | 34     | L       | 7                     | V            | V                | IV               | IV                     |
| 2    | F         | 23     | L       | 6                     | VI           | ND               | ND               | ND                     |
| 3    | F         | 24     | R       | 9                     | V            | ND               | ND               | ND                     |
| 4    | F         | 43     | L       | 204                   | VI           | VI               | VI               | V                      |
| 5    | M         | 19     | L       | 5                     | V            | V                | IV               | III                    |
| 6    | F         | 45     | R       | 4                     | V            | IV               | III              | III                    |
| 7    | F         | 35     | R       | 6                     | V            | IV               | III              | II                     |
| 8    | M         | 41     | R       | 9                     | VI           | V                | IV               | IV                     |
| 9    | F         | 46     | R       | 19                    | VI           | V                | III              | IV                     |
| 10   | M         | 54     | L       | 8                     | VI           | V                | III              | II                     |
| 11   | F         | 29     | R       | 24                    | V            | V                | IV               | III                    |
| 12   | F         | 53     | L       | 15                    | VI           | VI               | IV               | III                    |
| 13   | M         | 31     | L       | 6                     | VI           | ND               | ND               | ND                     |
| 14   | M         | 31     | L       | 9                     | VI           | VI               | IV               | III                    |
| 15   | M         | 28     | L       | 10                    | VI           | VI               | IV               | IV                     |
| 16   | M         | 32     | L       | 5                     | VI           | V                | IV               | III                    |
| 17   | F         | 24     | R       | 11                    | VI           | V                | IV               | III                    |
| 18   | M         | 48     | R       | 6.5                   | VI           | V                | IV               | III                    |
| 19   | F         | 43     | L       | 11                    | V            | V                | IV               | IV                     |
| 20   | M         | 41     | L       | 8                     | VI           | VI               | IV               | IV                     |
| 21   | F         | 24     | L       | 8                     | V            | V                | III              | III                    |
| 22   | F         | 42     | R       | 14                    | VI           | VI               | IV               | IV                     |
| 23   | M         | 24     | L       | 3.5                   | V            | V                | III              | III                    |

FP: Facial Paralysis; HB grade: House-Brackmann facial nerve grading system; ND: no data.

Fig. 2. Workflow and fMRI task design. A: Four tasks were designed and performed by the participants during fMRI examination, including closing the eyes normally, closing the eyes intensively, pursing the lips, and lifting the tongue up against the palate and down toward the floor of the mouth. In order to better show the action of tongue lifting, we use the schematic diagram in which the subject’s mouth is open, but in fact the subject keeps the mouth closed. B: The task design applied in this study is a block design, using 5 segments of 20 s off/20 s on for each block. C: Participant enrollments (HB grade evaluations, EPE and fMRI exams) in each visit. EPE: electrophysiological examination.
contraction potentials. Action potentials were recorded in the orbicularis oculi and orbicularis oris. Compound muscle action potentials (CMAPs) were recorded in the orbicularis oculi and orbicularis oris. Action potentials were recorded in the orbicularis oculi and orbicularis oris. Action potentials were recorded in the orbicularis oculi and orbicularis oris. Action potentials were recorded in the orbicularis oculi and orbicularis oris. Action potentials were recorded in the orbicularis oculi and orbicularis oris. Action potentials were recorded in the orbicularis oculi and orbicularis oris.

2.5. Acquisition of MRI data

MRI scans were conducted using a Siemens Prisma 3.0 T Magnetic Resonance Scanner (Siemens Healthineers, Erlangen, Germany) with a standard 20-channel head coil. Each neuroimaging session included one 3D T1-weighted structural scan and four scans of blood oxygen level-dependent functional MR imaging (fMRI). fMRI scans were performed using following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, voxel size = 3 x 3 x 3 mm, and number of slices = 30. For anatomical reference, a T1-weighted image was scanned before fMRI examination with TR = 2300 ms, TE = 2.32 ms, voxel size = 1 x 1 x 1 mm, and number of slices = 192.

Four designed tasks were performed by the participants during fMRI examinations, including closing eyes normally, closing eyes intensively, pursing lips, and lifting the tongue up against the palate and down to the floor of the mouth. The task design applied in this study was a block design task, using 5 segments of 20 s off/20 s on for each block (Fig. 2). Eight seconds of rest prior to the first block was designed to allow participants to adapt to the scanning environment. The experimental program was written with E-prime 2.0 (Psychology Software Tools, Inc., Pittsburgh, USA) and presented by a visual and auditory brain function stimulation system (Shenzhen Meide Medical Electronic Technology Co., Ltd., Shenzhen, China). The experimental program automatically commenced in synchronization with the scanning sequence.

Participants were trained to perform the tasks prior to the scanning procedure and were asked to stay relaxed or to execute the tasks without making head movements following the instructions presented on the projector. During the task of lip pursing, participants were asked to avoid moving their tongue and other facial muscles.

2.6. fMRI data preprocessing

Task-related functional image analysis was performed with the fMRI Expert Analysis Tool (FEAT, http://www.fmrib.ox.ac.uk/fsl) from FMRIB Software Library (FSL v. 6.0) (Jenkinson et al., 2012; Smith et al., 2004). Individual-level pre-processing included brain extraction, motion correction, spatial smoothing using a Gaussian kernel of full-width half-maximum of 6 mm, high-pass temporal filtering (60 s cutoff), and linear coregistration of functional images to their high-resolution T1-weighted structural image with FMRIB’s Linear Registration Tool (FLIRT). A univariate general linear model (GLM) was applied to estimate contrast images of each task condition. Task regressors were convolved with a double-gamma hemodynamic response function. To correct for multiple
comparisons, the resulting Z-statistic images were thresholded using clusters determined by \( z > 2.3 \) and a corrected cluster significance threshold of \( p = 0.05 \) (Jenkinson and Smith, 2001). All result images were warped and interpolated to Montreal Neurological Institute (MNI) standard space using the nonlinear warping matrix generated by the transformation of anatomical volume to standard space.

2.7. fMRI data analysis

One-sample t-tests were first used in second-level analysis to examine single-group average activations in the NC group and the patient group at different visits. Two-sample t-tests were conducted to reveal neural activity changes in patients prior to neurorrhaphy compared to the neural activity of NCs.

We conducted follow-up paired t-tests to characterize the direction of significant time effects after neurorrhaphy in comparisons of 1) pre-neurorrhaphy at 3–8 months (Post-V1) to preneurorrhaphy (Pre); 2) postneurorrhaphy at 9–17 months (Post-V2) to preneurorrhaphy; and 3) Post-V2 to Post V1 for each task.

Regions showing a ‘task-positive’ effect during tasks were considered for further analysis. The threshold of these comparisons was determined based on the magnitude (\( z > 3.1 \)) and extent (cluster significance \( P < 0.05 \)) of activation (Worsley et al., 2002).

2.8. Correlation analysis between neural activation and FN function

To examine the correlations between fMRI measures and FN function, we further conducted region of interest (ROI) analyses. ROIs were selected based on the group-mean activities in patients, with reference to previous facial-movement related areas (Rottler et al., 2014). Details are provided in section 3.4.

Pearson correlations of task-related activation with its corresponding continuously measured CMAPs and the Spearman correlation with HB grading scores were conducted in SPSS (version 20; IBM Corp., Armonk, NY, USA). All variables using in the correlation analyses have passed the normality test. We checked for the associations of brain activation with continuously measured CMAPs and the Spearman correlation with HB grading scale.

3. Results

3.1. FN function improvement

No significant difference in sex or age distribution was found between the patients and NCs. The overall results of clinical observation of FN function for the 23 patients are presented in Table 1 and Table 2a, Table 2b.

All patients were graded between V to VI in HB grading scale. Perceptible improvement in FN function was detected in 9 of the 23 patients at 3–8 months after neurorrhaphy (Post-V1). At 12–17 months after neurorrhaphy (Post-V2), 20 patients were successfully followed up, and all patients except 1 showed improved facial function. At the end of the follow-up period, two of the 20 patients reached grade II regarding HB FN function. Eleven other patients reached grade III, and six others reached grade IV. No synkinesis was found in any of these patients during eating, drinking or speaking. Statistical analysis showed that a significant difference in the HB grade when comparing the patients’ data prior to neurorrhaphy to data from Post-V1 (p = 0.003) and Post-V2 patients (p < 0.001). A significant difference was also found when comparing data from Post-V1 patients to data from Post-V2 patients (p < 0.001).

We also analyzed the relationship between the duration of facial paralysis from onset to neurorrhaphy and recovery of lost facial nerve function, and found that the duration was correlated with the HB grade at the first postoperative follow-up time point (r = 0.59, P = 0.006).

3.2. Electrophysiological examinations

Before neurorrhaphy, no CMAPs were found in any of the patients when the mandibular angle was electro-stimulated. Fibrillation potentials and positive sharp waves in the facial muscles were frequently detected at rest in all the patients, confirming their denervation.

After neurorrhaphy, CMAPs were detected in 19 patients except one when the FN was electro-stimulated in the mandibular angle at Post-V1. Muscle action potentials were detected in 18 patients in the orbicularis oris and in 8 patients in the orbicularis oculi when they voluntarily contracted their facial muscles. At these time points, we detected muscle action potentials in the orbicularis oris and in 8 patients in the orbicularis oculi when they voluntarily contracted their facial muscles. At these time points, we detected muscle action potentials in the orbicularis oris and orbicularis oris muscles of the patients when they lifted their tongue strongly against the palate, confirming the innervation of the facial muscles by hypoglossal motorneurons. The amplitude of the CMAPs and the muscle contraction potentials increased over time in these patients (Table 1). In contrast, the spontaneous activity waves gradually decreased as the muscle underwent nerve reinnervation. By monitoring the frequency of fibrillation potentials and positive sharp waves, an examiner assessed the extent and progress of denervation into the grades of none, +, ++, ++++, or ++++. More + indicates severer denervation. At the second postoperative follow-up visit, significant differences were established for CMAPs at the orbicularis oculi muscle (Post-V2: 0.56 ± 0.53 mV vs Preoperative: 0.16 ± 0.22 mV, \( p = 0.019 \)) and orbicularis oris muscle (Post-V2: 0.65 ± 0.41 mV vs Preoperative: 0.13 ± 0.18 mV, \( p = 0.002 \)) and for the fibrillation potential which could represent muscle denervation at the orbicularis oculi (p = 0.003) and oris (p = 0.01) muscles compared to the values prior to neurorrhaphy. We classified recruitment muscle contraction potentials as 0, 1, 2, 3 or 4 if there were no contraction potentials, a small number of action potentials, potentials in a single phase, a
combination of single-phase and mixed-phase potentials, and exclusively mixed-phase potentials, respectively. Statistical analysis showed that significant recovery was found in the orbicularis oculi muscle \( (p = 0.017) \) and orbicularis oris muscle \( (p = 0.008) \) based on a comparison of values between the preoperative timepoint and the second follow-up visit. Fig. 3 shows the improvement in FN function and electrophysiological outcomes in one patient.

Fig. 3. Case 1 with right facial paralysis. A, B, C: Facial expressions in the Pre, Post-V1 and Post-V2 periods, respectively. The top pictures show that the patient had complete paralysis of the right side of the face (A). The lower pictures show that his FN function improved to HB grade III 1 year after neurorrhaphy (C). D, E, F: Facial electrophysiological examination of the right side in the Pre, Post-V1 and Post-V2 periods, respectively. CMAPs were recorded from the frontalis muscle, orbicularis oculi muscle, great zygomatic muscle and orbicularis oris muscle (D, E, F). No CMAPs were detected in the right paralyzed facial muscle before neurorrhaphy. Slight signals were detected in the right orbicularis oculi, orbicularis oris and great zygomatic muscle 4 months after neurorrhaphy; the signals then improved over time (E, F). Muscle action potentials were detected in the orbicularis oris and orbicularis oculi when the patients voluntarily contracted his facial muscles or strongly contracted his tongue muscles to lift his tongues against his palates. This picture shows that the muscle action potentials and spontaneous potentials recorded from the right orbicularis oculi and orbicularis oris muscle recovered over time. FM: frontalis muscle; GZM: great zygomatic muscle; SP: spontaneous potential; MCP: muscle contraction potential.

A: Intensive eye closing

B: Lip pursing

Fig. 4. Changes in motor-task-related activation after neurorrhaphy in patients. A: BOLD activation maps of patients at different follow-up time points and normal subjects on intensive eye closing task. B: BOLD activation maps of patients at different follow-up time points and normal subjects on lip pursing task.

3.3. Changes in motor-task-related activation after neurorrhaphy in patients evaluated using pairwise comparisons

Mean activation maps related to the intensive eye closing and lip pursing are shown in Fig. 4 across groups. In the patient group, the surgery alleviated the widespread overactivation in motor cortices during the intensive eye closing and lip pursing tasks, and the effects lasted until the second follow-up visit. However, we did not detect significant changes across visits during normal eye closing and tongue lifting, which activation maps are provided in supplementary s-Fig. 1.

Pairwise comparisons between post-neurorrhaphy versus pre-
Neurorrhaphy data revealed a significant reduction in overactivation in the motor cortex in both the intensive eye closing and lip pursing tasks after surgery. For both tasks, reduction in regional activation was detected after neurorrhaphy (Fig. 5), with the largest effect size in the bilateral motor cortices, including the postcentral gyrus, the precentral gyrus, and the supplementary motor area (SMA). We did not detect activation changes during the normal eye closing and tongue lifting tasks.

During the intensive eye closing task, Post-V1 data showed decreased activation in the SMA extending to the paracingulate and anterior cingulate gyrus, inferior frontal gyrus and the frontal pole compared to the pre-neurorrhaphy group. This pattern of activation reduction overlapped with the areas of altered activation in preoperative patients compared to NCs (Fig. 5, Table 3).

During the lip pursing task, a similar activation reduction pattern was observed after neurorrhaphy. Patients showed decreased activation Post-V1 after neurorrhaphy in the SMA and further decreased activation in Post-V2 period in multiple brain regions, including in the right superior frontal gyrus, postcentral gyrus, parietal operculum cortex, and frontal pole. This activation reduction pattern also matched the areas of overactivation in preoperative patients compared to controls (Fig. 5, Table 3).

We did not detect significant changes from Post-V1 to Post-V2 in any of the four tasks.

3.4. Associations between brain area functional activation and nerve functions

Based on the group-mean activation, the regions listed below were selected as regions of interest (ROIs) in correlation analyses for the relevant tasks.

1. Intensive eye closing: the left middle temporal gyrus, left inferior frontal gyrus, left postcentral gyrus, left insula, SMA extending to the paracingulate, left motor cortex, and right motor cortex.

2 Lip pursing: the left superior temporal gyrus, right putamen, left motor cortex, right motor cortex and the supplementary cortex.

The overall results of ROI analyses in the intensive eye closing task and the lip pursing task are shown in Fig. 6. Pronounced overactivation occurred in almost all motor-related regions during both tasks before neurorrhaphy but gradually decreased to normal after neurorrhaphy.

After correcting for multiple comparisons with \( p < 0.01 \), intensive eye closing-related activation in the left postcentral gyrus was positively

Fig. 5. Changes in motor-task-related activation after neurorrhaphy in patients. A: The decreased activation area in the Post-V1 period compared to the activation in the Pre-period in the intensive eye closing task. B: top row: the decreased activation area in the Post-V1 period compared to the activation in the Pre-period in the lip pursing task; bottom row: the decreased activation area in the Post-V2 period compared to the activation in the Pre period in the lip pursing task.
correlated with CMAPs in the orbicularis oculi after 3–6 months ($r = 0.609, p = 0.007$). Another cluster of the left motor cortex, including the precentral gyrus, the postcentral gyrus, and the posterior division of the middle frontal gyrus, showed a marginally significant correlation with CMAPs in the orbicularis oculi at the Post-V1 period ($r = 0.499, p = 0.035$) and a marginal prediction effect on oculi CMAPs at the Post-V1 period by pre-neurorrhaphy activation ($r = 0.473, p = 0.047$). Interestingly, left insula activation during the intensive eye closing task showed a marginal negative correlation with HB grading after surgery ($r = -0.484, p = 0.036$). We also found the predictive effect of pre-activation during the intensive eye closing task to FN function changes. The left motor cortex and right motor cortex activation at Pre showed a marginally positive correlation with CMAPs changes in the orbicularis oculi from Pre to Post-V1 respectively ($r = 0.473, p = 0.047$) ($r = 0.496, p = 0.036$). The Pre-activation in left insula during the intensive eye closing task showed a marginal negative correlation with HB change from Pre to Post-V1 ($r = -0.500, p = 0.029$).

In the lip pursing task, a marginal positive correlation was detected between activation in the left superior temporal gyrus and the CMAPs of the orbicularis oris ($r = 0.556, p = 0.017$). Plots of the above correlations are shown in Fig. 7.

### 3.5. Brain activation in the more-affected and less-affected hemisphere

With flipped fMRI data of patients whose symptoms were on the right side to left, group mean activation in patients are bilateral and symmetric tested by a paired two-sample t-test on the current data to its x-axis flipped replicates. This result implies that the current bilateral facial motor task did not induce difference between the MAH and LAH. Thus,

| Cluster No. | Region                                      | Z max | Peak voxel in MNI space (x, y, z) | Cluster size |
|-------------|---------------------------------------------|-------|-----------------------------------|--------------|
| #1          | Supplementary motor cortex, paracingulate gyrus, anterior cingulate (bilateral) | 4.07  | -9, 23, 37                       | 2025         |
| #2          | Pars opercularis, frontal operculum cortex, pars triangularis (left) | 4.53  | -46, 18, 7                       | 1502         |
| #3          | Frontal pole (left)                         | 5.12  | -34, 52, 27                      | 1210         |
| #1          | Supplementary motor cortex (bilateral)      | 4.41  | -6, 2, 57                        | 2004         |
| #1          | Supplementary motor cortex, paracingulate gyrus, superior frontal gyrus (bilateral) | 4.46  | 0, 6, 46                         | 2563         |
| #2          | Postcentral gyrus, supramarginal gyrus, parietal operculum cortex (right) | 4.37  | 47, -31, 29                      | 2488         |
| #3          | Frontal pole (right)                        | 4.3   | 17, 72, 26                       | 2222         |

**Table 3**

Regions of changed activation after neurorrhaphy.

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**Fig. 6.** ROI analyses in the intensive eye closing task (A, B) and the lip pursing task (C, D). LST: left superior temporal; LMT: left middle temporal (posterior division); LPT: left pars triangularis; LPoC: left postcentral gyrus; SMA: supplementary motor cortex; ParaC: paracingulate; LPreC: left precentral gyrus; LMF: left middle frontal gyrus; RPreC: right precentral gyrus; RPoC: right postcentral gyrus. *: $p < 0.1$; **: $p < 0.05$. 

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we did not take the effect of paralysis side into consideration in further statistics.

4. Discussion

Peripheral nerve injuries not only result in paralysis of the corresponding target tissue, but also induce central functional modifications (Kaas, 1991; Kaas and Collins, 2003; Wall et al., 2002). The subsequent functional deficits can be restored by reinnervation of denervated targets and related reorganization of the CNS after nerve reconstruction (Drubach et al., 2004). Activity-dependent stimulation, sensory or motor intensive training programs, and stimulation to modulate excitability within cortico-subcortical connections are able to guide reorganization so as to help recover lost function (Marqueste et al., 2004; Tankéré et al., 2000). In the present study, we showed that the reconstructing surgery transferred overactivations in surrounding areas of the precentral gyrus to a more accurate region corresponding to facial motor function that was followed by functional improvement.

Cortical reorganization to allow for the adoption of a new task has been demonstrated previously after ectopic neurorrhaphy between the musculocutaneous and intercostal nerves for traumatic cervical root avulsion in patients (Mano et al., 1995). TMS studies showed that biceps muscle cortical control was transferred to clusters of neurons corresponding to the intercostal muscle cortical area after such neurorrhaphy. Activation coupling between these two muscle groups was shown by recording subthreshold rhythmic depolarizations of biceps motor units during respiration. Moreover, the original biceps area regained access to the biceps muscle via the intercostal nerve associated with improved independence of biceps contraction from respiration over time. In this study, we observed that ectopic control of the paralyzed facial muscle by hypoglossal neurons gradually shifted to voluntary facial movement in the patients after hypoglossal-facial neurorrhaphy. A similar functional reorganization in the cortex also occurred in the patients, transferring from tongue muscle-related to facial muscle-related areas.

Regarding the 8 defined functional areas of the cortex in this study, we found that the detected brain activations related to facial motor function were consistent with well-known findings, including the primary motor area, the ventral lateral promotor cortex, the SMA and the cingulate motor area (Morecraft et al., 2004). The frontal lobe and insula were also suggested to play an important role in sensorimotor function as an ipsilateral pathway (Kobayashi et al., 2003). Overactivation in motor cortices in patients with FN injury has been sufficiently substantiated (Calistri et al., 2020; Xiao et al., 2015). When performing facial motor tasks, patients must pay more attention to facial movements and use more facial motor imagery than healthy people. In this study, we found increased neural activation in patients not only within the facial executive regions but also in the surrounding cortical areas. Among these regions, the SMA is a vital area that showed significantly decreased activity after neurorrhaphy in both eye and lip movement. The SMA is believed to contribute to motor planning and to modulate complex movement impulses transferred from the primary motor cortex to the motor nuclei within the brainstem (Dresel et al., 2006). It also coordinates voluntary movements as well as involuntary movements (Nachev et al., 2008; Salardini et al., 2012). Increased activity of the SMA could be associated with the suppression of involuntary movements during the execution of self-paced movements (Pinis et al., 2013).

Fig. 7. The relationships between ROI activation and nerve function. A: ROI analyzed in intensive eye closing. a: Correlation between Post-V1 fMRI activation in LPoC and Post-V1 orbicularis oculi CMAPs. b: Correlation between Post-V1 fMRI activation in LPreC + LPoC + LMF and Post-V1 orbicularis oculi CMAPs. c: Correlation between Pre fMRI activation in left insular and the Post-V1 HB grade. d: Correlation between Pre fMRI activation in LPreC + LPoC + LMF and Post-V1 orbicularis oculi CMAPs. e: Correlation between Pre fMRI activation in LPreC + LPoC + LMF and CMAPs changes in the orbicularis oculi of Post-V1 compared with Pre. f: Correlation between Pre fMRI activation in RPreC + RPoC and CMAPs changes in the orbicularis oculi of Post-V1 compared with Pre. g: Correlation between Pre fMRI activation in left insular and HB grade change of Post-V1 compared with Pre. B: ROI analyzed in lip pursing task; Correlation between Post-V1 fMRI activation in LST + LMT and Post-V1 orbicularis oris CMAPs.
According to a study in patients with Meige’s syndrome, overactivation of the SMA during facial movement could be partly reversed by botulinum toxin treatment (Dresel et al., 2006). Therefore, we inferred that the overactivation in SMA in patients may relate to the control of inappropriate movements and increased awareness of imminent facial expressions. With progressive recovery of facial paraesthesia after neurosurgery, the activation of the SMA progressively returned to normal levels.

In addition to the SMA, the other two regions showing similar time courses were the bilateral pre- and postcentral gyrus. As representations of eye and lip motor function, the decreased activation in the pre- and postcentral gyrus after surgery may suggest a lack of effort for patients to complete the tasks in the recovery stage (Romeo et al., 2013).

It is worth noting that a predictive relationship was detected of preoperative brain activation in the left insular and bilateral motor cortex with postoperative motor function. Higher activation level in the bilateral motor cortex and the insula is related to better facial nerve function after the surgery evaluated by both clinical symptoms scaling and a subjective electromyographic method. We infer that the higher activation in these areas may imply more usable neural resources and higher plasticity in patients with facial paralysis. Given that repetitive TMS is a promising noninvasive method for the management of facial paralysis, these regions may be potential targets for future TMS treatment to assist cortical reorganization and rehabilitation. Although the small sample size in the current study is hard to support for an independent validation of the predictive effect, we believe the findings have provided some valuable outcomes.

We infer that the bilateral motor-related fMRI tasks may limit observations on differences between the more-affected side and the less-affected side. A study with unilateral eye closing and smiling tasks demonstrated that facial movements on either side caused more intensive activation of the SMA on the contralateral side of the affected face than those of the unaffected side (Wang et al., 2018). Further studies with unilateral tasks may be more sensitive to reveal the different post-surgery changes between the two hemispheres. Unfortunately, we were not able to cover the cerebellum when collecting these fMRI data considering the total scan time. Later experiments will be optimized with multiband fMRI to collect data from a larger FOV covering the whole brain and higher time resolution. Another limitation of the current study is that the patients were not able to revisit us post-surgery in a consistent time point, largely due to the travel distance.

In conclusion, in this study, we observed that the extent of facial function recovery was closely related to cortical reorganization in patients after hypoglossal-facial neurosurgery. The regained facial function had a positive correlation with enhanced activity in motor cortices before surgery, and this activity gradually diminished to normal levels after surgery. This is the first study to map activation change in motor cortices at multiple time points before and after treatment to repair the FN. The findings of these related central functional areas and the relationships between these areas and their changes provide may indicate potential treatment targets in the CNS when using adjuvant therapies such as repetitive TMS to further enhance functional recovery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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