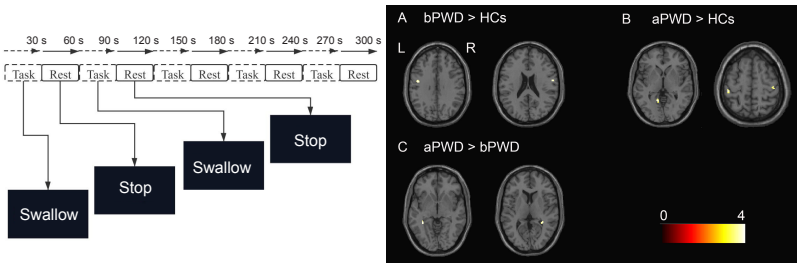
Research Article

Increased activation of the caudate nucleus and parahippocampal gyrus in Parkinson's disease patients with dysphagia after repetitive transcranial magnetic stimulation: a case-control study

https://doi.org/10.4103/1673-5374.324863
Date of submission: March 29, 2021
Date of decision: July 3, 2021
Date of acceptance: July 29, 2021
Date of web publication: September 17, 2021

Pei-Ling Huang[1, #], Song-Jian Wang[2, #], Rui-Feng Sun[3], Zi-Man Zhu[3], Xiao-Ling Li[3], Wen-Shan Li[3], Meng-Yue Wang[2], Meng Lin[2], Wei-Jun Gong[1, *]

Graphical Abstract *Brain activation in Parkinson's disease patients with dysphagia during the saliva-swallowing task before and after repetitive transcranial magnetic stimulation*



Abstract

Repetitive transcranial magnetic stimulation (rTMS) has been shown to effectively improve impaired swallowing in Parkinson's disease (PD) patients with dysphagia. However, little is known about how rTMS affects the corresponding brain regions in this patient group. In this case-control study, we examined data from 38 PD patients with dysphagia who received treatment at Beijing Rehabilitation Medicine Academy, Capital Medical University. The patients received high-frequency rTMS of the motor cortex once per day for 10 successive days. Changes in brain activation were compared via functional magnetic resonance imaging in PD patients with dysphagia and healthy controls. The results revealed that before treatment, PD patients with dysphagia showed greater activation in the precentral gyrus, supplementary motor area, and cerebellum compared with healthy controls, and this enhanced activation was weakened after treatment. Furthermore, before treatment, PD patients with dysphagia exhibited decreased activation in the parahippocampal gyrus, caudate nucleus, and left thalamus compared with healthy controls, and this activation increased after treatment. In addition, PD patients with dysphagia reported improved subjective swallowing sensations after rTMS. These findings suggest that swallowing function in PD patients with dysphagia improved after rTMS of the motor cortex. This may have been due to enhanced activation of the caudate nucleus and parahippocampal gyrus. The study protocol was approved by the Ethics Committee of Beijing Rehabilitation Hospital of Capital Medical University (approval No. 2018bkky017) on March 6, 2018 and was registered with Chinese Clinical Trial Registry (registration No. ChiCTR 1800017207) on July 18, 2018.

Key Words: brain regions; caudate; clinical trial; dysphagia; functional magnetic resonance imaging; parahippocampal gyrus; Parkinson's disease; precentral gyrus; repetitive transcranial magnetic stimulation; saliva swallowing task

Chinese Library Classification No. R445.2; R741; R454

Introduction

Dysphagia is a common symptom of Parkinson's disease (PD), with an incidence of 82% (Takizawa et al., 2016). The onset is insidious, and the severity is progressive in PD with dysphagia (PWD). In total, 95–100% of patients with early-stage PD exhibit dysphagia, and most develop moderate to severe dysphagia and have difficulty swallowing 10–11 years after motor symptoms appear (Luchesi et al., 2015; Takizawa et al., 2016). Abnormal motor patterns, decreased coordination, and common oropharynx symptoms are characteristic of

[1]Department of Neurological Rehabilitation, Beijing Rehabilitation Hospital of Capital Medical University, Beijing, China; [2]Beijing Key Laboratory of Fundamental Research on Biomechanics in Clinical Application, School of Biomedical Engineering, Capital Medical University, Beijing, China; [3]Department of Neurological Rehabilitation, Beijing Rehabilitation Medicine Academy, Capital Medical University, Beijing, China
*Correspondence to: Wei-Jun Gong, PhD, gwj197104@ccmu.edu.cn.
https://orcid.org/0000-0002-9134-8218 (Wei-Jun Gong)
#Both authors contributed equally to this article.

Funding: *This work was supported by the Beijing Municipal Science and Technology Commission Capital Clinical Feature Applied Research Project of China, No. Z181100001718205 (to WJG and PLH).*
How to cite this article: *Huang PL, Wang SJ, Sun RF, Zhu ZM, Li XL, Li WS, Wang MY, Lin M, Gong WJ (2022) Increased activation of the caudate nucleus and parahippocampal gyrus in Parkinson's disease patients with dysphagia after repetitive transcranial magnetic stimulation: a case-control study. Neural Regen Res 17(5):1051-1058.*

PD-associated dysphagia (PAD), and are known to cause malnutrition, social impairment, anxiety and depression, a high risk of aspiration/inhalation pneumonia, and a reduction in the effects of therapeutic treatments (Kim et al., 2015; Matsushima et al., 2016; Suttrup and Warnecke, 2016; van Hooren et al., 2016; Chang et al., 2020). Therefore, dysphagia seriously reduces the effects of rehabilitation and quality of life in PD patients (Lee et al., 2017).

In a double-blind randomized controlled study, 33 patients with PWD were randomly chosen to receive sham or real repetitive transcranial magnetic stimulation (rTMS; 20 Hz; 90% of the resting motor threshold) over the hand area of each motor cortex for 3 months. The researchers found that real rTMS improved the Arabic-Dysphagia Handicap Index score and dysphagia as measured via video-fluoroscopy (Khedr et al., 2019). Thus, rTMS is a valuable non-invasive technology that can be used to effectively treat PAD. It is generally believed that rTMS elicits neuroplasticity, which can stimulate neurons in different periods, including the refractory period. rTMS also excites horizontal connections among neurons, produces summation of the excitatory postsynaptic potential, and balances excitatory-inhibitory links in the cortex (Martin-Harris et al., 2005). High-frequency (> 1 Hz) rTMS may increase the local metabolic level, affect long-term facilitation, and produce excitatory effects (Troche et al., 2013). Another study found that rTMS could induce alterations in neuronal cell plasticity by mediating gene expression and neural regulation (Silbergleit et al., 2012). As few studies have focused on PAD, little is known about how rTMS influences brain activity in individuals with PWD.

Functional magnetic resonance imaging (fMRI) has been used to observe changes and connections in functional activity among brain regions during different physical states. The spatial resolution of fMRI (within 2 mm) is much higher than that of positron emission tomography (within 6 mm), which can facilitate investigations of swallowing activity (Hamdy et al., 1999; Lang et al., 2015; Benzagmout et al., 2019). Considering the low level of movement coordination and high risk of aspiration in PWD patients, few studies have examined this patient group, and none have used task-state fMRI. Accordingly, the characteristic neuronal activation in PWD patients and the effect of rTMS on corresponding brain regions during swallowing in this population are unknown. To address this in the present study, we explored rTMS-induced neuroplasticity in PWD patients by comparing activation among healthy controls (HCs) and individuals with PWD.

Participants and Methods

Participants

This was a case-control study. Between January 2019 and December 2019, the recruitment information was released in China through WeChat official accounts, chat groups, websites, leaflets, and face-to-face briefings with patients, family members, and doctors. A total of 84 PD patients were recruited, of whom 7 PD patients without dysphagia were excluded. Forty-seven individuals with PWD completed all of the examinations and treatments, but nine MRI datasets were of poor quality. Finally, the study included 38 PWD patients (23 men, 15 women, aged 60.32 ± 8.03 years, disease duration 6.89 ± 2.77 years, Hoehn-Yahr stage 2.13 ± 0.52, Unified Parkinson's Disease Rating Scale Part III (UPDRS-III) 26.76 ± 11.81, Montreal Cognitive Assessment (MoCA) 23.92 ± 4.40). Thirty-three healthy participants aged 40–80 years were recruited as controls (HCs). As three MRI datasets were of poor quality, we included data from 30 healthy participants (11 men, 19 women, aged 56.23 ± 9.73 years). The experiment was conducted at the Beijing Rehabilitation Hospital of Capital Medical University.

The inclusion criteria for PWD were as follows: i) patients fulfilled the Movement Disorder Society clinical diagnostic criteria for PD (Postuma et al., 2015); ii) patients were considered to have dysphagia and met one or more of the following criteria via videofluoroscopic swallowing examination (VFSE) (Mosier et al., 1999): a) oral transport time > 1.5 seconds; b) pharyngeal transport time > 1.0 second; c) pharyngeal delay time: under 60 years > 0.36 second, over and equal to 60 years > 0.24 second; d) upper esophageal sphincter opening time > 0.51 second; e) pharyngeal cavity residue (epiglottis valley, piriform sinus) > 25%; and f) Leakage Aspiration Scale score > 2; iii) patients were aged between 40 and 80 years. The inclusion criteria for HCs were good health and age between 40 and 80 years.

The study exclusion criteria were: i) a history of other diseases affecting swallowing function (e.g., gastrointestinal diseases after radiotherapy for head and neck tumors); ii) severe pneumonia, renal or cardiac dysfunction; iii) current indwelling nasogastric tube or gastrostomy; iv) cardiac pacemaker, nerve stimulator, metal artery clamp, and other magnetic resonance examination or rTMS contraindications found *in vivo*; and v) cognitive impairment (a Mini-mental State Examination score ≤ 17 reflects illiteracy, ≤ 20 reflects a primary school level, ≤ 24 reflects a middle school and secondary school level; MoCA score < 26).

Withdrawal was defined using the following criteria: i) incomplete rTMS treatment or lack of cooperation with fMRI examination; ii) incomplete fMRI data or unmet data processing requirements; and iii) lack of informed consent or incomplete experiments. The study protocol was approved by the Ethics Committee of Beijing Rehabilitation Hospital of Capital Medical University (approval No. 2018bkky017) on March 6, 2018 (**Additional file 1**). All participants were volunteers and provided written informed consent (**Additional file 2**) prior to engaging in the study. All study protocols were in accordance with the *Declaration of Helsinki* of 1975 and the applicable revisions at the time of the investigation. This study was registered with the Chinese Clinical Trial Registry (registration No. ChiCTR 1800017207) on July 18, 2018.

Assessment

Patients with PWD were evaluated using the UPDRS-III, Hoehn-Yahr stage and VFSE while in their best condition after taking medicine ("ON" period). The PWD patients underwent the dysphagia handicap index (DHI), Mr. Tengdao's swallowing curative effect evaluation of swallowing (MTSCEEOS), and a complete fMRI examination before and after treatment. The HCs underwent a task state fMRI examination. All examinations were conducted by two experienced doctors. The UPDRS-III is the third part of the Movement Disorder Society-sponsored revision of the UPDRS (MDS-UPDRS), published in 2008 (Goetz et al., 2008). It is used to evaluate movement function and contains 33 items with 0–4 points each for a total score of 132. A higher score indicates worse function. The Hoehn-Yahr Scale, which comprises levels 0–5, was used to record the degree of motor dysfunction in the PD patients (Goetz et al., 2008). A higher level on the scale was associated with a greater degree of dysfunction. The DHI includes three components with a total of 25 items. The items comprise nine physiological and functional aspects, respectively, and seven emotional aspects, for a total score of 0–100 (Khedr et al., 2019). A higher score was associated with a worse subjective evaluation. The MTSCEEOS scores were divided into 10 grades, ranging from 1–10 to indicate more severe to less severe swallowing difficulty (Wang et al., 2012).

rTMS intervention

The PWD patients received high frequency rTMS (OSF-6/T; OSF Medical Technology Limited Company, Wuhan, China). The rTMS protocol was as follows: intensity = 90% motion threshold, frequency = 10 Hz, train duration = 2.00 seconds, interval time

= 8 seconds, train pulse = 20 pulses, number of trains = 60, total pulses per session = 1200, total length of session = 10 minutes, figure-of-eight coil TMS device, alternately over the left or right M1 region once a day for 10 days. The levodopa equivalent daily doses were adjusted to the best conditions and remained the same throughout the experimental process. No intervention was delivered in the HC group.

Task state fMRI

We used a block design to test brain activation related to the saliva-swallowing task. Five task blocks and five rest blocks were alternately carried out (**Figure 1**). Each block lasted 30 seconds. Chinese sentences and words were presented in red on a black background on a paper screen. During the scanning task, when "repeat swallowing, press the button after each swallow" appeared on the screen, the subjects swallowed saliva (lip closure, flat tongue at the bottom of the mouth, upper hyoid lift and circumpharyngeal muscle contraction). After each swallowing action, the subjects were required to press the button. Once they pressed the button, "stop" appeared on the screen, and the subjects rested for 30 seconds. During the task, the subjects kept their head motionless to concentrate on completing the swallowing task. Stimuli were presented using an image projector and a paper screen located in front of the subjects' feet. The subjects viewed the screen through a 45° angled mirror attached to the head coil of the MRI setup. The subjects were trained before scanning to ensure their cooperation and ability to complete the task. We used a General Electric signal 3.0T magnetic resonance scanner (General Electric Company, Boston, MA, USA) with an 8-channel head coil with foam filling and earplugs to limit patient head movements and reduce noise. All subjects underwent a routine scan to identify unrelated intracranial organic lesions. The whole brain was scanned using three-dimensional T1 bravo sequences. The scanning line was consistent with the T2 fluid attenuated inversion recovery sequence. The scanning parameters were as follows: repetition time = 8.1 ms, echo time = 3.1 ms, flip angle = 90°, field of view = 30 cm × 30 cm, matrix = 300 × 300, slices = 164, thickness = 1 mm. For task state fMRI, we adopted a gradient echo planar imaging sequence. The scanning line was consistent with the T2 fluid attenuated inversion recovery sequence, and the scanning parameters were as follows: repetition time = 2000 ms, repetition time = 30 ms, flip angle = 90°, field of view = 28 cm × 28 cm, matrix = 94 × 32, slices = 40, thickness = 4 mm, space = 1 mm.

Data analysis

Preprocessing

The imaging data were analyzed using statistical parametric mapping software (SPM12; Wellcome Department of Cognitive Neurology, London, UK) in the Matlab environment (version 7.8; The MathWorks Inc., Natick, MA, USA). Preprocessing of functional scans consisted of slice timing (sinc interpolation-reference slice 20, i.e., middle of the repetition time). Subsequently, the images were spatially realigned to the mean image due to head motion. Participants whose head movements exceeded 6 mm on any axis or rotations greater than 6° were withdrawn. After the correction, coregistration was conducted of the T1 image to the mean echo planar imaging (normalized mutual information), and of these images to the SPM average T1 image. Then, normalization was performed in East Asian brains of the international consortium for brain mapping space template to reduce morphing errors (Zhang et al., 2017). Finally, the normalized images were smoothed with a Gaussian kernel of 8-mm full-width half-maximum.

Task activation and regions of interest

Statistical parametric maps were calculated in the first-level analysis for each subject with a general linear model, and parameters for the swallowing fMRI paradigm model specification (http://www.fil.ion.ucl.ac.uk/spm) were introduced. After model estimation, a matrix was obtained for each subject showing higher brain activation conditions compared to the control condition (activation > control). These resulting 'combined' images from each group were entered into second-level one-sample *t*-tests to yield group-level activation. These resulting 'combined' images from each group were entered into the second-level to yield group-level activation. One-way analysis of variance test ($P < 0.05$, family wise error corrected for multiple comparisons) were used to assess the average fMRI activity during task in each group with SPM12 (Díez-Cirarda et al., 2017). Furthermore, a two-sample *t*-test was carried out to explore the differences in activation between HCs and PWD or before and after rTMS treatment in PWD (Díez-Cirarda et al., 2017). Finally, on the basis of a statistical parametric map for an *F*-test with three groups, regions of interest were created with a radius of 8 mm centered at the voxels with the local maxima of T values with SPM12. The signal change was analyzed for each group.

Statistical analysis

Demographic and clinical variables were analyzed using SPSS 22.0 (IBM, Armonk, NY, USA). Differences in DHI and MTSCEEOS scores before *versus* after treatment in the PWD group were tested using the Wilcoxon rank-sum test. We tested differences in the average frequency of button presses during the 30 seconds among HCs, and before and after treatment in the PWD group using a one-way analysis of variance. The least significant difference (LSD) test was used to compare inter-group variables. The significance level was defined as $\alpha = 0.05$ with $P < 0.05$.

Results

Sociodemographic, clinical, and behavioral characteristics of the PWD group relative to rTMS treatment

The sociodemographic characteristics of the subjects are shown in **Table 1**. After treatment, those in the PWD group had a lower DHI ($z = -5.38$, $P < 0.05$) and a higher MTSCEEOS score ($z = -3.31$, $P < 0.05$) compared with before treatment (**Table 2**).

Table 1 | **Demographic data from Parkinson's disease with dysphagia patients and healthy controls**

Item	Parkinson's disease with dysphagia patients (*n* = 38)	Healthy controls (*n* = 30)
Gender (male/female)	23/15	11/19
Age (yr)	60.32±8.03	56.23±9.73
Disease duration (yr)	6.89±2.77	NA
Hoehn-Yahr stage	2.13±0.52	NA
UPDRS-III	26.76±11.81	NA
MoCA	23.92±4.40	NA

Data are expressed as mean ± SD, except for gender, which are expressed as number. MoCA: Montreal Cognitive Assessment; NA: not applicable; UPDRS-III: Unified Parkinson's Disease Rating Scale-III.

Table 2 | **Comparison of DHI and MTSCEEOS scores before and after repetitive transcranial magnetic stimulation in Parkinson's disease with dysphagia patients**

Item	Before	After	*d*	*z*	*P*
DHI	25.39±10.86	16.87±6.20	8.53±6.18	–5.38	0.00
Physiology	8.71±5.01	4.79±2.85	3.92±3.39	–5.18	0.00
Function	10.34±3.74	7.37±2.34	2.97±2.32	–5.12	0.00
Emotion	6.50±3.03	4.71±1.97	1.79±1.56	–4.92	0.00
MTSCEEOS score	8.89±1.25	9.76±0.49	0.87±1.30	–3.31	0.00

Data are expressed as mean ± SD (*n* = 38), and were analyzed by Wilcoxon rank-sum test. DHI: Dysphagia handicap index; MTSCEEOS: Mr. Tengdao's swallowing curative effect evaluation of swallowing.

Behavioral performance during the fMRI in the PWD group relative to rTMS treatment

The average button press frequencies among HCs during the 30 seconds, and those before and after rTMS treatment in the PWD group were 5.93 ± 1.66, 5.94 ± 2.43, and 6.02 ± 2.09, respectively. No significant differences were found among the HCs or the PWD before and after rTMS treatment ($P > 0.05$).

Activated brain regions in the PWD group relative to rTMS treatment

The activated brain regions in the HCs, as well as in the PWD group before versus after rTMS treatment are shown in **Table 3** and **Figure 2** (corrected at the cluster level of $P < 0.05$ with family wise error). Compared with the HCs, the PWD group had enhanced activation in the precentral gyrus (PCG; left BA6, right BA4) before rTMS treatment and enhanced activation in the PCG (right BA4), postcentral gyrus (left BA1), and lingual gyrus (left BA19) after rTMS treatment, as shown in **Table 4** and **Figure 3** (uncorrected, $P < 0.001$, $k > 10$).

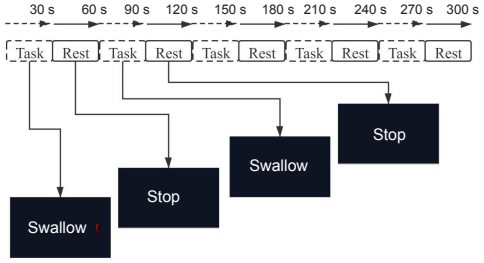


Figure 1 | **Task-state functional magnetic resonance imaging procedure.**
Five task blocks and rest blocks were presented alternately. The Chinese sentences in each task block said "repeat swallowing, press the button after each swallowing action" and the Chinese word in each rest block said "stop". In each task block, the subjects swallowed saliva repeatedly. After each swallowing action, the subjects were prompted to press the button. Then, "stop" appeared on the screen, and the subjects rested until the next trial. The experiment lasted 5 minutes.

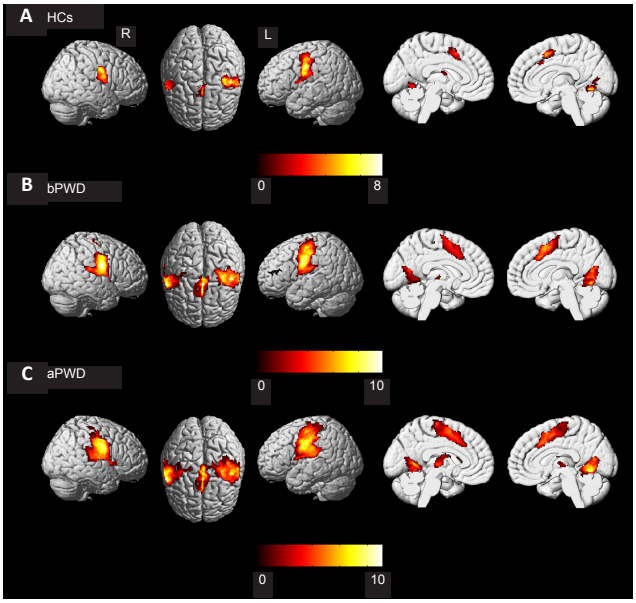


Figure 2 | **Functional magnetic resonance imaging showing changes in activation in individuals with Parkinson's disease with dysphagia and healthy controls during the saliva-swallowing task.**
(A) Healthy controls. (B, C) Parkinson's disease with dysphagia patients before (B) and after (C) repetitive transcranial magnetic stimulation treatment. Regions in which brain activation changed are shown in red or yellow. Results are corrected at the cluster level of $P < 0.05$ with family wise error. L: Left; R: right.

Activation in the right caudate and left parahippocampal gyrus (PHG; left BA19) was enhanced after rTMS in the PWD group, as shown in **Figure 3** (uncorrected, $P < 0.001$, $k > 10$).

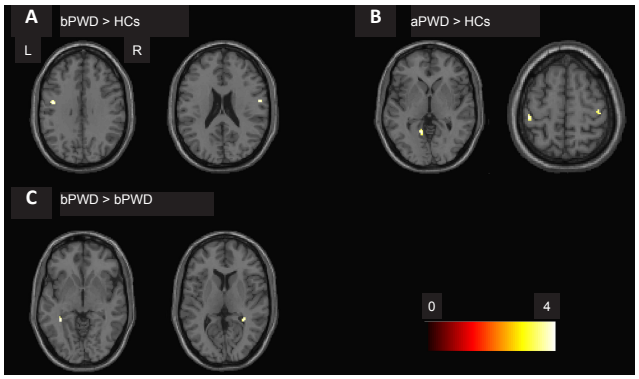


Figure 3 | **Functional magnetic resonance imaging of brain activation changes in patients with Parkinson's disease with dysphagia and healthy controls during a saliva-swallowing task.**
Regions in which brain activation changed are shown in red and yellow. Results show significant activation (uncorrected, $P < 0.001$, $k > 10$). (A) Enhanced activation in the precentral gyrus (left BA6, right BA4) between the HCs and bPWD group. (B) Enhanced activation in the precentral gyrus (right BA4), postcentral gyrus (left BA1), and lingual gyrus (left BA19) between HCs and the aPWD group. (C) Enhanced activation in the right caudate and left parahippocampal gyrus between the bPWD and aPWD groups. aPWD: Parkinson's disease with dysphagia after treatment; bPWD: Parkinson's disease with dysphagia before treatment; HCs: healthy controls; L; left; R: right.

For the PWD group, activation intensity of the bilateral PCG, supplementary motor area (SMA), and cerebellum was higher after *versus* before rTMS, and higher than that in the HCs at both time points. The opposite was observed in the PHG, caudate, and left thalamus. Moreover, the activation intensity of the right thalamus in the PWD group was lower after rTMS versus before rTMS (**Figures 4** and **5**).

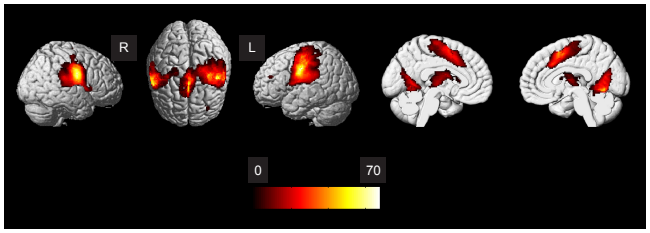


Figure 4 | **Effect of repetitive transcranial magnetic stimulation on the brain regions activated during the saliva-swallowing task in individuals with Parkinson's disease with dysphagia.**
Regions in which the brain activation changed after treatment are shown in red and yellow. Results were corrected at the cluster level of $P < 0.05$ with family wise error. aPWD: Parkinson's disease with dysphagia after treatment; bPWD: Parkinson's disease with dysphagia before treatment; HCs: healthy controls; L; left; R: right.

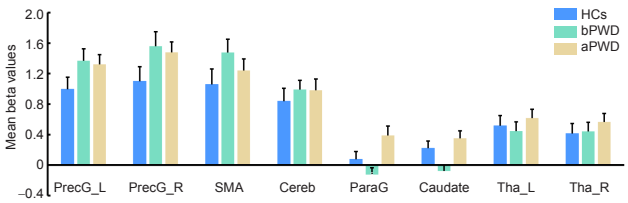


Figure 5 | **Effect of repetitive transcranial magnetic stimulation on the signal intensities of activated brain regions in individuals with Parkinson's disease with dysphagia.**
aPWD: Parkinson's disease with dysphagia after treatment; bPWD: Parkinson's disease with dysphagia before treatment; Cereb: cerebellum; HCs: healthy controls; ParadeG: parahippocampal gyrus; PreG_L: precentral gyrus left; PreG_R: precentral gyrus right; SMA: Supplementary motor area; Tha_L: thalamus left; Tha_R: thalamus right.

Table 3 | **Summarized activation in Parkinson's disease patients with dysphagia and healthy controls**

	Cluster size (voxels)	Hemisphere	Anatomical region	Brodmann area	*t* score	Montreal Neurological Institute Coordinates		
						x	*y*	*z*
Healthy controls	1388	Left	Precentral gyrus	4	7.25	–40	–12	46
	226	Right	Culmen	*	6.18	8	–66	–10
		Left	Culmen of vermis	*	6.08	–2	–64	–6
	609	Right	Precentral gyrus	6	5.99	54	–4	32
		Right	Postcentral gyrus	43	5.31	60	–10	18
	384	Left	Medial frontal gyrus	32	5.67	–2	8	46
		Right	Superior frontal gyrus	6	5.47	8	12	48
	39	Right	Cingulate gyrus	32	5.31	10	20	34
	46	Left	Thalamus	*	4.92	–10	–8	16
Parkinson's disease patients with dysphagia								
Before repetitive transcranial magnetic stimulation treatment	2884	Left	Precentral gyrus	6	9.84	–44	–8	34
		Left	Middle frontal gyrus	6	7.51	–44	0	52
		Left	Postcentral gyrus	43	7.44	–60	–8	20
	1946	Right	Precentral gyrus	4	9.37	58	–4	20
		Right	Insula	13	5.83	38	–2	10
		Right	Postcentral gyrus	40	5.37	56	–24	18
	1543	Right	Superior frontal gyrus	6	9.32	2	8	54
		Right	Cingulate gyrus	32	7.37	12	20	34
		Left	Cingulate gyrus	32	5.96	–8	16	34
	1368	Right	Culmen	*	8.54	16	–64	-10
		Left	Cuneus	30	6.38	–8	–70	10
	55	Left	Insula	13	5.45	–32	16	10
	31	Left	Thalamus	*	5.01	–12	–18	2
	28	Right	Insula	13	4.85	36	16	4
After repetitive transcranial magnetic stimulation treatment	6214	Left	Precentral gyrus	6	9.04	–50	–8	32
		Left	Postcentral gyrus	3	8.31	–60	–10	24
		Left	Superior frontal gyrus	6	8.2	0	10	54
	3261	Right	Precentral gyrus	6	8.32	52	–4	32
	1673	Right	Culmen of Vermis	*	7.37	4	–62	–6
		Left	Culmen	*	7.31	–12	–52	–2
	870	Left	Thalamus	*	7.15	–12	–16	4
		Left	Insula	13	6.34	–32	14	10
		Right	Insula	13	5.75	32	–10	16
	74	Right	Thalamus	*	5.73	12	–16	0
	21	Right	Caudate	*	5.13	12	–6	16

Data were analyzed by one-way analysis of variance test followed by the least significant difference test and all results were corrected at the cluster level of $P < 0.05$ family wise error. * Indicates the brain area is not noted in the way of Brodmann area.

Table 4 | **Comparison of activated brain regions between groups**

	Cluster size (voxels)	Hemisphere	Anatomical region	Brodmann area	*t* score	Montreal Neurological Institute Coordinates		
						x	*y*	*z*
bPWD-aPWD	13	Left	Parahippocampal gyrus	19	3.63	–36	–42	–4
	11	Right	Caudate	*	3.58	32	–42	8
bPWD-HCs	26	Left	Precentral gyrus	6	3.58	–44	–6	32
	15	Right	Precentral gyrus	4	3.55	58	–6	22
aPWD-HCs	18	Right	Precentral gyrus	4	3.92	40	–22	62
	30	Left	Lingual gyrus	19	3.92	–12	–54	0
	20	Left	Postcentral gyrus	1	3.84	–44	–28	60

aPWD: PWD after treatment; bPWD: PWD before treatment; HCs: healthy controls. * indicates the brain area is not noted in the way of Brodmann area.

Discussion

Only two previous studies have used fMRI to examine PAD (Suntrup et al., 2013; Gao et al., 2019): one used magnetoencephalography and the other used resting-state fMRI. To the best of our knowledge, the present study is the first to use task-state fMRI to study rTMS-induced changes in activation in PAD patients using the saliva-swallowing task and not the autonomous water-swallowing task or the reflex water-swallowing task (Perry et al., 2018; Kober et al., 2019). The latter two tasks are difficult to accomplish in PWD patients who are restricted by recumbency. In addition, considering that decreased coordination between the oral and pharyngeal phases causes salivation (Pfeiffer, 2018), saliva swallowing was safer and closer to the pathological state of PWD patients. Our

data indicate that brain region activation was more consistent between the HCs and PWD group before rTMS treatment, and that it was roughly the same as that observed in previous autonomous water-swallowing and water-swallowing reflex tests in healthy participants (Perry et al., 2018; Kober et al., 2019). Brain activation was mostly concentrated in the cortical sensory motor area (CSMA), premotor area, SMA, basal ganglia, insula, cerebellum, and other brain regions, indicating that saliva swallowing can be used as a task paradigm for PWD patients (Perry et al., 2018; Kober et al., 2019).

No previous studies have published rTMS protocol for dysphagia in PD patients. The rTMS protocols used in clinical settings are generally based on existing protocols (such as those for dysphagia in stroke patients) and are designed on an individualized basis. However, unlike stroke, the brain sites involved in PDW are often bilateral, unfixed, extensive, and progressive (Kober et al., 2019). Kikuchi et al. (2013) and Gao et al. (2019) suggested that there was hemispheric imbalance in PDW. During autonomous swallowing, the CSMA is the largest and most stable activated area, and it exhibits the strongest signal (Hamdy et al., 1999; Mosier et al., 1999; Suntrup et al., 2013; Maidan et al., 2017). This was in line with the present results. Thus, stimulation of the bilateral cortex could help to improve the observed imbalance, and this would be consistent with the pathological changes observed in PDW. The CSMA (including the PCG) was activated in the HC, pre-rTMS PDW, and post-rTMS PDW groups, which coincided with previous results (Hamdy et al., 1999; Mosier et al., 1999; Suntrup et al., 2013; Maidan et al., 2017). This is supported by previous studies that identified sensory and motor neurons related to facial, oral, and throat muscles in this region, as these were activated when saliva entered the throat from the mouth during our study. Furthermore, the CSMA participates in autonomous action (e.g., autonomous swallowing), and might be the highest center for initiating swallowing.

The front part of the premotor area, which stores motor memory, is an advanced center for planning and selecting motor programs, as well as guiding and regulating the swallowing process. The posterior part of the premotor area, which is located near the primary motor area, has two-way connections and overlapping functions (Hamdy et al., 1999; Mosier et al., 1999). The primary motor area accepts movement planning information (e.g., swallowing) from the front part of the premotor area, and implements the movement plan (e.g., swallowing) through the fiber connections from the posterior part of the premotor area. Together, the SMA and the premotor area form Brodmann area 6 (Hamdy et al., 1999; Mosier et al., 1999). The SMA plays an important role in complex temporal movement and in movement initiation and execution (Hamdy et al., 1999; Mosier et al., 1999). The insula, which is the main taste cortex, is associated with the ventral posterolateral thalamus (the sensory representative area of the face and mouth, and the termination replacement relay station of first stage taste afferent neurons) through the anterior thalamus (Hamdy et al., 1999; Mosier et al., 1999).

Through positron emission tomography technology, Kikuchi et al. (2013) found that glucose metabolism was reduced in the SMA (BA6) and anterior cingulate gyrus in PWD patients compared with normal controls. Furthermore, they found that the bilateral medial frontal lobe, medial cingulate cortex, thalamus, and upper, middle, and lower orbital frontal lobe were hypometabolic 3 years after a PWD diagnosis. Compared with HCs, they observed enhanced activation in PWD patients before and after rTMS in the PCG (BA4, 6) and lingual gyrus (BA19). This indicates that swallowing function was weakened in these patients such that an increased activation volume and intensity were needed to maintain swallowing function. These results are consistent with the findings of the present study. Gao et al. (2019) found that PWD patients (n = 13) exhibited enhanced functional connectivity in the left cerebellar tonsil, cerebellum (BA8, 9), and fusiform compared with a normal control group (n = 10). According to these two studies, PWD patients maintain a baseline swallowing state by enhancing connections of the left cerebellar tonsil, cerebellum (BA8, 9), and fusiform gyrus in the quiet state (i.e., when no swallowing action is performed). Enhanced activation of the PCG, lingual gyrus, and other brain regions occurs in a compensatory manner after initiating a swallowing action.

Previous neuroimaging and pathophysiological studies on dopamine loss in the striatum have suggested that the pattern of dopamine loss in the basal ganglia is inhomogeneous (Winogrodzka et al., 2003; Pasquini et al., 2019). In other words, the dopaminergic neurotransmitters binding with the striatal neurons in the shell nucleus were asymmetrically reduced, and that in comparison, the ones in the head of the caudate body were retained. The gradient of dopaminergic loss is largely preserved in all PD patients (Pasquini et al., 2019). Im et al. (2018) and Kim et al. (2019) showed that caudate damage can increase the risk of aspiration and prolong the recovery time of swallowing. Hence, caudate injury is likely involved in the occurrence of dysphagia in PD patients and is potentially associated with gradient changes in dopaminergic loss. In this study, we found no significant pre-rTMS caudate activation in the PWD group compared with the HCs, while the PWD group exhibited post-rTMS improvements in swallowing quality and enhanced caudate activation compared with the HCs. This confirmed the previous hypothesis that the caudate is associated with the occurrence of dysphagia in PWD patients. High-frequency rTMS can stimulate the release of neurotransmitters in the caudate of healthy persons and PWD patients, leading to enhanced neuroplasticity (Strafella et al., 2001; Sacheli et al., 2019). Therefore, it is possible that a caudate-associated abnormal dopaminergic damage gradient could inhibit the ability of the caudate to perform normal compensatory functions, and thus participates in the pathophysiological processes that underlie impaired swallowing in PAD patients. High-frequency rTMS may promote homeostasis in caudate-associated dopamine levels by altering neurotransmitter release, which in turn could improve swallowing function.

The DHI assesses swallowing function using three aspects and can be greatly affected by the subjective feelings of patients. The PHG is part of the limbic system and is closely related to emotion. Activation of the PHG has been found to increase with exercise and positive events (Loeffler et al., 2018; Loprinzi, 2019). In this study, transient saliva swallowing activity did not enhance PHG activation. However, rTMS might have enhanced pleasure by promoting PHG activation, which in turn improved subjective feelings of swallowing.

Differences in the intensity of brain activation among the three groups might be related to the degree of injury in each region, compensatory ability, and the selectivity of the rTMS effect on specific brain regions. Braak proposed that pathological changes spread from the peripheral to the central nervous system, but not all types of PD patients conform to this hypothesis (Jellinger, 2019). The diversity of symptoms in PWD reflects the complexity of location, extent, and compensatory capacity in PD. All patients included in this study had a Hoehn-Yahr stage below 3. Thus, their condition may not have developed to the point of involving the substantia nigra, midbrain, or deep anterior cerebral nuclei. According to Braak's hypothesis, the neocortex was also not likely to be involved in these patients. Hulme et al. (2013) found that the ability or mechanism of neurons to express plasticity might be recruited in non-specific ways under pathological conditions,

which could explain the compensatory enhancement of the PCG, SMA, and cerebellar activation intensity in the PWD patients before and after rTMS treatment.

The activation intensity of the PHG and caudate was significantly reduced in the PWD group before rTMS treatment, indicating that the PHG and caudate were not the main compensatory mechanisms, but that they might be related to the occurrence and progression of dysphagia in PD. After treatment, the activation intensity of the PHG and caudate increased. This was associated with rTMS-induced reduction in the inhibition state of the PHG and caudate, likely via neurotransmitter regulation. Dysphagia is associated with thalamic injury (Kooshkabadi et al., 2013). However, deep brain stimulation of the subthalamic nucleus restored some motor patterns in the pharyngeal phase to performance levels approximating those of "normal" swallowing but did not improve the degree of hyoid bone excursion or oral phase measures in PD patients (Ciucci et al., 2008). Thalamus metabolism in PWD patients gradually decreased as onset time increased (Kikuchi et al., 2013). The changes in the thalamic activation intensity observed in the three groups in this study might be related to the short duration of disease in the PWD patients and relative functional retention of the thalamus.

There were three limitations in this study. First, the sample was relatively small. Second, PD patients without dysphagia were not included. Finally, we did not use objective evaluation methods such as VFSE after treatment. However, that activation of the right caudate and left parahippocampal gyrus was enhanced in PD patients with dysphagia reflects that neuroplasticity was induced by high-frequency rTMS. Thus, these regions may be potential therapeutic targets for precise treatment. Finally, our data indicate that the task paradigm was safe and effective for patients with a high risk of aspiration.

In conclusion, the saliva-swallowing task appears to be a safe and effective experimental paradigm for assessing patients with a high risk of aspiration such as those with PWD. Enhanced activation of the PCG, postcentral gyrus, and lingual gyrus functions in a compensatory manner after initiating swallowing action in PWD. rTMS treatment led to improved subjective swallowing sensations and enhanced activation of the caudate and PHG in PAD patients, providing evidence for rTMS-induced neuroplasticity and a potential treatment for PWD.

Author contributions: *Study design and guidance, volunteer recruitment and manuscript draft: PLH; WJG; fMRI parameter design, interpretation and data analysis: SJW, MYW, ML; data acquisition including scale and image: PLH, RFS, XLL, ZMZ, WSL. All authors read and approved the final manuscript.*
Conflicts of interest: *There are no conflicts of interest to declare.*
Financial support: *This work was supported by the Beijing Municipal Science and Technology Commission Capital Clinical Feature Applied Research Project of China, No. Z181100001718205 (to WJG and PLH). The funding source had no role in study conception and design, data analysis or interpretation, paper writing or deciding to submit this paper for publication.*
Institutional review board statement: *The study protocol was approved by the Ethics Committee of Beijing Rehabilitation Hospital of Capital Medical University (approval No. 2018bkky017) on March 6, 2018.*
Declaration of patient consent: *The authors certify that they have obtained all appropriate patient consent forms from the patients. In the forms, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.*
Reporting statement: *The writing and editing of the article were performed in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement.*
Biostatistics statement: *The statistical methods of this study were reviewed by the epidemiologist of Capital Medical University, China.*
Copyright license agreement: *The Copyright License Agreement has been signed by all authors before publication.*
Data sharing statement: *No individual deidentified participant data (including data dictionaries) will be shared.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewer: *Haewon Byeon, Honam University, Korea.*
Additional files:
Additional file 1: *Hospital ethics approval (Chinese).*
Additional file 2: *Informed consent form (Chinese).*
Additional file 3: *STROBE checklist.*

References

Benzagmout M, Boujraf S, Alami B, Amadou HA, El Hamdaoui H, Bennani A, Jaafari M, Rammouz I, Maaroufi M, Magoul R, Boussaoud D (2019) Emotion processing in Parkinson's disease: a blood oxygenation level-dependent functional magnetic resonance imaging study. Neural Regen Res 14:666-672.

Chang KH, Tzeng YT, Wey JH, Liu YJ, Lin YN, Chung WK (2020) Pneumonia in Parkinson's disease: barium aspiration in videofluoroscopic swallowing study. Respirol Case Rep 8:e00546.

Ciucci MR, Barkmeier-Kraemer JM, Sherman SJ (2008) Subthalamic nucleus deep brain stimulation improves deglutition in Parkinson's disease. Mov Disord 23:676-683.

Díez-Cirarda M, Ojeda N, Peña J, Cabrera-Zubizarreta A, Lucas-Jiménez O, Gómez-Esteban JC, Gómez-Beldarrain M, Ibarretxe-Bilbao N (2017) Increased brain connectivity and activation after cognitive rehabilitation in Parkinson's disease: a randomized controlled trial. Brain Imaging Behav 11:1640-1651.

Gao J, Guan X, Cen Z, Chen Y, Ding X, Lou Y, Wu S, Wang B, Ouyang Z, Xuan M, Gu Q, Xu X, Huang P, Zhang M, Luo W (2019) Alteration of brain functional connectivity in Parkinson's disease patients with dysphagia. Dysphagia 34:600-607.

Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stern MB, Dodel R, Dubois B, Holloway R, Jankovic J, Kulisevsky J, Lang AE, Lees A, Leurgans S, LeWitt PA, Nyenhuis D, Olanow CW, et al. (2008) Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord 23:2129-2170.

Hamdy S, Mikulis DJ, Crawley A, Xue S, Lau H, Henry S, Diamant NE (1999) Cortical activation during human volitional swallowing: an event-related fMRI study. Am J Physiol 277:G219-225.

Hulme SR, Jones OD, Abraham WC (2013) Emerging roles of metaplasticity in behaviour and disease. Trends Neurosci 36:353-362.

Im I, Jun JP, Hwang S, Ko MH (2018) Swallowing outcomes in patients with subcortical stroke associated with lesions of the caudate nucleus and insula. J Int Med Res 46:3552-3562.

Jellinger KA (2019) Is Braak staging valid for all types of Parkinson's disease? J Neural Transm (Vienna) 126:423-431.

Khedr EM, Mohamed KO, Soliman RK, Hassan AMM, Rothwell JC (2019) The effect of high-frequency repetitive transcranial magnetic stimulation on advancing Parkinson's disease with dysphagia: double blind randomized clinical trial. Neurorehabil Neural Repair 33:442-452.

Kikuchi A, Baba T, Hasegawa T, Kobayashi M, Sugeno N, Konno M, Miura E, Hosokai Y, Ishioka T, Nishio Y, Hirayama K, Suzuki K, Aoki M, Takahashi S, Fukuda H, Itoyama Y, Mori E, Takeda A (2013) Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study. BMJ Open 3:e002249.

Kim JH, Oh SH, Jeong HJ, Sim YJ, Kim DG, Kim GC (2019) Association between duration of dysphagia recovery and lesion location on magnetic resonance imaging in patients with middle cerebral artery infarction. Ann Rehabil Med 43:142-148.

Kim YH, Oh BM, Jung IY, Lee JC, Lee GJ, Han TR (2015) Spatiotemporal characteristics of swallowing in Parkinson's disease. Laryngoscope 125:389-395.

Kober SE, Grössinger D, Wood G (2019) Effects of motor imagery and visual neurofeedback on activation in the swallowing network: a real-time fMRI study. Dysphagia 34:879-895.

Kooshkabadi A, Lunsford LD, Tonetti D, Flickinger JC, Kondziolka D (2013) Gamma Knife thalamotomy for tremor in the magnetic resonance imaging era. J Neurosurg 118:713-718.

Lang XY, Shao GL, Sun JJ, Shi L, Fan LY (2015) Construction of rabbit models of radiation-induced brain injury and selection of magnetic resonance parameters. Zhongguo Zuzhi Gongcheng Yanjiu 19:4299-4303.

Lee JM, Derkinderen P, Kordower JH, Freeman R, Munoz DG, Kremer T, Zago W, Hutten SJ, Adler CH, Serrano GE, Beach TG (2017) The search for a peripheral biopsy indicator of α-synuclein pathology for Parkinson disease. J Neuropathol Exp Neurol 76:2-15.

Loeffler LAK, Radke S, Habel U, Ciric R, Satterthwaite TD, Schneider F, Derntl B (2018) The regulation of positive and negative emotions through instructed causal attributions in lifetime depression- A functional magnetic resonance imaging study. Neuroimage Clin 20:1233-1245.

Loprinzi PD (2019) The effects of physical exercise on parahippocampal function. Physiol Int 106:114-127.

Luchesi KF, Kitamura S, Mourão LF (2015) Dysphagia progression and swallowing management in Parkinson's disease: an observational study. Braz J Otorhinolaryngol 81:24-30.

Maidan I, Rosenberg-Katz K, Jacob Y, Giladi N, Hausdorff JM, Mirelman A (2017) Disparate effects of training on brain activation in Parkinson disease. Neurology 89:1804-1810.

Martin-Harris B, Brodsky MB, Michel Y, Ford CL, Walters B, Heffner J (2005) Breathing and swallowing dynamics across the adult lifespan. Arch Otolaryngol Head Neck Surg 131:762-770.

Matsushima A, Matsushima J, Matsumoto A, Moriwaka F, Honma S, Itoh K, Yamada K, Shimohama S, Ohnishi H, Mori M (2016) Analysis of resources assisting in coping with swallowing difficulties for patients with Parkinson's disease: a cross-sectional study. BMC Health Serv Res 16:276.

Mosier K, Patel R, Liu WC, Kalnin A, Maldjian J, Baredes S (1999) Cortical representation of swallowing in normal adults: functional implications. Laryngoscope 109:1417-1423.

Pasquini J, Durcan R, Wiblin L, Gersel Stokholm M, Rochester L, Brooks DJ, Burn D, Pavese N (2019) Clinical implications of early caudate dysfunction in Parkinson's disease. J Neurol Neurosurg Psychiatry 90:1098-1104.

Perry BJ, Martino R, Yunusova Y, Plowman EK, Green JR (2018) Lingual and jaw kinematic abnormalities precede speech and swallowing impairments in ALS. Dysphagia 33:840-847.

Pfeiffer RF (2018) Gastrointestinal dysfunction in Parkinson's disease. Curr Treat Options Neurol 20:54.

Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, Obeso J, Marek K, Litvan I, Lang AE, Halliday G, Goetz CG, Gasser T, Dubois B, Chan P, Bloem BR, Adler CH, Deuschl G (2015) MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 30:1591-1601.

Sacheli MA, Neva JL, Lakhani B, Murray DK, Vafai N, Shahinfard E, English C, McCormick S, Dinelle K, Neilson N, McKenzie J, Schulzer M, McKenzie DC, Appel-Cresswell S, McKeown MJ, Boyd LA, Sossi V, Stoessl AJ (2019) Exercise increases caudate dopamine release and ventral striatal activation in Parkinson's disease. Mov Disord 34:1891-1900.

Silbergleit AK, LeWitt P, Junn F, Schultz LR, Collins D, Beardsley T, Hubert M, Trosch R, Schwalb JM (2012) Comparison of dysphagia before and after deep brain stimulation in Parkinson's disease. Mov Disord 27:1763-1768.

Strafella AP, Paus T, Barrett J, Dagher A (2001) Repetitive transcranial magnetic stimulation of the human prefrontal cortex induces dopamine release in the caudate nucleus. J Neurosci 21:Rc157.

Suntrup S, Teismann I, Bejer J, Suttrup I, Winkels M, Mehler D, Pantev C, Dziewas R, Warnecke T (2013) Evidence for adaptive cortical changes in swallowing in Parkinson's disease. Brain 136:726-738.

Suttrup I, Warnecke T (2016) Dysphagia in Parkinson's disease. Dysphagia 31:24-32.

Takizawa C, Gemmell E, Kenworthy J, Speyer R (2016) A systematic review of the prevalence of oropharyngeal dysphagia in stroke, Parkinson's disease, Alzheimer's disease, head injury, and pneumonia. Dysphagia 31:434-441.

Troche MS, Brandimore AE, Foote KD, Okun MS (2013) Swallowing and deep brain stimulation in Parkinson's disease: a systematic review. Parkinsonism Relat Disord 19:783-788.

van Hooren MR, Baijens LW, Vos R, Pilz W, Kuijpers LM, Kremer B, Michou E (2016) Voice- and swallow-related quality of life in idiopathic Parkinson's disease. Laryngoscope 126:408-414.

Wang DC, chen WL, Li JH, Wang ZQ, Luo HL (2012) A clinical study of low-dose pramipexole for swallowing disorders in patients with Parkinson's disease. Zhongguo Yiyao Zhinan 10:399-400.

Winogrodzka A, Bergmans P, Booij J, van Royen EA, Stoof JC, Wolters EC (2003) [(123)I]beta-CIT SPECT is a useful method for monitoring dopaminergic degeneration in early stage Parkinson's disease. J Neurol Neurosurg Psychiatry 74:294-298.

Zhang W, Li C, Chen L, Xing X, Li X, Yang Z, Zhang H, Chen R (2017) Increased activation of the hippocampus during a Chinese character subvocalization task in adults with cleft lip and palate palatoplasty and speech therapy. Neuroreport 28:739-744.

P-Reviewer: Byeon H; C-Editor: Zhao M; S-Editors: Yu J, Li CH; L-Editors: Yu J, Song LP; T-Editor: Jia Y

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page，line
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	P3，63
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	P3，58-77
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4，84-111
Objectives	3	State specific objectives, including any prespecified hypotheses	P5，110-111
Methods			
Study design	4	Present key elements of study design early in the paper	P6，116
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P6，116-126
Participants	6	(a) 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	P6，128-150
		(b) For matched studies, give matching criteria and the number of controls per case	Not used
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P7，152-166
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	P7，152-166
Bias	9	Describe any efforts to address potential sources of bias	P7，153-154，157，173-174 P0，201-212
Study size	10	Explain how the study size was arrived at	P5，115
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P10，196-232 P21，487-491
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	P10，196-237
		(b) Describe any methods used to examine subgroups and interactions	P10，221-232，236
		(c) Explain how missing data were addressed	P6，116-120
		(d) If applicable, explain how matching of cases and controls was addressed	Not used
		(e) Describe any sensitivity analyses	P11，221-222
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for	P6，115-125

		eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	P6，116-120
		(c) Consider use of a flow diagram	Not used
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P11，239 P21，482-503
		(b) Indicate number of participants with missing data for each variable of interest	P6，116-120
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	P7，153
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	P23，495
		(b) Report category boundaries when continuous variables were categorized	Not used
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not used
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not used
Discussion			
Key results	18	Summarise key results with reference to study objectives	P11，234
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	P18，370-372
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P17，366-371
Generalisability	21	Discuss the generalisability (external validity) of the study results	P13,265-274
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	P1,29

*Give information separately for cases and controls.

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 http://www.strobe-statement.org.