RESEARCH ARTICLE

Neural Representations of the Voice Tremor Spectrum

Laura de Lima Xavier, MD,[1,2] and Kristina Simonyan, MD, PhD, Dr med[1,2,3*]

[1]Department of Otolaryngology – Head and Neck Surgery, Massachusetts Eye and Ear, Boston, Massachusetts, USA
[2]Department of Otolaryngology – Head and Neck Surgery, Harvard Medical School, Boston, Massachusetts, USA
[3]Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA

ABSTRACT: **Objectives:** Voice tremor is a common movement disorder that manifests as involuntary oscillations of laryngeal muscles, leading to rhythmic alterations in voice pitch and loudness. Differential diagnosis of essential tremor of voice (ETv) is often challenging and includes dystonic tremor of voice (DTv), which is characterized by irregular, isometric contractions of laryngeal muscles during dystonic activity. Although clinical characteristics of voice tremor are well described, the pathophysiology underlying its heterogeneous phenomenology remains limited.

Methods: We used a multimodal approach of functional magnetic resonance imaging for assessment of brain activity during symptomatic speech production, high-resolution magnetic resonance imaging for the examination of cortical thickness and gray matter volume, and diffusion-weighted imaging for evaluation of white matter integrity to identify disorder-specific neural alterations and their relationships with the symptomatology of ETv and DTv.

Results: We found a broad overlap between cortical alterations in ETv and DTv, involving sensorimotor regions responsible for the integration of multisensory information during speech production, such as primary sensorimotor, inferior/superior parietal, and inferior temporal cortices. In addition, ETv and DTv showed unique patterns of abnormalities in regions controlling speech motor preparation, which were localized in the cerebellum in ETv and the premotor cortex, insula, and superior temporal gyrus in DTv. Neural alterations in superior parietal and inferior temporal cortices were correlated with ETv severity, whereas changes in the left premotor cortex were associated with DTv severity.

Conclusions: Our findings point to the pathophysiological spectrum underlying ETv and DTv and favor a more heterogeneous rather than dichotomous diagnostic classification of these voice tremor disorders. © 2020 International Parkinson and Movement Disorder Society

Key Words: voice tremor; brain imaging; symptom severity

Voice tremor is a common movement disorder that manifests as involuntary oscillations of laryngeal muscles, leading to rhythmic alterations in pitch and loudness during active and passive tasks, such as speaking and breathing.[1] Voice tremor presents either in isolation or in combination with tremor of upper extremities, under the umbrella of essential tremor (ET).[2] Differential diagnosis of essential tremor of voice (ETv) is often challenging and includes other movement disorders.[3] Among these is dystonic tremor of voice (DTv), which is observed in about one-third of patients with laryngeal dystonia (LD) and is characterized by irregular, isometric contractions of laryngeal muscles during dystonic activity, selectively affecting speech production.[4]

Clinically, both similarities and differences between patients with ETv and DTv have been described. DTv, in combination with LD, shows intermediate age and sex distributions between ETv and LD without tremor.[5] Botulinum toxin injections yield a better therapeutic response in LD without tremor, intermediate in DTv, and worse in ETv.[6-8] In contrast, alcohol is more beneficial for symptom improvement in ETv, followed by DTv and LD without tremor.[9-11] Despite this refined understanding of

---

*__Correspondence to:__ Dr. Kristina Simonyan, Department of Otolaryngology–Head and Neck Surgery, Massachusetts Eye and Ear, Harvard Medical School, 243 Charles Street, Suite 421, Boston, MA 02114 USA; E-mail: kristina_simonyan@meei.harvard.edu

Relevant conflicts of interest/financial disclosures: Nothing to report.

Funding agencies: This study was supported by the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (grant R01DC012545 to K.S.).

Received: 8 April 2020; **Revised:** 4 August 2020; **Accepted:** 6 August 2020

DOI: 10.1002/mds.28259

clinical characteristics and therapeutic outcomes of voice tremor disorders, the current knowledge of their pathophysiological traits is very limited.

Our recent studies investigated alterations in brain structure and function in LD versus DTv and suggested that these disorders may be at the different ends of the same pathophysiological spectrum, with common changes in the basal ganglia, sensorimotor, and parietal cortical regions responsible for speech control.[12,13] However, how abnormalities in DTv overlap or separate from those in ETv remains unknown, thus hindering the broader understanding of the pathophysiology underlying the heterogeneous phenomenology of voice tremor.

In this study, we used a comprehensive multimodal brain imaging approach of functional magnetic resonance imaging (fMRI) for assessment of brain activity during symptomatic speech production, high-resolution MRI for examination of cortical thickness (CT) and gray matter volume, and diffusion-weighted imaging for evaluation of white matter integrity to identify neural alterations in ETv and DTv and define their relationships with symptomatology of these voice tremor disorders. Because DTv always occurs in combination with LD, DTv-specific neural signatures were separated from those associated with LD by an additional contrast between patients with LD/DTv and a separate group of patients with LD without any forms of tremor. Given previous evidence of similar alterations in the sensorimotor and cerebello-thalamo-cortical networks in ET and dystonia,[14,15] we hypothesized the presence of overlapping abnormalities within these circuitries in ETv and DTv. We further hypothesized that additional, segregated alterations differentiating ETv from DTv would be present in brain regions involved in multisensory integration and motor preparation for voice and speech production, thus underlying disorder-specific symptomatology in these related but clinically distinct forms of voice tremor.

Subjects and Methods

Subjects

Ninety-three subjects participated in this study (Table 1). The patient cohort included 18 patients with ETv (age 62.5 ± 12.2 years, 15 women/3 men), of whom 9 had isolated voice tremor (age 64.1 ± 10 years, 7 women/2 men) and 9 had voice tremor combined with hand tremor (age 60.8 ± 14.5 years, 7 women/2 men); 25 patients with LD/DTv (age 60.2 ± 10.8 years, 22 women/3 men); and 25 patients with LD without DTv or any other tremor (age 53.7 ± 9.5 years, 22 women/3 men). Control subjects were 25 age- and sex-matched healthy individuals (age 54.2 ± 8.5 years, 18 women/7 men).

The diagnosis of tremor was established based on a recommended multidisciplinary approach, including a detailed case history, acoustic perceptual voice evaluation, and neurological and laryngological examinations.[16,17] Exclusion criteria for both patients and control subjects were any neurological, psychiatric, or laryngeal disorders, except for ETv, DTv, or LD in the patient cohorts. All subjects were right-handed, as determined by the Edinburgh Handedness Inventory,[18] and native English speakers with a normal cognitive status as determined by the Montreal Cognitive Assessment or Mini-Mental State Examination. None had any known verified gene mutations, including *TOR1A*/DYT1, *TUBB4A*/DYT4, *THAP1*/DYT6, or *GNAL*/DYT25. All patients were fully symptomatic at the time of study participation. None of the subjects were taking any centrally acting medications. All subjects abstained from alcohol and caffeine for 24 hours before study participation. Botulinum toxin was used as a treatment in 2 patients with ETv, 10 patients with LD/DTv, and 17 patients with LD (Table 1). Due to the different number of patients in each group who received botulinum toxin injections, this treatment might have represented a confounding factor in the between-group analysis. However, all patients participated in the study at least 3 months after their last injection, when they were fully symptomatic. Moreover, no significant differences in the duration of botulinum toxin treatment or the time since the last injection were found between the groups (all $P \geq 0.15$), thus further minimizing the possibility of confounding effects of botulinum toxin on brain activity.

Clinical information on the symptom onset, duration, and severity was obtained based on the multidisciplinary approach for evaluation of voice tremor and related disorders.[16,17] Voice and speech of patients were recorded during production of sustained vowels, repeated syllables, and a set of 20 symptom-provoking sentences in 15 patients with ETv and 24 patients with DTv. Recordings in three patients with ETv and one patient with DTv were missing because of the technical issues. The tremor severity was perceptually evaluated using a visual analogue scale (0 = no tremor, 100 = most severe tremor).[19]

All subjects gave written informed consent before study participation, which was approved by the Institutional Review Boards of Icahn School of Medicine at Mount Sinai and Mass General Brigham.

Imaging Data Collection

All subjects underwent high-resolution structural and functional brain MRI on a 3.0 T Philips scanner equipped with an eight-channel head coil. Whole-brain fMRI data were obtained using a gradient-weighted echo-planar imaging (EPI) pulse sequence and blood oxygen level–dependent contrast with sparse-sampling event-related design (effective repetition time [TR], 10.6 s; echo time

TABLE 1. Demographics of study participants

Demographics	Essential Tremor of Voice (n = 18)	Dystonic Tremor of Voice (n = 25)	Laryngeal Dystonia (n = 25)	Healthy Controls (n = 25)	Corrected *P* Value
Sex (female/male)	15/3	22/3	22/3	19/6	≥0.9
Age, y (mean ± SD)	62.5 ± 12.2	60.2 ± 10.8	53.7 ± 9.5	54.1 ± 8.5	≥0.3
Age at onset, y (mean ± SD)	51.7 ± 17.1	46.7 ± 13.1	40.2 ± 11.2	N/A	≥0.3
Duration of disease, y (mean ± SD)	10.9 ± 9.4	13.5 ± 11.3	13.5 ± 7.7	N/A	≥0.4
Tremor severity (mean ± SD)	61.3 ± 20.8	50.1 ± 20.5	N/A	N/A	0.12
Dystonia subtype	N/A	17 AD/VT 8 AB/VT	17 AD/8 AB	N/A	N/A
Botulinum toxin treatment (n)	2 of 18	10 of 25	17 of 25	N/A	N/A
Duration, y (mean ± SD)	19.5 ± 20.5	9.5 ± 7.0	8.9 ± 7.2	N/A	≥0.17
Time since last injection, mo (mean ± SD)	4.5 ± 0.7	17.1 ± 26.0	6.0 ± 2.7	N/A	≥0.15
Centrally acting medications or agents	None				N/A
Handedness (Edinburgh Inventory)	Right				N/A
Cognitive status (MMSE/MoCA)	>27/20				N/A
Genetic status	No mutations for *TOR1A*/DYT1, *TUBB4A*/DYT4, *THAP1*/DYT6, or *GNAL*/DYT25				N/A

Statistical comparisons were made between each patient group and control subjects, as well as between patient groups using two-sample *t* test for continuous variables and the chi-square test for categorical variables, corrected for multiple comparisons.
Abbreviations: SD, standard deviation; N/A, not applicable; AD, adductor; AB, abductor; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

[TE], 30 ms; flip angle [FA], 90°; field of view [FOV] 240 mm; voxel size $3.75 \times 3.75 \times 4$ mm), which minimized scanning artifacts because of possible orofacial movements and neutralized the scanner noise interference with acoustic stimulus presentation. The experimental task included 10 English symptom-provoking sentences (eg, "Jack ate eight apples," "Tom is in the army") and a resting condition as a baseline, which were presented in a pseudorandomized order. This experimental design was extensively used in previous studies and demonstrated to activate voice- and speech-related brain regions and capture alterations associated with DTv and LD.[20-24]

High-resolution whole-brain T1-weighted images were acquired with 3D-magnetization prepared rapid acquisition gradient echo sequence (TR, 7.5 ms; TE, 2 ms; inversion time [TI], 1000 ms; FA, 8°; FOV, 240 mm; slice thickness, 1.0 mm) and used as an anatomical reference for brain activation and for analysis of CT and gray matter volume.

Whole-brain diffusion-weighted images were acquired using a single-shot spin-echo EPI sequence along 60 noncollinear directions and one volume without diffusion encoding (b0 image) (TR, 13,000 ms; FOV, 240 mm; matrix 96×96 mm zero-filled to 256×256 mm; slice thickness, 2.4 mm; b, 1000 s/mm^2).

fMRI Analysis

Analysis was performed using the *afni_proc.py* processing pipeline of AFNI software. In brief, following the removal of the first two volumes to account for the magnetization equilibrium, time series were registered to the volume collected closest in time to the anatomical scan using heptic polynomial interpolation; aligned to the anatomical scan; spatially normalized to the MNI space; spatially smoothed with a 4-mm Gaussian filter; and normalized to the percent signal change. A regressor for the task was convolved with a canonical hemodynamic response function and entered into a multiple regression model to derive the blood oxygen level–dependent response.

Control for motion artifacts included regression of motion parameters, censoring of TRs, and censoring of outlier TRs. Regression of motion parameters was based on six motion parameter estimates calculated during the realignment of the EPI volumes that were included as covariates of no interest and three quadratic polynomials that were used to model baseline drifts for each imaging run. Censoring of TRs excluded TRs where the Euclidean norm of the motion derivative was ≥1.0; this cutoff was set based on simulations of motion artifacts at the presence of a slow effective TR of 10.6 s. Because outliers may capture residual motion in some cases where the motion parameters do not, additional censoring of outlier TRs was performed to ensure the stringent removal of TRs containing residual motion artifacts. Ten subjects with more than 25% of censored TRs were excluded from final analysis. The final fMRI cohort included 14 patients with ETv (age 60 ± 12.4 years, 11 women/3 men), 25 patients with LD/DTv (age 60.2 ± 10.8 years, 22 women/3 men), 21 patients with LD without DTv or any other tremor (age 54.5 ± 9.2 years, 18 women/3 men), and 24 healthy control subjects (age

54.6 ± 8.3 years, 18 women/6 men). Their Euclidean norm motion values were within the acceptable limits for ETv (0.35 ± 0.13), LD/DTv (0.38 ± 0.11), LD (0.43 ± 0.12), and healthy controls (0.36 ± 0.13). One-way analysis of variance found no statistically significant differences in the Euclidean norm motion parameters between the groups ($F_{1,83} = 2.63$; $P = 0.11$).

CT Analysis

MRI data from all 68 patients and 25 control subjects were used for the analysis of brain structural alterations because motion artifacts did not impact structural images. T1-weighted images underwent preprocessing using the standard pipeline of FreeSurfer software. To calculate the surface-based anatomical measures, we reconstructed models of white and gray matter surfaces from T1 volumes. CT was measured as the distance between these surfaces at each vertex. Careful visual inspection of all MRIs and manual corrections of reconstructed surfaces were made, as needed, including the correction of erroneous skull striping by adjusting watershed parameters, manual editing of skull tissue, and an addition of control points to normalize intensity for white matter surface reconstruction. The resultant CT maps were spatially normalized to the MNI space.

Gray Matter Volume Analysis

The voxel-based morphometry analysis was conducted using the Computational Anatomy Toolbox (CAT12) running on MATLAB version 9.2. Whole-brain structural data were segmented into gray matter, white matter, and cerebrospinal fluid. Bias correction was performed to remove intensity nonuniformities. Gray matter probability maps were nonlinearly registered to the MNI space and smoothed using an 8-mm full-width half-maximum of the Gaussian kernel. The total intracranial volume of each subject was calculated as a covariate for group analysis.

White Matter Integrity Analysis

Diffusion-weighted images were processed using FSL software. Eddy current distortions and motion artifacts were corrected using affine registration to the b0 reference. Fractional anisotropy (FA), which reflects the white matter tract integrity, was calculated by fitting the diffusion model at each voxel. Using the tract-based spatial statistics pipeline, individual FA maps were registered to the MNI space, and the alignment-invariant tract representation known as the mean FA skeleton was generated at the threshold of 0.2. Each subject's FA map was projected onto the mean FA skeleton, resulting in a 4D skeletonized FA volume.

Statistical Analysis

First, we contrasted patients with LD/DTv with a separate group of patients with LD without DTv or any other form of tremor to separate neural alterations in DTv from those in co-occurring LD. This was performed using a two-tailed independent *t* test for each imaging modality at family-wise error (FWE)-corrected $P \le 0.05$, voxelwise threshold $P \le 0.01$, and a minimum cluster size of 343 mm^3, as determined by 3dClustSim program of AFNI software.

To examine the disorder-specific changes in brain activity, CT, gray matter volume, and white matter integrity, we used two-tailed independent *t* tests to assess differences in each patient group (ETv, DTv) compared with the same group of healthy control subjects. Subjects' age and sex were included as covariates of no interest. In voxel-based morphometry analysis, the total intracranial volume was included as an additional covariate. The overall statistical significance was set at FWE-corrected $P \le 0.05$, voxelwise threshold $P \le 0.01$, and a minimum cluster size of 715 mm^3, according to 3dClustSim. To visualize the spatial distribution of overlapping and distinct alterations in ETv and DTv from the normal baseline in each imaging modality, we performed conjunction analyses between the *a priori* statistically thresholded parametric maps of ETv versus controls and DTv versus controls. To examine distinct abnormalities in ETv and DTv, we performed direct group comparisons between these cohorts using independent *t* tests at FWE-corrected $P \le 0.05$, voxelwise threshold $P \le 0.01$, and a minimum cluster size of 120 mm^3, according to 3dClustSim.

Brain regions showing statistically significant abnormalities were examined for their relationship with the clinical features of voice tremor, including the age of onset, disease duration, and symptom severity. Voxelwise Spearman rank correlation coefficients were computed between abnormal regions and clinical characteristics at corrected $P \le 0.05$ using 3dTcorr1D program of AFNI software.

In a pilot study, the ETv cohort was stratified into patients with isolated ET of voice (n = 9) and patients with combined ET of voice and hand (n = 9). Independent *t* tests assessed differences between these two subgroups at FWE-corrected $P \le 0.05$, voxelwise threshold $P \le 0.01$, and a 3dClustSim-defined minimum cluster size of 154 mm^3.

Results

Common Abnormalities in Patients With ETv and DTv Compared With Healthy Individuals

Compared to healthy control subjects, patients with ETv and DTv showed commonly increased functional activity during speech production in the left primary somatosensory cortex and decreased activity in the left inferior parietal lobule at FWE-corrected $P \leq 0.05$ (Fig. 1A.I and Table 2). Common structural alterations included bilateral CT increases in the bilateral superior parietal lobule, extending to the bilateral primary somatosensory cortex and left primary motor cortex, as well as increased gray matter volume in the left inferior temporal gyrus at FWE-corrected $P \leq 0.05$ (Fig. 1B.I–C.I and Table 2). No statistically significant changes in white matter integrity were found in patients with ETv and DTv compared with control subjects.

Distinct Abnormalities in ETv Versus DTv

In addition, patients with ETv were characterized by functional changes in the right cerebellum (lobule VIII), whereas patients with DTv had alterations in the right insula and superior temporal gyrus (area TE 3) at FWE-corrected $P \leq 0.05$ (Fig. 1A.II and Table 2). Structural differences in patients with ETv included CT changes in the right inferior temporal gyrus, whereas patients with DTv showed CT alteration in the left primary motor cortex and gray matter volumetric changes in the left premotor cortex extending to the bilateral supplementary motor area (SMA) at FWE-corrected $P \leq 0.05$ (Fig. 1B.II–C.II and Table 2). Again, no statistically significant differences in white matter integrity were found between the two patient groups.

Distinct Abnormalities in Isolated ET of Voice Versus Combined ET of Voice and Hand

CT in the left superior and inferior parietal lobules and right superior temporal gyrus was increased in patients with combined ET of voice and hand compared with those with isolated ET of voice at FWE-corrected $P \leq 0.05$ (Fig. 1D.I). No statistically significant differences in brain activity, gray matter volume, or white matter integrity were found between these groups at FWE-corrected $P \leq 0.05$.

Relationship Between Disorder Clinical Characteristics and Neural Alterations

ETv severity was negatively associated with CT changes in the bilateral superior parietal lobule (left: $R_s = -0.68$, $P = 0.006$; right: $R_s = -0.69$, $P = 0.005$) and gray matter volume in the right inferior temporal gyrus ($R_s = -0.68$, $P = 0.005$) (Fig. 2A). In patients with DTv, a significant negative correlation was found between symptom severity and decreased left gray matter volume in the left premotor cortex ($R_s = -0.43$, $P = 0.036$) (Fig. 2B).

Discussion

Our findings suggest the presence of a broad overlap between cortical alterations in ETv and DTv that involve brain regions responsible for the integration of multisensory information during speech production. Concurrently, focal subcortical versus cortical abnormalities in regions controlling motor preparation to speech production differentiate ETv from DTv (Fig. 3).

Among commonly altered brain regions in both forms of voice tremor were the primary sensorimotor cortex, superior/inferior parietal lobules, and inferior temporal gyrus. The role of the primary sensorimotor cortex in the pathophysiology of these movement disorders is apparent. Abnormal activity, functional connectivity, and structural organization relevant to dystonia- or tremor-affected body representations within this region have been widely reported in the literature and characterized as disorder-relevant impairments.[14,15] Relevant to ETv and DTv, alterations in the bilateral primary somatosensory and left primary motor cortices point to a similar pathophysiological mechanism leading to abnormal coupling between the sensory input and motor output specifically during speech production.

Because the sensory system plays an important role in driving the motor system, these sensory alterations further extended into parietal and temporal cortical regions, adding deficiencies in processing of action-oriented spatial guidance, movement sequencing, and sensorimotor integration to the common pathophysiology of ETv and DTv. Changes in the parietal region have been previously shown to instigate the top-down alterations within the sensorimotor network, being linked to both polygenic and extrinsic risks for the development of dystonia and tremor.[13,20,25,26] It was also shown that parietal alterations, together with primary sensorimotor abnormalities, may represent an important biomarker for accurate diagnostic classification of these patients from healthy individuals.[27,28] Alterations in the inferior temporal cortex suggest the presence of similarly aberrant pattern recognition for lexical and semantic processing during speech production[29] in patients with ETv and DTv. Together, our findings indicate that the sensorimotor cortical control of processing and execution of complex movement sequences is commonly compromised across the voice tremor spectrum disorders.

The prominence of bilateral superior parietal and right inferior temporal alterations in patients with ETv is further evident from their significant negative correlations with symptom severity, indicating that patients with more severe ETv may reverse the abnormal increases of CT and gray matter volume in these

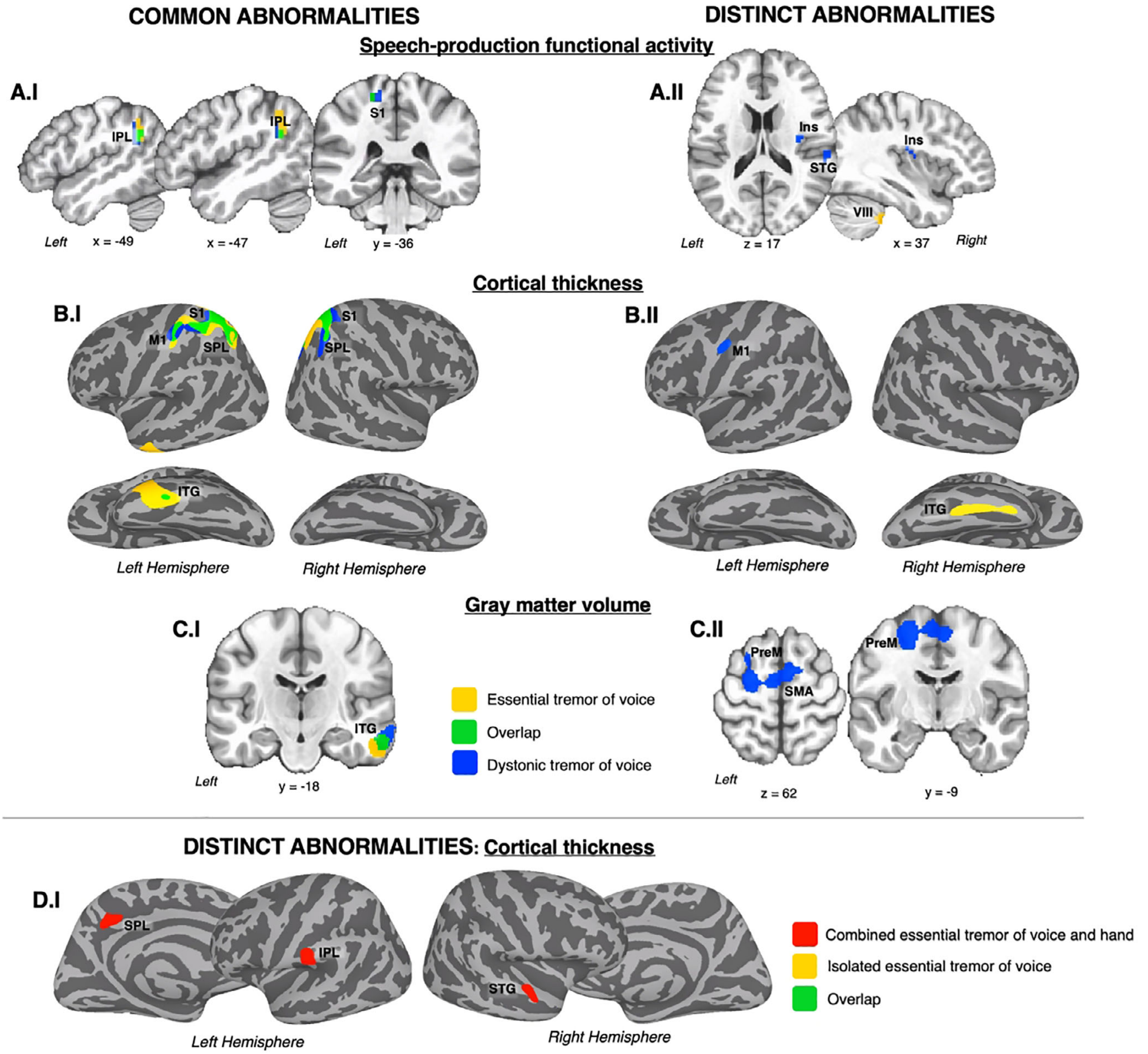

FIG. 1. Common and distinct neural abnormalities in essential tremor of voice and dystonic tremor of voice in (**A.I** and **A.II**) brain activity during symptomatic speech production, (**B.I** and **B.II**) cortical thickness, and (**C.I** and **C.II**) gray matter volume. Common changes are shown in green; distinct disorder-specific changes are shown in yellow for essential tremor of voice and blue for dystonic tremor of voice. (**D.I**) Distinct changes in cortical thickness in a pilot comparison of isolated essential tremor of voice versus combined essential tremor of voice and hand (shown in red). Ins, insula; IPL, inferior parietal lobule; ITG, inferior temporal gyrus; M1, primary motor cortex; PreM, premotor cortex; S1, primary somatosensory cortex; SMA, supplementary motor area; SPL, superior parietal lobule; STG, superior temporal gyrus; VIII, cerebellum lobule.

regions. However, abnormalities in these regions did not appear to return to the levels observed in healthy individuals (Fig. 2), pointing to a rather pathophysiologically compensatory cause of these structural changes. In contrast, although patients with DTv exhibited similarly increased CT and gray matter volume in parietal and temporal regions compared with healthy controls, they did not show correlations between these alterations and their tremor characteristics, suggesting an alternative mechanism of these changes in DTv compared with ETv.

Furthermore, distinct changes of the preparatory components of the speech motor control were characterized by alterations in the cerebellum in ETv and the SMA–premotor cortex, insula, and superior temporal gyrus in DTv. The cerebellum has long been considered to be primarily impaired in ET, likely giving rise to aberrant oscillations within the

TABLE 2. Differences in brain function and structure in patients with ETv and DTv compared with healthy control subjects

Anatomical Reference		Cluster Size (mm^3)	Cluster Peak *t* Value	Cluster Peak x, y, z
Common Abnormalities				
Functional activity during speech production				
Patients > controls				
L. primary somatosensory cortex	ETv	86	3.1	−19, −36, 55
	DTv	556	3.8	−12, −39, 55
Patients < controls				
L. inferior parietal lobule	ETv	599	−3.48	−47, −53, 27
	DTv	471	−3.3	−47, −50, 20
Cortical thickness				
Patients > controls				
L. superior parietal lobule extending to primary somatosensory and primary motor cortex	ETv	1373	5.9	−16, −47, 66
	DTv	1535	4.7	−13, −49, 65
R. superior parietal lobule extending to primary somatosensory cortex	ETv	1094	5.2	22, −42, 69
	DTv	1011	4.6	17, −44, 68
L. inferior temporal gyrus	ETv	595	4.5	−29, −13, −63
	DTv	16	3	−30, −16, −61
Gray matter volume				
Patients > control				
R. inferior temporal gyrus	ETv	801	4.5	58, −24, −27
	DTv	2291	6.3	58, −26, −26
Distinct Abnormalities				
Functional activity during speech production				
ETv > DTv patients				
R. cerebellum (lobule VIII)		343	−4.8	37, −43, −43
DTv > ETv patients				
R. insula		343	3.3	37, −12, 17
R. superior temporal gyrus		300	3.1	61, −29, −13
Cortical thickness				
ETv > DTv patients				
R. inferior temporal gyrus		596	4.7	46, −28, −25
DTv > ETv patients				
L. primary motor cortex		156	3.9	−41, −12, 42
ET voice + hand > ET voice				
L. inferior parietal lobule		193	−5.6	−57, −26, 24
L. superior parietal lobule		207	−4.2	−11, −41, 41
R. superior temporal gyrus		170	−4.9	59, −7, −3
Gray matter volume				
DTv > ETv patients				
L. premotor cortex extending to bilateral supplementary motor area		3460	4.1	−24, −14, 64

Abbreviations: L., left; ETv, essential voice tremor; DTv, dystonic tremor of voice; R., right; ET, essential tremor.

cerebello-thalamo-cortical network.[30,31] Specific to ETv, we found focal functional alterations in the cerebellar lobule VIII, which is known to be functionally coupled with the sensorimotor cortex.[32,33] This region has been previously shown to coordinate the production of various motor behaviors, including verb generation, temporal sequencing, and ordering of syllables, as well as the timing of speech motor commands.[32,34,35] The more significant cerebellar role in the ETv pathophysiological axis may thus involve altered refinement of internal speech motor models and abnormal modulation of motor sequences for speech output (Fig. 3).

Conversely, DTv was characterized by functional and structural alterations in cortical regions that are responsible for motor planning, selection, and execution of complex sequences during speech production. Among these, abnormalities in the SMA and dorsal premotor cortex may be linked to those in the superior parietal and primary motor cortex and contribute to the altered temporal representation of motor sequences and higher-order processing of subsequently ordered motor programs relevant to speech production.[36-39] Notably, DTv severity showed a significant negative correlation with changes in the gray matter volume of the premotor cortex, suggesting that patients with more severe symptoms had decreases of volumetric changes in this region. Again, because the overall premotor volume remained increased in patients with DTv compared with healthy controls and patients with ETv, we suggest that the observed severity-dependent decreases in this

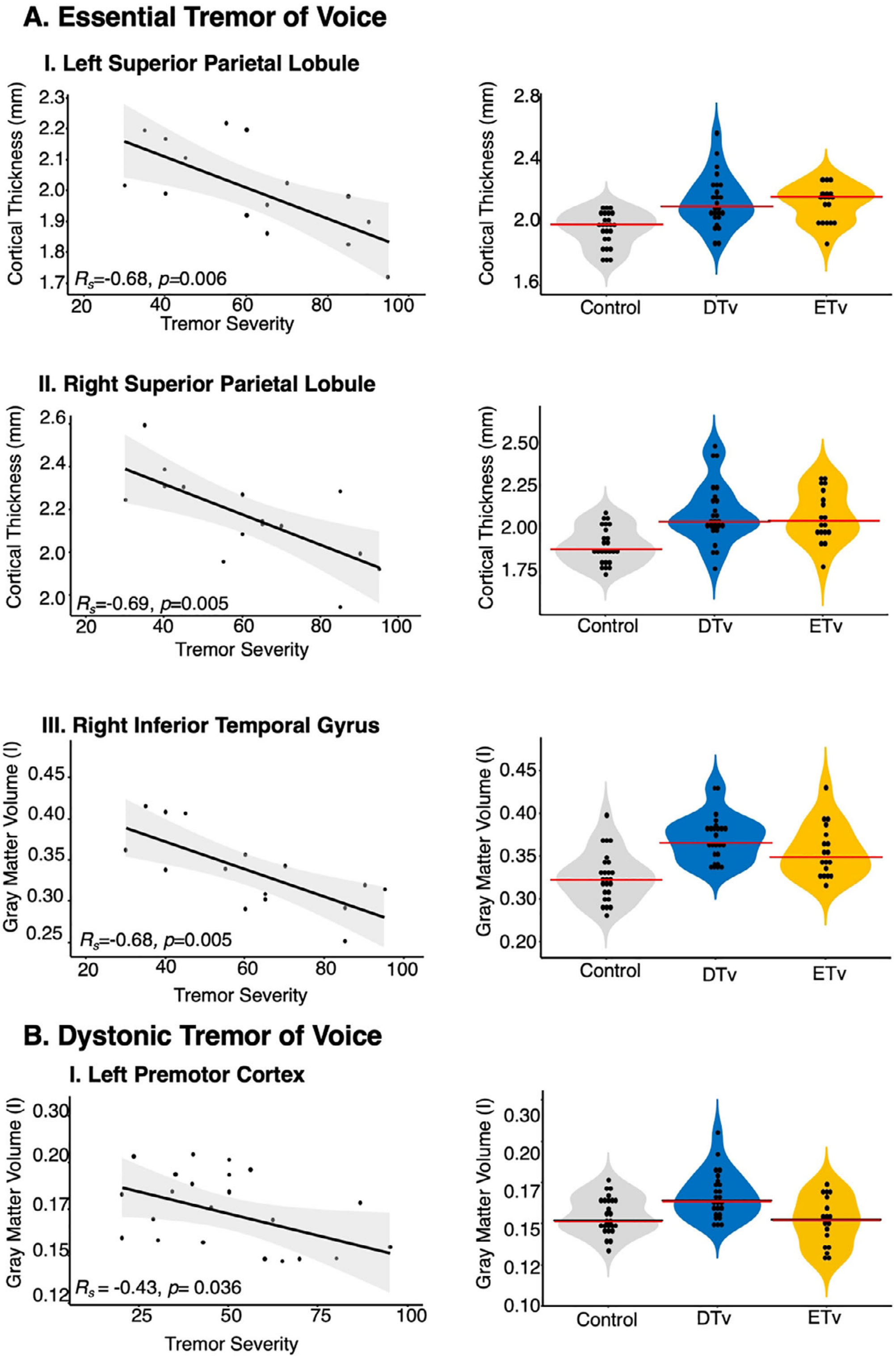

FIG. 2. Clinical correlates of neural abnormalities in essential tremor of voice and dystonic tremor of voice. Significant correlations between structural abnormalities in gray matter volume and cortical thickness and the severity of (**A**) essential tremor of voice (ETv) and (**B**) dystonic tremor of voice (DTv). The corresponding violin plots show the distribution of abnormal regional values in each patient group and healthy controls.

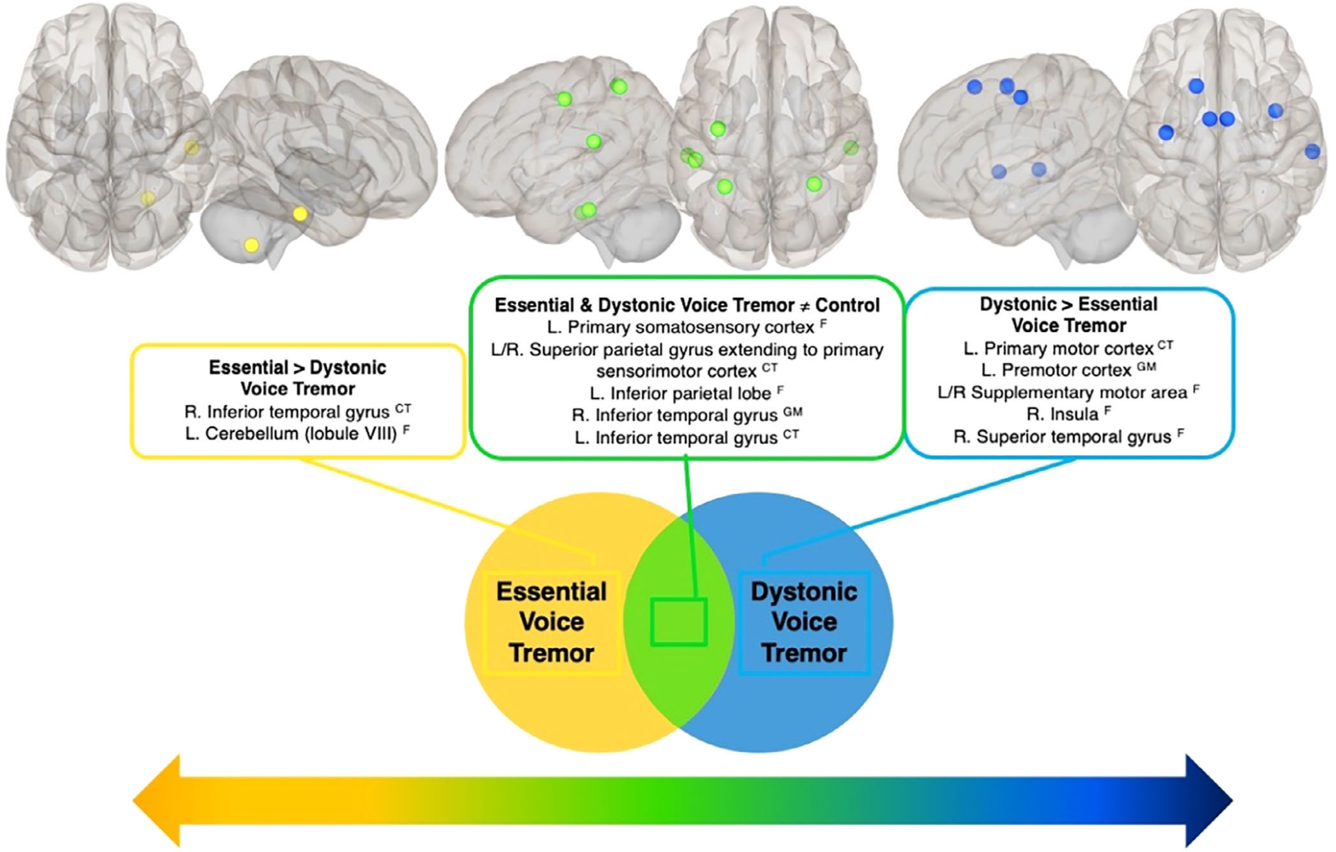

FIG. 3. Schematic of neural alterations in the spectrum of voice tremor disorders. A diagram summarizes the main findings of functional and structural abnormalities in patients with essential tremor of voice and dystonic tremor of voice. The left and right panels list abnormalities that are characteristic to essential and dystonic voice tremor, respectively, whereas the middle panel lists regions where alterations of brain function and structure are present in both disorders. Above each panel, 3D brain reconstructions illustrate the approximate location of the peak cluster values for each cortical and subcortical abnormality. The arrow signifies the presence of shared abnormalities among the voice tremor phenotypes and an increasing compromise of the neural architecture in each form of voice tremor toward the end of the spectrum. CT, abnormal cortical thickness; F, functional abnormality; GM, abnormal gray matter volume; L., left; R., right.

region may be secondary to the gray matter remodeling as an adaptation to increased symptom severity. Further DTv-distinct abnormalities in the superior temporal gyrus were localized in the higher-order auditory cortex (area TE 3), which is involved in the spectrotemporal analysis of auditory input.[40,41] Alterations in this region are likely to be associated with deficient monitoring of auditory feedback during speech production, leading to an inability to adjust and modulate pitch perturbations in patients with DTv. Finally, the insula is an important sensorimotor relay structure converging speech motor planning, auditory temporal processing, internal speech movement representations, and cognitive control of speech production.[42-46] Its selective alterations in DTv, but not ETv, may suggest the presence of task-specific interruptions of neural information flow between different components of the speech controlling system,[21] placing DTv closer to the pathophysiological axis of dystonia[46,47] (Fig. 3). Taken together, our findings of DTv-specific neural alterations point to the faulty processing of motor commands as a result of more significant deficits at the planning and preparatory stages for task production.

A somewhat unexpected finding of this study was the absence of significant white matter alterations in patients with ETv and DTv. This is a discrepancy from previous studies that reported white matter changes in the cerebellum, corpus callosum, thalamocortical visual pathways in ET,[48-50] as well as the posterior limb of the internal capsule in DTv.[12] Possible explanations may include the stringent criteria for selection of homogeneous patient populations and the methodological constraints, such as differences in diffusion-weighted imaging sequence acquisition and a stringent statistical thresholding employed in this study that might have excluded smaller clusters of between-group differences reported in the previous literature.

Our preliminary finding of distinct parietal and temporal changes in CT in patients with combined ET of voice and hand compared with isolated ET of voice point to potentially increased complexity of neural changes associated with the presence of additional symptoms. Notably, the areas of alterations in patients with combined ET of voice and hand did not overlap with those found in isolated ETv or DTv, suggesting that abnormalities identified in the main study were relevant to voice tremor characteristics rather than being contaminated by the presence of tremor in other body regions. A detailed investigation of the differences between various clinical phenotypes of ET is warranted in future studies.

In conclusion, this study is among the first to our knowledge to demonstrate common and distinct functional and structural brain abnormalities in patients with voice tremor disorders, ETv and DTv. Combined with previous studies in DTv and LD,[12,13] our findings point to the presence of a spectrum of clinical and pathophysiological characteristics across these disorders, favoring a more heterogeneous rather than dichotomous diagnostic classification of ETv and DTv. Ultimately, a refined characterization of disorder-specific abnormalities at distinct pathophysiological levels will aid the objective differential diagnosis of these disorders and help future identification of specific therapeutic targets for patients with various forms of voice tremor. ■

Acknowledgments: This study was supported by the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (grant R01DC012545 to K.S.). We thank Steven J. Frucht, MD, and Andrew Blitzer, MD, DDS, for patient referral and clinical evaluations.

References

1. Blitzer A, Brin MF, Ramig LO. Neurologic Disorders of the Larynx. New York - Stuttgart: Thieme; 2011.
2. Barkmeier-Kraemer JM. Isolated voice tremor: a clinical variant of essential tremor or a distinct clinical phenotype? Tremor Other Hyperkinet Mov (N Y) 2020;10. https://doi.org/10.7916/tohm.v0.738. eCollection 2020.
3. Merati AL, Heman-Ackah YD, Abaza M, Altman KW, Sulica L, Belamowicz S. Common movement disorders affecting the larynx: a report from the neurolaryngology committee of the AAO-HNS. Otolaryngol Head Neck Surg 2005;133(5):654–665.
4. Blitzer A, Brin MF, Stewart CF. Botulinum toxin management of spasmodic dysphonia (laryngeal dystonia): a 12-year experience in more than 900 patients. Laryngoscope 2015;125(8):1751–1757.
5. Patel PN, Kabagambe EK, Starkweather JC, et al. Defining differences in patient characteristics between spasmodic dysphonia and laryngeal tremor. Laryngoscope 2019;129(1):170–176.
6. Brin MF, Blitzer A, Stewart C. Laryngeal dystonia (spasmodic dysphonia): observations of 901 patients and treatment with botulinum toxin. Adv Neurol 1998;78:237–252.
7. Gurey LE, Sinclair CF, Blitzer A. A new paradigm for the management of essential vocal tremor with botulinum toxin. Laryngoscope 2013;123(10):2497–2501.
8. Sulica L, Louis ED. Clinical characteristics of essential voice tremor: a study of 34 cases. Laryngoscope 2010;120(3):516–528.
9. Kirke DN, Frucht SJ, Simonyan K. Alcohol responsiveness in laryngeal dystonia: a survey study. J Neurol 2015;262(6):1548–1556.
10. Guglielmino G, Moraes BTD, Villanova LC, Padovani M, Biase NGD. Comparison of botulinum toxin and propranolol for essential and dystonic vocal tremors. Clinics 2018;73:e87.
11. Junker J, Brandt V, Berman BD, et al. Predictors of alcohol responsiveness in dystonia. Neurology 2018;91(21):e2020–e2026.
12. Kirke DN, Battistella G, Kumar V, Rubien-Thomas E, Choy M, Rumbach A, Simonyan K. Neural correlates of dystonic tremor: a multimodal study of voice tremor in spasmodic dysphonia. Brain Imaging Behav 2017;11(1):166–175.
13. Battistella G, Simonyan K. Top-down alteration of functional connectivity within the sensorimotor network in focal dystonia. Neurology 2019;92(16):e1843–e1851.
14. Nieuwhof F, Panyakaew P, van de Warrenburg BP, Gallea C, Helmich RC. The patchy tremor landscape: recent advances in pathophysiology. Curr Opin Neurol 2018;31(4):455–461.
15. Simonyan K. Neuroimaging applications in dystonia. Int Rev Neurobiol 2018;143:1–30.
16. Ludlow CL, Domangue R, Sharma D, et al. Consensus-based attributes for identifying patients with spasmodic dysphonia and other voice disorders. JAMA Otolaryngol Head Neck Surg 2018;144(8):657–665.
17. Ludlow CL, Adler CH, Berke GS, et al. Research priorities in spasmodic dysphonia. Otolaryngol Head Neck Surg 2008;139(4):495–505.
18. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9(1):97–113.
19. Rumbach AF, Blitzer A, Frucht SJ, Simonyan K. An open-label study of sodium oxybate in spasmodic dysphonia. Laryngoscope 2017;127(6):1402–1407.
20. de Lima Xavier L, Simonyan K. The extrinsic risk and its association with neural alterations in spasmodic dysphonia. Parkinsonism Relat Disord 2019;65:117–123.
21. Fuertinger S, Horwitz B, Simonyan K. The functional connectome of speech control. PLoS Biol 2015;13(7):e1002209.
22. Fuertinger S, Simonyan K. Connectome-wide phenotypical and genotypical associations in focal dystonia. J Neurosci 2017;37(31):7438–7449.
23. Kirke DN, Battistella G, Kumar V, Rubien-Thomas E, Choy M, Rumbach A, Simonyan K. Neural correlates of dystonic tremor: a multimodal study of voice tremor in spasmodic dysphonia. Brain Imaging Behav 2017;11(1):166–175.
24. Simonyan K, Berman BD, Herscovitch P, Hallett M. Abnormal striatal dopaminergic neurotransmission during rest and task production in spasmodic dysphonia. J Neurosci 2013;33(37):14705–14714.
25. Putzel GG, Battistella G, Rumbach AF, Ozelius LJ, Sabuncu MR, Simonyan K. Polygenic risk of spasmodic dysphonia is associated with vulnerable sensorimotor connectivity. Cereb Cortex 2018;28(1):158–166.
26. DeSimone JC, Archer DB, Vaillancourt DE, Wagle Shukla A. Network-level connectivity is a critical feature distinguishing dystonic tremor and essential tremor. Brain 2019;142(6):1644–1659.
27. Serrano JI, Ignacio Serrano J, Romero JP, Rocon E, Louis ED, Benito-León J. A data mining approach using cortical thickness for diagnosis and characterization of essential tremor. Sci Rep 2017;7(1). https://doi.org/10.1038/s41598-017-02122-3
28. Battistella G, Fuertinger S, Fleysher L, Ozelius LJ, Simonyan K. Cortical sensorimotor alterations classify clinical phenotype and putative genotype of spasmodic dysphonia. Eur J Neurol 2016;23(10):1517–1527.
29. Indefrey P, Levelt WJ. The spatial and temporal signatures of word production components. Cognition 2004;92(1–2):101–144.
30. Gallea C, Popa T, García-Lorenzo D, et al. Intrinsic signature of essential tremor in the cerebello-frontal network. Brain 2015;138(Pt 10):2920–2933.
31. Louis ED, Faust PL. Essential tremor pathology: neurodegeneration and reorganization of neuronal connections. Nat Rev Neurol 2020;16(2):69–83.
32. Stoodley CJ, Valera EM, Schmahmann JD. Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study. Neuroimage 2012;59(2):1560–1570.

33. Stoodley CJ, Schmahmann JD. Functional topography of the human cerebellum. Handb Clin Neurol 2018;154:59–70.

34. Ackermann H. Cerebellar contributions to speech production and speech perception: psycholinguistic and neurobiological perspectives. Trends Neurosci 2008;31(6):265–272.

35. Bohland JW, Guenther FH. An fMRI investigation of syllable sequence production. Neuroimage 2006;32(2):821–841.

36. Ghosh SS, Tourville JA, Guenther FH. A neuroimaging study of premotor lateralization and cerebellar involvement in the production of phonemes and syllables. J Speech Lang Hear Res 2008;51(5): 1183–1202.

37. Matsuzaka Y, Aizawa H, Tanji J. A motor area rostral to the supplementary motor area (presupplementary motor area) in the monkey: neuronal activity during a learned motor task. J Neurophysiol 1992;68(3):653–662.

38. Shima K, Tanji J. Both supplementary and presupplementary motor areas are crucial for the temporal organization of multiple movements. J Neurophysiol 1998;80(6):3247–3260.

39. Simonyan K, Ostuni J, Ludlow CL, Horwitz B. Functional but not structural networks of the human laryngeal motor cortex show left hemispheric lateralization during syllable but not breathing production. J Neurosci 2009;29(47):14912–14923.

40. Zachlod D, Rüttgers B, Bludau S, Mohlberg H, Langner R, Zilles K, Amunts K. Four new cytoarchitectonic areas surrounding the primary and early auditory cortex in human brains. Cortex 2020;128; 1–21.

41. Tourville JA, Reilly KJ, Guenther FH. Neural mechanisms underlying auditory feedback control of speech. Neuroimage 2008;39(3): 1429–1443.

42. Baldo JV, Wilkins DP, Ogar J, Willock S, Dronkers NF. Role of the precentral gyrus of the insula in complex articulation. Cortex 2011; 47(7):800–807.

43. Guenther FH. Cortical interactions underlying the production of speech sounds. J Commun Disord 2006;39(5):350–365.

44. Dronkers NF. A new brain region for coordinating speech articulation. Nature 1996;384(6605):159–161.

45. Fedorenko E, Fillmore P, Smith K, Bonilha L, Fridriksson J. The superior precentral gyrus of the insula does not appear to be functionally specialized for articulation. J Neurophysiol 2015;113(7): 2376–2382.

46. Battistella G, Kumar V, Simonyan K. Connectivity profiles of the insular network for speech control in healthy individuals and patients with spasmodic dysphonia. Brain Struct Funct 2018;223(5): 2489–2498.

47. Hanekamp S, Simonyan K. The large-scale structural connectome of task-specific focal dystonia. Hum Brain Mapp 2020.41(12):3252–3265.

48. Juttukonda MR, Franco G, Englot DJ, et al. White matter differences between essential tremor and Parkinson disease. Neurology 2019;92(1):e30–e39.

49. Pietracupa S, Bologna M, Bharti K, et al. White matter rather than gray matter damage characterizes essential tremor. Eur Radiol 2019; 29(12):6634–6642.

50. Nestrasil I, Svatkova A, Rudser KD, et al. White matter measures correlate with essential tremor severity-a pilot diffusion tensor imaging study. Brain Behav 2018;8(8):e01039.