Short- and Long-term Central Action of Botulinum Neurotoxin Treatment in Laryngeal Dystonia

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Abstract

Background and Objectives
Laryngeal dystonia (LD) is isolated task-specific focal dystonia selectively impairing speech production. The first choice of LD treatment is botulinum neurotoxin (BoNT) injections into the affected laryngeal muscles. However, whether BoNT has a lasting therapeutic effect on disorder pathophysiology is unknown. We investigated short-term and long-term effects of BoNT treatment on brain function in patients with LD.

Methods
A total of 161 participants were included in the functional MRI study. Statistical analyses examined central BoNT effects in patients with LD who were stratified based on the effectiveness and duration of treatment.

Results
Patients with LD who were treated and benefited from BoNT injections had reduced activity in the left precuneus compared with BoNT-naive and treatment nonbenefiting patients. In addition, BoNT-treated patients with adductor LD had decreased activity in the right thalamus, whereas BoNT-treated abductor patients with LD had reduced activity in the left inferior frontal cortex. No statistically significant differences in brain activity were found between patients with shorter (1–5 years) and longer (13–28 years) treatment durations. However, patients with intermediate treatment duration of 6–12 years showed reduced activity in the right cerebellum compared with patients with both shorter and longer treatment durations and reduced activity in the right prefrontal cortex compared with patients with shorter treatment duration.

Discussion
Our findings suggest that the left precuneus is the site of short-term BoNT central action in patients with LD, whereas the prefrontal-cerebellar axis is engaged in the BoNT response in patients with intermediate treatment duration of 6–12 years. Involvement of these structures points to indirect action of BoNT treatment on the dystonic sensorimotor network through modulation of motor sequence planning and coordination.

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Glossary

ABLD = abductor LD; **ADLD** = adductor LD; **ANCOVA** = analysis of covariance; **BFMDRS** = Burke-Fahn-Marsden Dystonia Rating Scale; **BOLD** = blood oxygen level–dependent; **BoNT** = botulinum neurotoxin; **EPI** = echo-planar imaging; **FA** = flip angle; **FOV** = field of view; **FWE** = family-wise error; **HC** = healthy control; **LD** = laryngeal dystonia; **SNAP** = soluble N-ethylmaleimide-sensitive factor attachment protein; **TE** = echo time; **TR** = repetition time.

Laryngeal dystonia (LD) is isolated focal task-specific dystonia characterized by involuntary spasms in the laryngeal muscles that occur selectively during speech production. LD negatively affects the quality of life because patients are limited in their daily and professional activities because of inability to communicate, which leads to lower socioeconomic status, poor self-perception, psychiatric comorbidities, and suicidal behaviors.[1,2]

Despite its chronic, debilitating impact, LD pathophysiology is unclear, and, consequently, the therapeutic options remain limited. Similar to other forms of dystonia, the first choice of LD therapy is botulinum neurotoxin type A (BoNT) injections into the affected muscle. However, BoNT efficacy highly depends on the type of LD, with approximately 90% of patients with adductor LD (ADLD) reporting 90% benefits but only 10% of patients with abductor LD (ABLD) receiving approximately 70% benefits.[3] In patients who do respond to the treatment, BoNT benefits are seen for only approximately 30% of the injection cycle, with more than half of patients experiencing a common side effect of excessive breathiness on an average of 10 days postinjection.

BoNT molecular mode of action includes extracellular binding to glycoprotein structures on cholinergic nerve terminals, cleavage of the components of the soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor complex, intracellular blockade of acetylcholine release, and neuromuscular transmission, leading to alleviation of dystonic muscle contractions. BoNT injections must be repeated every 3–4 months in patients with LD because their effectiveness wears off over time due, in part, to the regeneration of the SNAP-25 protein complex in the laryngeal muscles.[4] The diminishing benefits of BoNT treatment may also be due to the presence of neutralizing antibodies against BoNT[5] or transient modulation of pathophysiologically abnormal brain function.

To that end, the current literature[6] on the central effects of BoNT therapy in LD and other forms of focal dystonia reports inconsistent findings that vary from none[7,8] to moderate[9] to substantial[10] modulation of brain activity at the peak of treatment benefits, that is, around 1–1.5 months postinjection (Figure 1, eTable 1, links.lww.com/WNL/C151). The extent of BoNT-based neuromodulation of disorder pathophysiology outside of this narrow time window of peak efficacy is unknown, which limits our understanding of the full range of factors contributing to both short-term (within the treatment cycle) and long-term (over the years of treatment) therapeutic outcomes.

We conducted a series of studies to systematically investigate short-term or long-term effects of BoNT injections on pathophysiologically altered brain function in LD. We hypothesized that the temporary effectiveness of BoNT treatment is partly because of its insufficient modulation of activity in key sensorimotor brain regions involved in the output of dystonic speech in patients with LD.

Methods

Study Participants

Study participants were recruited between August 2012 and September 2019 through online advertisements and referrals by treating physicians from tertiary hospitals across the United States. The inclusion criteria included a confirmed diagnosis of LD, right-handedness, native English language, and normal cognitive status. The exclusion criteria included any history of other neurologic conditions (including other forms of dystonia but excluding dystonic tremor of voice in patients) or psychiatric disorders (including anxiety and depression), significant radiologic findings on brain MRI, recent history (within 1 year) of voice and speech therapy, any centrally acting medications, non-MRI compatible tattoos or ferromagnetic implants, and pregnancy or breastfeeding at the time of study participation.

Following these criteria, a total of 161 participants participated in the study, including 111 patients with LD (90 female participants/21 male participants, age 55.9 ± 12.9 years) and 50 healthy controls (HCs, 32 female participants/18 male participants, age 51.0 ± 10.1 years) (Table 1). LD diagnosis in patients and the absence of laryngeal and other neurologic problems in all participants were confirmed using a combined approach of a case history, laryngeal and neurologic examinations, and voice/speech perceptual evaluation.[11] Dystonic tremor of voice was present in 41% of patients with LD as a characteristic feature of this disorder.[11] The absence of psychiatric history in all participants was established based on a combination of participant's reports of the absence of psychiatric problems, no formal diagnosis by a psychiatrist documented in the participant's chart, and no history of use of psychotropic medications.

All treated patients received BoNT type A injections. The efficacy of treatment was established based on the review of patient's medical information from treating physicians, including history, physical, laryngeal, speech-language pathology,

Figure 1 Graphical Review of the Current Literature Examining BoNT Central Effects in LD and Other Forms of Focal Dystonia
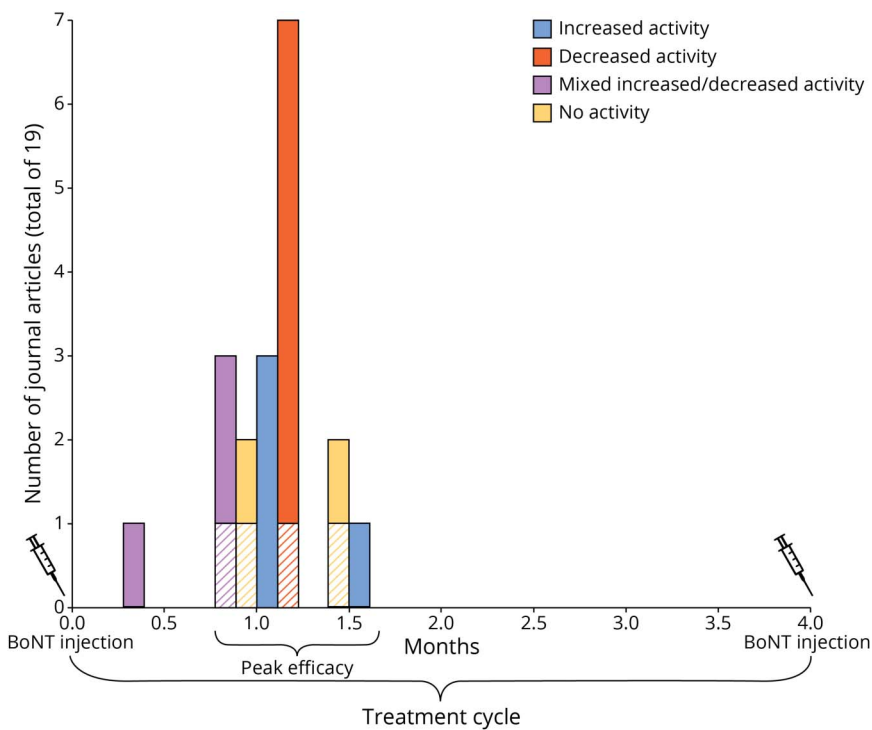
A review of 19 neuroimaging studies investigating the effects of BoNT treatment on brain function reveals the timeline of their treatment cycle at which patients were assessed in each study (see eTable 1, links.lww.com/WNL/C151). Most of the studies recruited patients around the peak efficacy of BoNT injections at 1–1.5 months posttreatment; the short-and long-term effects have not yet been investigated. The bars show the total number of studies per BoNT modulatory effect on brain activity. Studies involving patients with LD are shown with striped color bars; studies involving other forms of focal dystonia (blepharospasm, orofacial dystonia, cervical dystonia, hand dystonia) are shown in solid color bars. BoNT = botulinum neurotoxin; LD = laryngeal dystonia.

or neurologic examinations, and by questioning each patient about their treatment timelines and perceived benefits using a structured questionnaire. Our patient cohort matched typical clinical demographics of LD including more female patients and patients with ADLD who have greater benefits from BoNT injections.[3] All patients participated in the study when fully symptomatic, at least 3 months after last injection, to match HCs and patients who did not receive BoNT treatment. This study design allowed the assessment of short-term (at the end of the treatment cycle) and long-term (over the years of treatment) central effects of BoNT injections on disorder pathophysiology outside of the narrow time window (1–1.5 months) of clinically significant symptom improvement.r

Primary Experimental Groups

Participants were assigned to 4 groups for examination of different aspects of central response to BoNT treatment (Figure 2A, Table 1).

1. Overall brain function in patients with LD compared with HCs was assessed as a first step to reproduce previously reported functional alterations in this disorder. We selected 57 patients with LD (42 female participants/15 male participants age 54.7 ± 13.4 years) from a larger cohort of 111 patients with LD to create an age-and sex-balanced design compared with 50 HCs (32 female participants/18 male participants age 51.0 ± 10.0 years) (Figure 2A.a). The LD group included patients who were

BoNT-naive, BoNT-treated, BoNT-benefiting, and BoNT–non-benefiting.

2. Short-term effects on brain function in BoNT-naive vs BoNT-treated patients with LD were examined in 29 patients who never received BoNT treatment (BoNT-naive, 21 female participants/7 male participants age 53.9 ± 14.5 years) compared with 28 patients who received at least 1 BoNT injection (BoNT-treated, 20 female participants/8 male participants age 55.4 ± 12.8 years, 4.9 ± 6.2 treatment years, 17.0 ± 22.7 injections) (Figure 2A.b).

3. Short-term effects on brain function in BoNT-benefiting vs BoNT–non-benefiting patients with LD were investigated by further stratifying the BoNT-treated group into 14 patients who reported injection benefits (10 female participants/4 male participants, age 55.9 ± 13.9 years, 8.3 ± 7.3 treatment years, 29.9 ± 26.4 injections) and 14 patients who reported no BoNT benefits (10 female participants/4 male participants age 55.9 ± 12.0 years, 1.5 ± 0.9 treatment years, 4.1 ± 4.0 injections) (Figure 2A.c). Among those who did not benefit from BoNT injections, 13 patients were primary nonresponders whose symptoms did not improve from the very first and all subsequent injections (3.2 ± 2.2 injections). One patient was a secondary nonresponder who benefited from the first injection but not subsequent 15 injections.

4. Long-term effects of BoNT treatment on brain function were examined in 54 patients with LD who received BoNT

Table 1 Patient Demographics and Clinical Characteristics

LD patient cohorts for assessment of short-term BoNT central effects

	BoNT-naive (n = 29)	BoNT-treated (n = 28)	BoNT benefiting (n = 14)	BoNT nonbenefiting (n = 14)
Age, y, mean ± SD	54.1 ± 14.2	55.4 ± 12.8	54.9 ± 13.9	55.9 ± 12.0
Sex, female:male	22:7	20:8	10:4	10:4
Handedness	Right (Edinburgh Inventory)			
Language	Monolingual Native English			
Cognitive status	MMSE ≥27 points or MoCA ≥26 points			
Dystonia phenotype				
ADLD:ABLD	18:11	15:13	10:4	5:9
ADLD/DTv:ABLD/DTv	8:5	10:5	5:2	5:3
Dystonia duration, y, mean ± SD	13.2 ± 13.0	14.8 ± 10.8	14.9 ± 12.4	14.6 ± 9.0
Symptom severity (BFMDRS)	4.5 ± 3.1	5.5 ± 3.5	4.6 ± 2.7	6.4 ± 4.1
Dystonia onset, y, mean ± SD	40.9 ± 16.9	40.3 ± 13.7	39.4 ± 13.9	41.4 ± 14.6
No. of BoNT injections, mean ± SD	n/a	17.0 ± 22.7	29.9 ± 26.4	4.1 ± 4.0
Duration of BoNT treatment, y, mean ± SD	n/a	4.9 ± 6.2	8.3 ± 7.3	1.5 ± 0.9
Duration of BoNT treatment cycle, mo, mean ± SD	n/a	3.8 ± 2.1	3.5 ± 1.0	4.3 ± 3.5

LD patient cohorts for assessment of long-term BoNT central effects

	BoNT benefit 1–5 y (n = 18)	BoNT benefit 6–12 y (n = 19)	BoNT benefit 13–28 y (n = 17)
Age, y, mean ± SD	51.5 ± 12.4	59.2 ± 11.3	60.9 ± 11.9
Sex, female:male	15:3	16:3	17:0
Handedness	Right (Edinburgh Inventory)		
Language	Monolingual Native English		
Cognitive status	MMSE ≥27 points or MoCA ≥26 points		
Dystonia phenotype			
ADLD:ABLD	13:5	17:2	15:2
ADLD/DTv:ABLD/DTv	5:1	6:0	5:1
Dystonia duration, y, mean ± SD	9.4 ± 10.8	15.2 ± 10.5	23.9 ± 8.9
Symptom severity (BFMDRS)	5.1 ± 3.8	4.8 ± 2.1	4.0 ± 1.6
Dystonia onset, y, mean ± SD	43.2 ± 15.2	44.0 ± 14.0	37.1 ± 12.3
No. of BoNT injections, mean ± SD	7.4 ± 5.7	28.4 ± 14.6	66.1 ± 28.8
Duration of BoNT treatment, y, mean ± SD	2.8 ± 1.5	9.0 ± 2.3	19.9 ± 5.5
Duration of BoNT treatment cycle, mo, mean ± SD	4.2 ± 2.3	4.3 ± 2.2	3.9 ± 1.1

Patient stratification by LD phenotype for assessment of BoNT central effects

	BoNT-naive ADLD (n = 18)	BoNT-naive ABLD (n = 11)	BoNT-treated ADLD (n = 15)	BoNT-treated ABLD (n = 13)
Age, y, mean ± SD	53.9 ± 15.0	54.3 ± 13.5	58.1 ± 14.5	52.4 ± 10.1
Sex, female:male	15:3	7:4	12:3	8:5
Handedness	Right (Edinburgh Inventory)			
Language	Monolingual Native English			

Continued

Table 1 Patient Demographics and Clinical Characteristics *(continued)*

Patient stratification by LD phenotype for assessment of BoNT central effects

	BoNT-naive ADLD (n = 18)	BoNT-naive ABLD (n = 11)	BoNT-treated ADLD (n = 15)	BoNT-treated ABLD (n = 13)
Cognitive status	MMSE ≥27 points or MoCA ≥26 points			
Dystonia duration, y, mean ± SD	11.6 ± 12.7	15.8 ± 13.6	15.4 ± 12.2	14.2 ± 9.3
Symptom severity (BFMDRS)	4.9 ± 3.6	4.0 ± 2.0	5.2 ± 3.5	5.8 ± 3.7
Dystonia onset, y, mean ± SD	42.3 ± 17.0	38.5 ± 17.4	42.0 ± 15.4	38.3 ± 11.7
No. of BoNT injections, mean ± SD	n/a	n/a	25.5 ± 27.7	7.2 ± 8.6
Duration of BoNT treatment, y, mean ± SD	n/a	n/a	7.2 ± 7.5	2.2 ± 2.4
Duration of BoNT treatment cycle, mo, mean ± SD	n/a	n/a	3.9 ± 2.6	3.3 ± 0.8

Abbreviations: ABLD = abductor LD; ADLD = adductor LD; BFMDRS = Burke-Fahn-Marsden Dystonia Rating Scale; BoNT = botulinum toxin; DTv = dystonic tremor of voice; HC = healthy control; LD = laryngeal dystonia; MMSE = mini-mental state examination; MoCA = Montreal Cognitive Assessment; n/a = nonapplicable.
MMSE and MoCA were adjusted by age and education.

treatment for 1–5 years (N = 18; 15 female participants/3 male participants age 51.5 ± 12.4 years), 6–12 years (N = 19; 16 female participants/3 male participants age 59.2 ± 11.3 years), and 13–28 years (N = 17; all female participants age 60.9 ± 11.8 years) (Figure 2A.d). All patients received continuous treatment since their LD diagnosis, with injections administered every 4.1 ± 1.9 months (Table 1). Because there is no previous literature relevant to the stratification of patients with dystonia for the assessment of long-term treatment effects, our group subdivisions were based on the considerations that LD symptoms may progress within the first 2 years of disease onset, and BoNT treatment efficacy may not be established during the first injection cycle.[12] Therefore, the patient group with shortest treatment duration included those who received injections between 1 and 5 years to ensure that the treatment regimen in this group was fully established, and patients received consistent benefits. Subsequently, the patient groups with intermediate (6–12 years) and longer (13–28 years) treatment durations were composed to match the group with the shortest treatment duration (1–5 years) by cohort size, sex, disease characteristics, and duration of the BoNT injection cycle.

Secondary Experimental Groups

Given the distinctly greater benefits of BoNT treatment in patients with ADLD than ABLD,[3] a secondary study examined the short-term central effects of BoNT injections dependent on LD phenotype. Patients were grouped as follows (Figure 2B, Table 1):

1. Brain function in BoNT-naive but phenotypically different LD was examined in 18 ADLD BoNT-naive patients (15 female participants/3 male participants age 53.9 ± 15.0 years) vs 11 ABLD BoNT-naive patients (7 female participants/4 male participants age 54.3 ± 13.5 years) (Figure 2B.a).

2. Brain function in BoNT-treated but phenotypically different LD was assessed in 15 ADLD BoNT-treated patients (12 female participants/3 male participants age 58.1 ± 14.5 years, 7.2 ± 7.5 treatment years, 25.5 ± 27.7 injections) vs 13 ABLD BoNT-treated patients (8 female participants/5 male participants age 52.4 ± 10.1 years, 2.2 ± 2.4 treatment years, 7.2 ± 8.6 injections) (Figure 2B.b).

3. Brain function between BoNT-naive and treated but phenotypically same ADLD was compared in 18 BoNT-naive patients with ADLD (15 female participants/3 male participants age 53.9 ± 15.0 years) vs 15 BoNT-treated patients with ADLD (12 female participants/3 male participants age 58.1 ± 14.5 years, 7.2 ± 7.5 treatment years, 25.5 ± 27.7 injections) (Figure 2B.c).

4. Brain function between BoNT-naive and BoNT-treated but phenotypically same ABLD was examined in 11 BoNT-naive patients with ABLD (7 female participants/4 male participants age 54.3 ± 13.5 years) vs 13 BoNT-treated patients with ABLD (8 female participants/5 male participants age 52.4 ± 10.1 years, 2.2 ± 2.4 treatment years, 7.2 ± 8.6 injections) (Figure 2B.d).

Because of limited BoNT benefits in patients with ABLD,[3] phenotypical stratifications of our cohort for analysis of long-term central effects of treatment were not performed.

MRI Acquisition

Brain images were acquired on a 3.0 T Philips MRI scanner equipped with an 8-channel head coil. The participant's head was tightly cushioned inside the coil to reduce movements. All participants were trained on the fMRI study design and instructed to restrict movements during scanning. Participants were monitored through a 2-way audio communication system during scanning to ensure correct task performance.

Figure 2 Overview of Patient Stratification Depicts (A) Primary Experimental Groups and (B) Secondary Experimental Groups Used for Statistical Comparisons
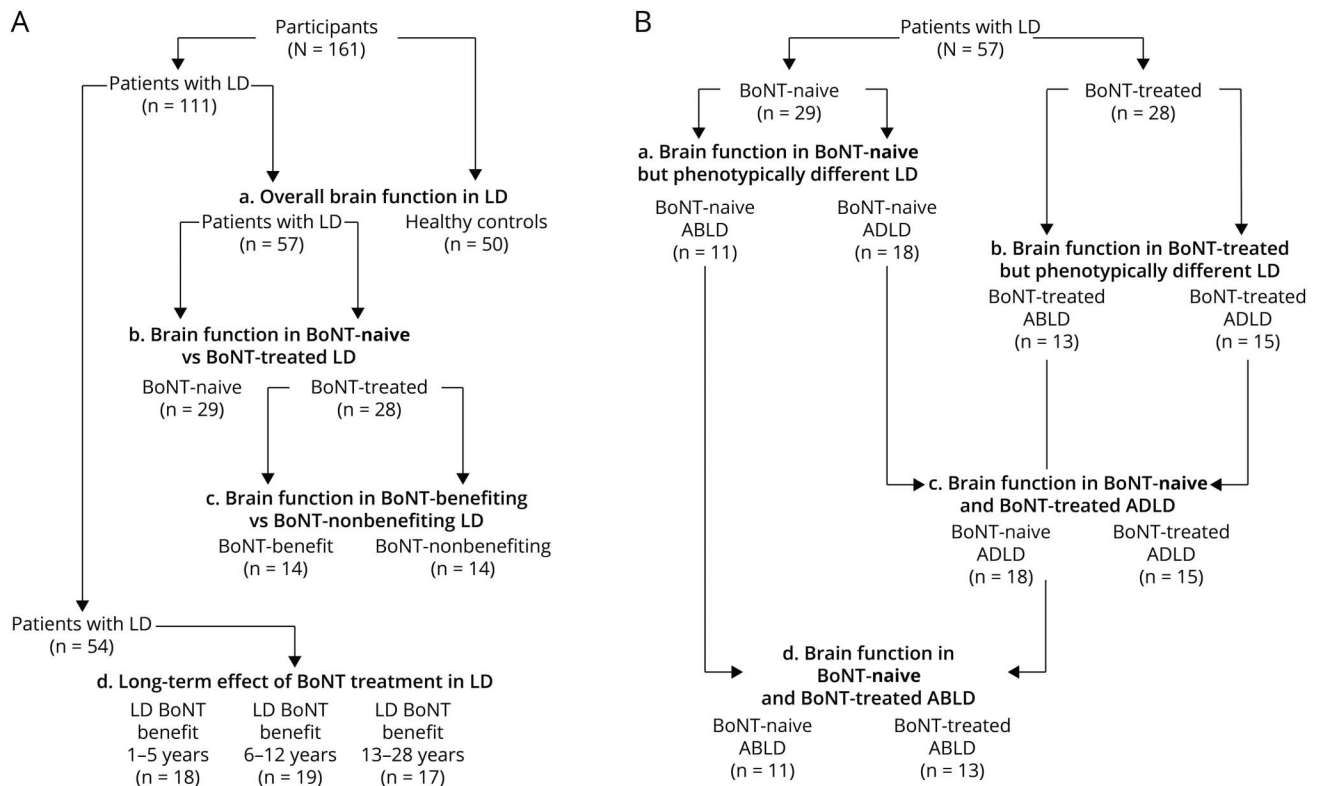
ABLD = abductor LD; ADLD = adductor LD; BoNT = botulinum neurotoxin; LD = laryngeal dystonia.

Functional images were collected using gradient-weighted echo-planar imaging (EPI) pulse sequence with blood oxygen level–dependent (BOLD) contrast and a sparse-sampling event-related design (repetition time [TR] 2 seconds per volume, 10.6 seconds between volumes, echo time [TE] 30 milliseconds, flip angle [FA] 90°, field of view [FOV] 240 mm, voxel size 3.5 × 3.5 mm, 4-mm slice thickness, 36 slices), which helped minimize motion artifacts because of orofacial movements during speech production and neutralize the scanner noise interference with acoustic stimulus presentation. The experimental task included 8 LD symptom-eliciting English sentences and a resting condition as a baseline. After acoustic presentation (3.6 seconds) of a sample sentence using the MR-compatible headphones, participants were cued with an arrow to produce the sentence (5 seconds) and then remain silent for volume acquisition (2 seconds). Task and resting conditions were pseudorandomized within and between functional runs and participants. Each participant completed 4 functional runs, including a total of 32 tasks and 16 rests.

A high-resolution T1-weighted MRI image was acquired as an anatomical reference using 3D magnetization–prepared rapid acquisition gradient echo sequence (TR 7.5 milliseconds, TE 3.4 milliseconds, FA 8°, FOV 210 mm, 1-mm slice thickness, 172 slices).
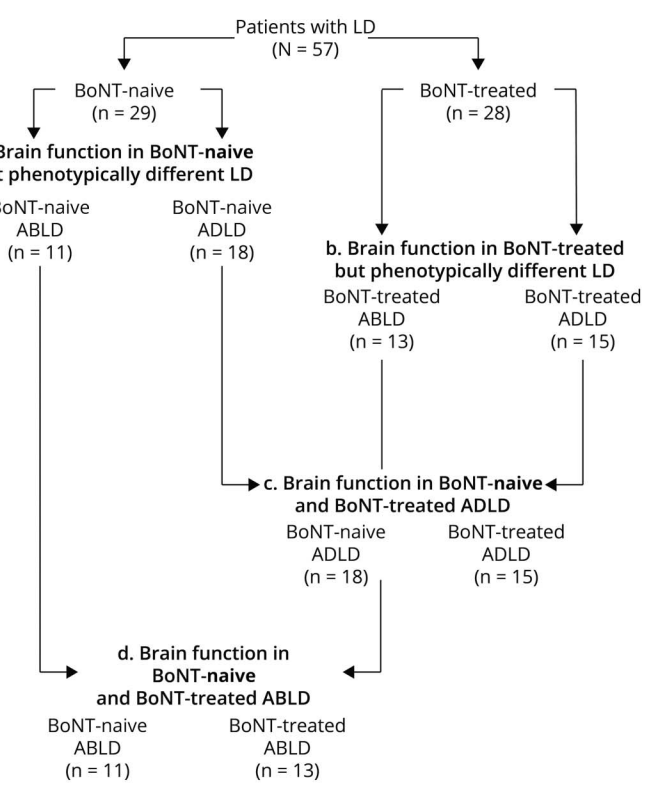
MRI Data Analysis

Images were analyzed using the standard afni_proc.py pipeline in AFNI software. The first 2 volumes were discarded to account for the magnetization equilibrium; time series were despiked and registered to the EPI volume using heptic polynomial interpolation. The resultant time series were spatially normalized to the AFNI standard Talairach-Tournoux space, smoothed with a 4-mm Gaussian filter, and scaled by voxelwise mean. The motion was corrected by regressing motion parameters, censoring TRs, and censoring outlier TRs. Six motion parameter estimates were included as covariates of no interest; 3 quadratic polynomials were used to model baseline drifts for each imaging run. TR censoring excluded TR pairs where the Euclidean norm of the motion derivative exceeded 1.0. Outlier censoring excluded TRs when more than 10% of the automasked brain was marked as an outlier. A regressor associated with the speech task was convolved with a canonical hemodynamic response function and used in a multiple regression model to predict the BOLD response.

Statistical Analysis

Two-tailed 2-sample t tests or χ^2 tests were used, as appropriate, to examine between-group differences in participant demographics (age, sex) and LD clinical characteristics (LD duration, age at onset, severity) at Bonferroni-corrected $p \leq 0.05$. Symptom severity was assessed using the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) at the

time of study participation. LD clinical characteristics were further examined for their relationship with mean percent BOLD signal change in regions of significant between-group differences (as determined below) using Spearman rank correlation coefficients at whole-brain voxelwise-corrected $p \leq 0.05$ with minimum cluster size ≥ 214 mm³ and $R_s \geq \pm 0.4$.

Primary analysis included statistical comparisons between groups as outlined in the Primary experimental groups section (Figure 2A, Table 1). One-way analysis of variance examined differences between patients with LD and HCs. Two separate 2-way analyses of covariance (ANCOVA), with the total number of injections and time (in months) from last injection as nuisance covariates, examined short-term effects of injections between BoNT-naive and BoNT-treated patients and between BoNT-benefiting and BoNT–non-benefiting patients with LD. Statistical significance was set at whole-brain family-wise error (FWE)–corrected $p \leq 0.05$ with minimum voxelwise and cluster-size thresholds as determined for each contrast by AFNI 3dClustSim program.

Long-term effects of BoNT treatment on brain function were computed using 2-way ANCOVA with 3 factors defined by the number of years the patient received and benefited from BoNT treatment. Sex differences in between-group comparisons of 13–28 vs 1–5 years or 6–12 years could not be assessed because the former group included only female patients. LD duration was significantly different between groups with 1–5 vs 13–28 years of treatment ($p = 0.002$, see Results). Therefore, sex and disease duration were modeled as covariates of no interest in addition to the total number of injections and time (in months) from last injection. Statistical significance was set at whole-brain voxelwise-corrected $p \leq 0.01$ with a minimum cluster size of ≥ 858 mm³.

Secondary analysis examined clinically distinct benefits of BoNT treatment in different LD phenotypes as outlined in the Secondary experimental groups section (Figure 2B, Table 1). In all comparisons, between-group statistically significant differences were determined using 2-way ANCOVA, with covariates of no interest including the total number of injections, time (in months) from last injection, and response to BoNT, as appropriate, at whole-brain voxelwise-corrected $p \leq 0.01$ with minimum cluster size ≥ 858 mm³.

Standard Protocol Approvals, Registrations, and Patient Consents
Standard protocol and informed consent were approved by the Institutional Review Boards of Mass General Brigham and Icahn School of Medicine at Mount Sinai. Written informed consent was obtained from all participants before study participation. Data from some participants at the peak of BoNT efficacy (1–1.5 months) were used in our previous study.[10]

Data Availability
Anonymized data not published within this article may be made available by request from any qualified investigator.

Results

No statistically significant differences were found between groups in age (all $p \geq 0.11$), sex (all $p \geq 0.47$), LD phenotype (ADLD vs ABLD, all $p \geq 0.08$), duration (all $p \geq 0.22$, except the expected long-term group comparison of 1–5 vs 13–28 years of treatment at $p = 0.002$), age at onset (all $p \geq 0.48$), or symptom severity (all $p \geq 0.09$).

Overall, patients with LD compared with HCs showed hyperactivity in bilateral primary sensorimotor, inferior frontal, and auditory cortex, cerebellum (lobule VI), left anterior insula, lentiform nucleus, and thalamus and abnormal hypoactivity in right inferior parietal cortex (Figure 3A.a, Table 2). LD duration was negatively correlated with activity in left auditory cortex ($R_s = -0.45$, $p = 0.0005$; Figure 4A.a).

Short-Term Central Effects of BoNT Treatment
We found that BoNT-treated patients had reduced activity in left superior parietal lobule (precuneus) compared with BoNT-naive patients who never received injections (Figure 3A.b, Table 2). Precuneus activity in BoNT-naive patients was positively correlated with the age at LD onset ($R_s = 0.42$, $p = 0.02$; Figure 4A.b) and negatively correlated with disease duration ($R_s = -0.65$, $p = 0.0001$; Figure 4A.b) and severity ($R_s = -0.53$, $p = 0.003$; Figure 4A.c). Moreover, patients with LD who did not benefit from BoNT injections maintained similar hyperactivity of precuneus compared with those who benefited from treatment (Figure 3A.c, Table 2).

Further examination of treatment effects in phenotypically stratified cohorts showed that BoNT-naive patients with ABLD had increased activity in left inferior parietal cortex compared with BoNT-naive patients with ADLD (Figure 3B.a, Table 2), whereas BoNT-treated patients with ABLD had increased activity in right cerebellum (lobule VI) compared with BoNT-treated patients with ADLD (Figure 3B.a, Table 2). Comparisons of treatment effects within the same phenotype showed that BoNT-naive patients with ADLD had increased activity in right thalamus (parietal subdivision) compared with BoNT-treated patients with ADLD (Figure 3B.c, Table 2), whereas BoNT-naive patients with ABLD had greater activity in left inferior frontal gyrus compared with BoNT-treated patients with ABLD (Figure 3B.d, Table 2). BoNT-naive patients with ADLD showed a negative correlation between right thalamic activity and disease duration ($R_s = -0.59$, $p = 0.01$; Figure 4B.a). Conversely, BoNT-treated patients with ADLD showed negative correlations between right cerebellar activity and disease onset ($R_s = -0.65$, $p = 0.009$; Figure 4B.b) and between right thalamic activity and symptom severity ($R_s = -0.79$, $p = 0.0005$; Figure 4B.c). On the other hand, BoNT-naive patients with ABLD had a negative correlation between activity of left inferior

Figure 3 Short-Term and Long-Term Central Effects of BoNT Treatment in Patients With LD
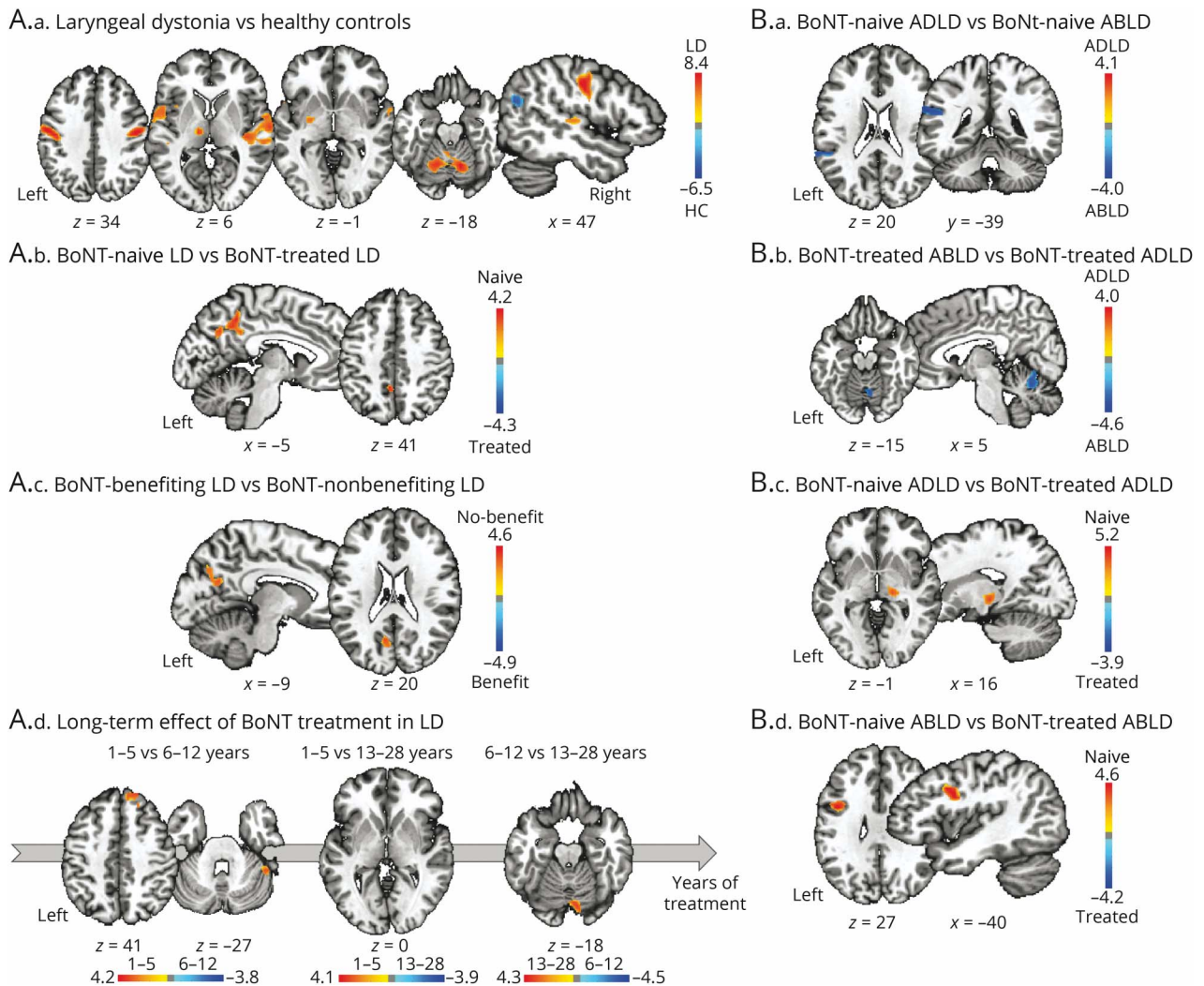
(A) Statistically significant differences in brain activity during speech production are shown between (a) an aggregate group of patients with LD and HCs (FWE-corrected $p \leq 0.05$ with minimum voxelwise threshold $p \leq 0.00001$ and cluster size ≥ 343 mm³), (b) BoNT-naive and BoNT-treated patients with LD (FWE-corrected $p \leq 0.05$ with minimum voxelwise threshold $p \leq 0.01$ and cluster size $\geq 1,715$ mm³), (c) patients with LD with and without benefits from BoNT treatment (whole-brain voxelwise-corrected $p \leq 0.01$ with a minimum cluster size of ≥ 858 mm³), and (d) long-term effects of BoNT treatment in patients receiving injections between 1 and 28 years (whole-brain voxelwise-corrected $p \leq 0.01$ with a minimum cluster size of ≥ 858 mm³). (B) In phenotypically stratified groups, statistically significant differences in brain activity during speech production are shown between (a) BoNT-naive patients with ADLD and patients with ABLD, (b) BoNT-treated patients with ADLD and patients with ABLD, (c) BoNT-naive and treated patients with ADLD, and (d) BoNT-naive and treated patients with ABLD. Statistical significance is set at whole-brain voxelwise-corrected $p \leq 0.01$ with minimum cluster size ≥ 858 mm³. Brain slices are shown in the AFNI standard Talairach-Tournoux space. Color bars represent the t scores. ABLD = abductor type LD; ADLD = adductor type LD; BoNT = botulinum neurotoxin; FWE = family-wise error; LD = laryngeal dystonia.

parietal cortex and symptom severity ($R_s = -0.87$, $p = 0.001$; Figure 4B.d), whereas BoNT-treated patients with ABLD established positive correlations between activity of left inferior frontal gyrus and disease duration ($R_s = 0.71$, $p = 0.007$; Figure 4B.d) as well as symptom severity ($R = 0.75$, $p = 0.003$; Figure 4B.d).

Long-Term Central Effects of BoNT Treatment

Patients with LD receiving BoNT injections for 1–5 years maintained increased activity in right middle frontal gyrus and cerebellum (Crus 1) compared with patients receiving treatment for 6–12 years (Figure 3A.d, Table 2). Patients with LD with 13–28 years of treatment had increased activity in right

cerebellum (lobule VI) compared with patients receiving BoNT injections for 6–12 years. This cerebellar activity was positively correlated with symptom severity ($R_s = 0.59$, $p = 0.01$; Figure 4A.d) in patients with 13–28 years of BoNT treatment. There were no statistically significant differences between patients with LD who had shortest (1–5 years) and longest (13–28 years) durations of BoNT treatments.

Discussion

In a series of studies in therapeutically and phenotypically diverse LD cohorts, we examined the central effects of BoNT treatment

**Table 2** Differences in Brain Activity During Symptomatic Speech Production

Anatomical regions	Cluster peak coordinates (x, y, z)	Cluster size (mm³)	Cluster peak *t* level
Laryngeal dystonia > healthy controls			
R primary sensorimotor cortex, extending to auditory cortex	47, −11, 34	9,818	8.4
L primary sensorimotor cortex	−51, −11, 38	4,244	8.1
L cerebellum (lobule VI)	−16, −57, −18	2,443	6.9
R cerebellum (lobule VI)	12, −57, −18	2,229	7.8
L inferior frontal gyrus	−47, −1, 6	1,501	5.6
L auditory cortex	−33, −32, 13	1,157	6.9
L thalamus	−12, −18, 6	600	6.9
L insula	−33, 10, 10	600	6.4
L lentiform nucleus	−23, −8, −1	385	6.5
Laryngeal dystonia < healthy controls			
R inferior parietal cortex	47, −60, 20	343	6.5
Short-term central effects of BoNT treatment			
LD BoNT-naive > LD BoNT-treated			
L superior parietal lobule (precuneus)	−5, −46, 41	1,757	3.9
LD BoNT benefit < LD BoNT nonbenefit			
L superior parietal lobule (precuneus)	−9, −60, 20	1,029	3.9
BoNT-naive ADLD < BoNT-naive ABLD			
L inferior parietal cortex	−58, −39, 20	986	4.0
BoNT-treated ADLD < BoNT-treated ABLD			
R cerebellum (lobule V)	5, −57, −15	1,157	3.5
BoNT-naive ADLD > BoNT-treated ADLD			
R thalamus	16, −22, −1	1,200	4.1
BoNT-naive ABLD > BoNT-treated ABLD			
L inferior frontal gyrus	−40, 3, 27	1,586	4.6
Long-term central effects of BoNT treatment			
LD BoNT benefit 1–5 y > LD BoNT benefit 6–12 y			
R middle frontal gyrus	19, 38, 34	1,243	3.7
R cerebellum (Crus 1)	47, −39, −25	1,157	3.2
LD BoNT benefit 13–28 y > LD BoNT benefit 6–12 y			
R cerebellum (lobule VI)	12, −81, −15	1,286	3.6

Abbreviations: ABLD = abductor type LD; ADLD = adductor type LD; BoNT = botulinum toxin; LD = laryngeal dystonia; L = left; R = right.

on pathophysiologically abnormal brain activity. Our findings of brain functional alterations in patients with LD compared with healthy individuals are in line with the previous literature,[13-15] confirming the robustness of our experimental design and reproducibility of findings as a prerequisite for reliable identification of central BoNT effects in these patients.

The current literature on the central action of BoNT in patients with focal dystonia, including LD, remains controversial. Some studies have shown post-BoNT decreases in the amplitude of precentral P22/N30 component of median nerve somatosensory evoked potentials and their association with decreased short-interval intracortical inhibition in cervical and limb dystonias[16,17]; temporary elimination of the facilitatory effect of paired associative

Figure 4 Relationships Between LD Clinical Features and Brain Functional Alterations
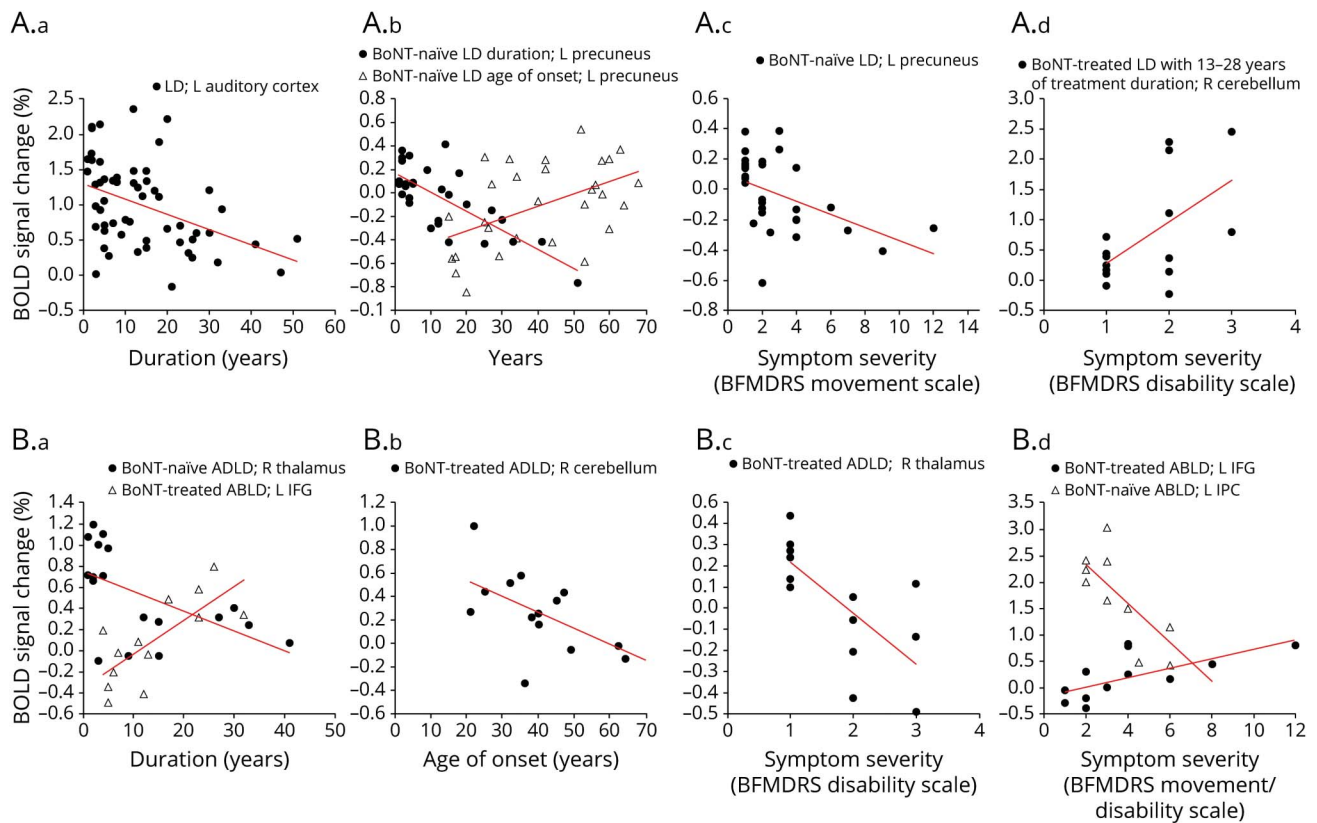
(A) Scatter plots show statistically significant correlations between regional mean percent BOLD signal change and (a) disorder duration in patients with LD, (b) duration and age at disorder onset in BoNT-naive patients with LD, (c) symptom severity in BoNT-naive patients with LD, and (d) symptom severity in BoNT-treated patients with LD with 13–28 years of treatment duration. (B) Scatter plots depict statistically significant correlations between regional mean percent BOLD signal change and (a) disorder duration in BoNT-naive patients with ADLD and BoNT-treated ABLD, (b) age at disorder onset in BoNT-treated patients with ADLD, (c) symptom severity in BoNT-treated patients with ADLD, and (d) symptom severity in BoNT-naive patients with ABLD and BoNT-treated patients with ABLD. Statistical significance is set at whole-brain voxelwise-corrected $p \leq 0.05$ with minimum cluster size ≥ 214 mm^3 and $R_s \geq \pm 0.40$. ABLD = abductor type LD; ADLD = adductor type LD; BFMDRS = Burke-Fahn-Marsden Dystonia Rating Scale; BoNT = botulinum neurotoxin; LD = laryngeal dystonia.

stimulation in cervical dystonia[18]; partial modulation of sensorimotor hyperactivity in laryngeal, orofacial, and cervical dystonias[9,19-21]; and even normalization of white matter hemispheric asymmetry in cervical dystonia.[22] Another line of research has found that BoNT treatment does not exert neuromodulatory influences on abnormal brain function, with no differences in neural activity before and after injections in patients with blepharospasm, focal hand, and laryngeal dystonias.[7,10,23-25] These opposing findings may, in part, be related to differences in recruited study participants and experimental designs, including clinically heterogeneous cohorts, examination of motor vs nonmotor vs resting conditions, and nonstandardized data acquisition and signal processing protocols. Notably, the major focus of previous studies has been on BoNT central effects at 1–1.5 months postinjection, which captured its immediate neuromodulatory action during peak efficacy but limited our understanding of its short-term and long-term action on disorder pathophysiology (Figure 1).

In this study of patients with LD at the end of their treatment cycle, we demonstrate that BoNT injections have a short-term neuromodulatory effect on left precuneus, whose activity is significantly decreased in BoNT-treated patients compared with patients who received treatment without clinically apparent benefits and to patients who never received injections. Among BoNT-naive patients, precuneus hyperactivity was found to be greater in those with later LD onset but lesser in those with milder symptoms and shorter disease duration, thus capturing individual patient variability associated with their disorder status and pointing to a clinical feature-dependent trait in this cohort.

The role of precuneus in the pathophysiology of dystonia remains elusive. This region has been, by and large, out of focus of studies investigating major basal ganglia-thalamo-sensorimotor and cerebellar impairments in this disorder. However, several recent investigations have noted abnormal precuneus function and structure in LD and other focal dystonias, such as blepharospasm, Meige syndrome, and cervical and musician's dystonias.[26-31] Evidence from these studies suggests that precuneus alterations may play a role in subtle deficits of cognitive function in patients with dystonia, including impaired visuospatial attention,[32,33] temporal discrimination,[29,34] motor imagery, or spatially guided behaviors.[35] It is plausible that restoration of precuneus activity with BoNT treatment, as shown in this study, might have an impact on

improved cognitive function in these patients. In line with this assumption, an earlier behavioral study in patients with blepharospasm has found that alleviation of dystonic symptoms with BoNT injections is associated with the improvement of sustained attention deficits on a time-controlled visual cancellation test.[36] Future neuroimaging studies are warranted to incorporate specific precuneus-driven cognitive paradigms to confirm the relationship between BoNT effectiveness, improved cognitive function, and precuneus activity in dystonia.

Although we did not find the overall group-level effects of BoNT treatment on primary sensorimotor activity, further stratification of our cohort based on LD phenotypes helped reveal additional involvement of cortical and subcortical sensorimotor regions that are conventionally implied in dystonia pathophysiology. Specifically, we found that right thalamic activity is attenuated in ADLD BoNT-treated vs ADLD BoNT-naive patients. The thalamus serves as a relay station of the basal ganglia and cerebellar loops, with thalamic functional and structural changes having widespread effects on dystonic network organization.[37,38] It is notable that thalamic differences were found in its parietal subdivision, pointing again to modulatory effects of BoNT on the parietal circuitry (potentially including precuneus) through thalamo-parietal connections. Furthermore, the negative relationship between thalamic activity and symptom severity in BoNT-treated patients with ADLD suggests greater modulation of this region in more severe patients.

Conversely, ABLD BoNT-treated patients had decreased inferior frontal activity compared with ABLD BoNT-naive patients. Alterations in this region have been previously linked to task specificity of speech impairment in LD[15,39] based on the prominent role of inferior frontal gyrus in speech motor preparation, articulatory timing, pitch, and tone control. However, the positive relationships of inferior frontal activity with symptom severity and disease duration in ABLD BoNT-treated patients indicate that the modulatory treatment effects may be lessened with the symptom progression.

Patients with LD with intermediate BoNT treatment duration of 6–12 years had reduced activity in right cerebellum compared with patients with either shorter (1–5 years) or longer (13–28 years) treatment duration and reduced activity in middle frontal gyrus compared with patients with shorter (1–5 years) treatment duration. These data suggest that patients with LD with intermediate treatment duration likely receive the highest level of neuromodulatory benefits from BoNT injections. Notably, there were no significant differences in brain activity between patients with shorter (1–5 years) and longer (13–28 years) treatment durations, suggesting similar levels of BoNT neuromodulation in these cohorts independent of either disease or treatment duration. Lesser modulatory effects of BoNT in patients with longer treatment duration may also contribute to clinically known diminishing BoNT efficacy over the years and secondary nonresponse.[12]

BoNT modulation of cerebellar activity in patients with LD with intermediate treatment duration aligns well with the involvement of this structure in dystonia pathophysiology.[40] Cerebellar changes are believed to contribute to abnormal sensorimotor integration and maladaptive plasticity in dystonia, partly because of deficient cerebellar output to the basal ganglia, leading to abnormal motocortical excitability.[41] BoNT effects on the cerebellar lobule VI are especially relevant to LD symptomatology given its well-documented role in speech production.[42,43] BoNT-induced attenuation of abnormal cerebellar activity may, thus, result in an indirect reduction of motocortical activity associated with speech symptom alleviation.

The right middle frontal gyrus was another region found to be modulated by BoNT in patients with LD with intermediate treatment duration. Its altered function and structure have been previously linked to abnormally elevated temporal discrimination thresholds, aberrant functional network kernel formation, and disease penetrance in LD.[34,44,45] In other forms of dystonia, increased gray matter volume and decreased functional connectivity of middle frontal gyrus have been reported in blepharospasm,[46,47] its reduced structural connectivity with the basal ganglia and increased activity after positive feedback learning found in writer's cramp,[48,49] and pretreatment homogeneity related to varied response to BoNT injections identified in cervical dystonia.[50] By modulating middle frontal activity, BoNT treatment in these patients may have a cascading effect on the frontoparietal network activity and, hence, the control of higher-order executive function during learning and coordination of the correct sequences of complex motor behaviors, such as speech production.

We acknowledge that our patient stratification approach for the evaluation of long-term central effects of BoNT treatment might have, in part, been arbitrary because of the lack of prior relevant guidelines. Future longitudinal studies of clinical characteristics and brain changes in patients with dystonia are needed for establishing recommendations for their objective stratifications.

Another study limitation may be related to clinically low efficacy of BoNT injections in patients with phenotypically rare ABLD.[3] Reduced availability of BoNT-benefiting patients with ABLD might have affected the power of secondary analysis of short-term central treatment effects and rendered phenotypical stratifications for analysis of long-term BoNT effects statistically not meaningful. Larger, multicenter studies may overcome this limitation in future investigations of central BoNT effects.

Typically, standardized and validated dystonia rating scales, such as BFMDRS, are not used clinically for the evaluation of BoNT efficacy in patients with LD, while other unified outcome measures of dystonic voice symptoms are not yet developed.[11] Most commonly, the clinician's perceptual acoustic evaluation of LD symptoms is used in combination with the patient's reports of injection benefits. Our reliance on this clinically driven definition of LD symptom improvement after BoNT injections may represent a study limitation. On the other hand, this approach

allowed us to incorporate both patient-subjective and clinician-objective evaluations of treatment effects and stratify patients to BoNT benefit/no-benefit groups by their clinical state of symptoms.

In summary, our findings show that left precuneus is the main site of short-term central effects of BoNT in treated and benefitting patients with LD, with an additional involvement of right thalamus in patients with ADLD and left inferior frontal gyrus in patients with ABLD. As for the long-term central effects of BoNT treatment, the prefrontal-cerebellar axis is primarily modulated in patients with intermediate treatment duration of 6–12 years compared with patients with shorter or longer treatment durations. Taken together, these data indicate that, through modulation of regions involved in speech motor sequence planning, coordination, and cognitive function, BoNT treatment has only indirect short-term or long-term neuromodulatory effects on primary sensorimotor regions involved in the output of dystonic speech in patients with LD. Insufficient modulation of activity of these patho-physiologically critical cortical sensorimotor regions may, in turn, contribute to the temporary effectiveness of BoNT treatment in patients with dystonia.

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Appendix (continued)

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