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- and long-term effects of BoNT treatment on brain function in LD patients.

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

Results: LD patients who were treated and benefited from BoNT injections had reduced activity in the left precuneus compared to BoNT-naïve and treatment non-benefiting patients. Additionally, BoNT-treated patients with adductor LD had decreased activity in the right thalamus, whereas BoNT-treated abductor LD patients 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 to 12 years showed reduced activity in the right cerebellum compared to patients with both shorter and longer treatment durations and reduced activity in the right prefrontal cortex compared to patients with shorter treatment duration.

Discussion: Our findings suggest that the left precuneus is the site of short-term BoNT central action in LD patients, 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

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points to indirect action of BoNT treatment on the dystonic sensorimotor network via modulation of speech motor sequence planning and coordination.

**Introduction**

Laryngeal dystonia (LD) is isolated focal task-specific dystonia characterized by involuntary spasms in the laryngeal muscles that occur predominantly during speech production. LD negatively affects the quality of life as patients are limited in their daily and professional activities due to inability to communicate, which leads to lower socio-economic 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 about 90% of adductor LD (ADLD) patients reporting 90% benefits but only 10% of abductor LD (ABLD) patients receiving about 70% benefits.\(^3\) In patients who do respond to the treatment, BoNT benefits are seen for only about 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 post-injection.

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, and 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 LD patients as 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\textsuperscript{5} or transient modulation of pathophysiologically abnormal brain function.

To that end, the current literature\textsuperscript{6} on the central effects of BoNT therapy in LD and other forms of focal dystonia reports inconsistent findings that vary from none\textsuperscript{7,8} to moderate\textsuperscript{9} to substantial\textsuperscript{10} modulation of brain activity at the peak of treatment benefits, i.e., around 1-1.5 months post-injection (Fig. 1, Supplementary Table 1). 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- 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 due to its insufficient modulation of activity in key sensorimotor brain regions involved in the output of dystonic speech in LD patients.

**Materials and 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 neurological conditions (including other forms of dystonia but excluding dystonic tremor of voice in patients) or psychiatric disorders (including anxiety and depression), significant radiological findings on brain MRI, recent history (within one 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 subjects participated in the study, including 111 LD patients (90 females/21 males, age 55.9±12.9 years) and 50 healthy controls (32 females/18 males, age 51.0±10.1 years) (Table 1). LD diagnosis in patients and the absence of laryngeal and other neurological problems in all subjects was confirmed using a combined approach of a case history, laryngeal and neurological examinations, and voice/speech perceptual evaluation\textsuperscript{11}. Dystonic tremor of voice was present in 41% of LD patients as a characteristic feature of this disorder\textsuperscript{11}. The absence of psychiatric history in all subjects was established based on a combination of subject’s reports of the absence of psychiatric problems, no formal diagnosis by a psychiatrist documented in the subject’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, or neurological 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 and ADLD patients who have greater benefits from BoNT injections\textsuperscript{3}. All patients participated in the study when fully symptomatic, at least three months after last injection, to match healthy controls 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.
Primary experimental groups

Subjects were assigned to four groups for examination of different aspects of central response to BoNT treatment (Fig. 2A, Table 1).

(I) Overall brain function in LD patients compared to healthy individuals was assessed as a first step to reproduce previously reported functional alterations in these patients. We selected 57 LD patients (42 females/15 males, age 54.7±13.4 years) from a larger cohort of 111 LD patients to create an age-and sex-balanced design compared to 50 healthy controls (32 females/18 males, age 51.0±10.0 years) (Fig. 2A-a). LD group included patients who were BoNT-naïve, BoNT-treated, BoNT-benefiting, and BoNT-non-benefiting.

(II) Short-term effects on brain function in BoNT-naïve vs. BoNT-treated LD patients were examined in 29 patients who never received BoNT treatment (BoNT-naïve, 21 females/7 males, age 53.9±14.5 years) compared to 28 patients who received at least one BoNT injection (BoNT-treated, 20 females/8 males, age 55.4±12.8 years, 4.9±6.2 treatment years, 17.0±22.7 injections) (Fig. 2A-b).

(III) Short-term effects on brain function in BoNT-benefiting vs. BoNT-non-benefiting LD patients were investigated by further stratifying the BoNT-treated group into 14 patients who reported injection benefits (10 females/4 males, 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 females/4 males, age 55.9±12.0 years, 1.5±0.9 treatment years, 4.1±4.0 injections) (Fig. 2A-c). Among those who did not benefit from BoNT injections, 13 patients were primary non-responders whose symptoms did not improve from the very first injection and all subsequent treatments (3.2±2.2 injections). One
patient was a secondary non-responder who benefited from the first injection but not subsequent 15 injections.

(IV) Long-term effects of BoNT treatment on brain function were examined in 54 LD patients who received BoNT treatment for 1 to 5 years (N=18; 15 females/3 males, age 51.5±12.4 years), 6 to 12 years (N=19; 16 females/3 males, age 59.2±11.3 years), and 13 to 28 years (N=17; all females, age 60.9±11.8 years) (Fig. 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 prior literature relevant to the stratification of dystonia patients for the assessment of long-term treatment effects, our group subdivisions were based on the considerations that LD symptoms may progress within the first two years of disease onset, and BoNT treatment efficacy may not be established during the first injection cycle\textsuperscript{15}. 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 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 ADLD than ABLD patients\textsuperscript{3}, a secondary study examined the short-term central effects of BoNT injections dependent on LD phenotype. Patients were grouped as follows (Fig. 2B, Table 1):

(I) Brain function in BoNT-naïve but phenotypically different LD was examined in 18 ADLD BoNT-naïve patients (15 females/3 males, age 53.9±15.0 years) vs. 11 ABLD BoNT-naïve patients (7 females/4 males, age 54.3±13.5 years) (Fig. 2B-a).
(II) *Brain function in BoNT-treated but phenotypically different LD* was assessed in 15 ADLD BoNT-treated patients (12 female/3 male, age 58.1±14.5 years, 7.2±7.5 treatment years, 25.5±27.7 injections) vs. 13 ABLD BoNT-treated patients (8 females/5 males, age 52.4±10.1 years, 2.2±2.4 treatment years, 7.2±8.6 injections) (Fig. 2B-b).

(III) *Brain function between BoNT-naïve and treated but phenotypically same ADLD* was compared in 18 BoNT-naïve ADLD patients (15 females/3 males, age 53.9±15.0 years) vs. 15 BoNT-treated ADLD patients (12 females/3 males, age 58.1±14.5 years, 7.2±7.5 treatment years, 25.5±27.7 injections) (Fig. 2B-c).

(IV) *Brain function between BoNT-naïve and BoNT-treated but phenotypically same ABLD* was examined in 11 BoNT-naïve ABLD patients (7 females/4 males, age 54.3±13.5 years) vs. 13 BoNT-treated ABLD patients (8 females/5 males, age 52.4±10.1 years, 2.2±2.4 treatment years, 7.2±8.6 injections) (Fig. 2B-d).

Because of limited BoNT benefits in ABLD patients\(^3\), 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 3.0T Philips MRI scanner equipped with an 8-channel head coil. The subject’s head was tightly cushioned inside the coil to reduce movements. All subjects were trained on the fMRI study design and instructed to restrict movements during scanning. Subjects were monitored through a two-way audio communication system during scanning to ensure correct task performance.

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 (TR 2s per volume, 10.6s between volumes, TE 30ms, FA 90°, FOV 240mm,
voxel size 3.5×3.5mm, 4-mm slice thickness, 36 slices) to minimize motion artifacts due to orofacial movements during speech production and to neutralize the scanner noise interference with acoustic stimulus presentation. The experimental task included eight LD symptom-eliciting English sentences and a resting condition as a baseline. Following acoustic presentation (3.6s) of a sample sentence via the MR-compatible headphones, subjects were cued with an arrow to produce the sentence (5s) and then remain silent for volume acquisition (2s). Task and resting conditions were pseudorandomized within and between functional runs and subjects. Each subject completed four functional runs, including a total of 24 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 (3D-MPRAGE: TR 7.5ms, TE 3.4ms, FA 8°, FOV 210mm, 1-mm slice thickness, 172 slices).

MRI data analysis

Images were analyzed using the standard afni_proc.py pipeline in AFNI software. The first two 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; three 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 two-sample \( t \)-tests or chi-square tests were used, as appropriate, to examine between-group differences in subject demographics (age, sex) and LD clinical characteristics (LD duration, age of 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’s rank correlation coefficients at whole-brain voxelwise-corrected \( p \leq 0.05 \) with minimum cluster size \( \geq 214 \text{ mm}^3 \) and \( R_s \geq \pm 0.4 \).

**Primary analysis** included statistical comparisons between groups as outlined in the Primary experimental groups section (Fig. 2A, Table 1). One-way analysis of variance (ANOVA) examined differences between LD patients and healthy controls. Two separate two-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-naïve and BoNT-treated patients and between BoNT-benefiting and BoNT-non-benefiting LD patients. 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 two-way ANCOVA with three factors defined by the number of years patient received and benefited from BoNT treatment. Sex differences in between-group comparisons of 13-28 years 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 years 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 $\geq 858 \text{ mm}^3$.

Secondary analysis examined clinically distinct benefits of BoNT treatment in different LD phenotypes as outlined in the Secondary experimental groups section (Fig. 2B, Table 1). In all comparisons, between-group statistically significant differences were determined using two-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 $\geq 858 \text{ mm}^3$.

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 subjects before study participation. Data from some subjects 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 years vs. 13-28 years of treatment at $p = 0.002$), age of onset (all $p \geq 0.48$), or symptom severity (all $p \geq 0.09$).

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

Short-term central effects of BoNT treatment

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

Further examination of treatment effects in phenotypically stratified cohorts showed that BoNT-naïve ABLD patients had increased activity in left inferior parietal cortex compared to BoNT-naïve ADLD patients (Fig. 3B-I, Table 2), whereas BoNT-treated ABLD patients had increased activity in right cerebellum (lobule VI) compared to BoNT-treated ADLD patients (Fig. 3B-II, Table 2). Comparisons of treatment effects within the same phenotype showed that
BoNT-naïve ADLD patients had increased activity in right thalamus (parietal subdivision) compared to BoNT-treated ADLD patients (Fig. 3B-III, Table 2), whereas BoNT-naïve ABLD patients had greater activity in left inferior frontal gyrus compared to BoNT-treated ABLD patients (Fig. 3B-IV, Table 2). BoNT-naïve ADLD patients showed a negative correlation between right thalamic activity and disease duration ($R_s = -0.59, p = 0.01$; Fig. 4B-I). Conversely, BoNT-treated ADLD patients showed negative correlations between right cerebellar activity and disease onset ($R_s = -0.65, p = 0.009$; Fig. 4B-II) and between right thalamic activity and symptom severity ($R_s = -0.79, p = 0.0005$; Fig. 4B-III). On the other hand, BoNT-naïve ABLD patients had a negative correlation between activity of left inferior parietal cortex and symptom severity ($R_s = -0.87, p = 0.001$; Fig. 4B-IV), whereas BoNT-treated ABLD patients established positive correlations between activity of left inferior frontal gyrus and disease duration ($R_s = 0.71, p = 0.007$; Fig. 4B-IV) as well as symptom severity ($R = 0.75, p = 0.003$; Fig. 4B-IV).

*Long-term central effects of BoNT treatment*

LD patients receiving BoNT injections for 1 to 5 years had increased activity in right middle frontal gyrus and cerebellum (Crus 1) compared to patients receiving treatment for 6 to 12 years (Fig. 3A-IV, Table 2). LD patients with 13 to 28 years of treatment had increased activity in right cerebellum (lobule VI) compared to patients receiving BoNT injections for 6 to 12 years. This cerebellar activity was positively correlated with symptom severity ($R_s = 0.59, p = 0.01$; Fig. 4A-IV) in patients with 13-28 years of BoNT treatment. There were no statistically significant differences between LD patients 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 on pathophysiologically abnormal brain activity. Our findings of brain functional alterations in LD patients compared to healthy individuals are in line with the previous literature\textsuperscript{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\textsuperscript{16, 17}, temporary elimination of the facilitatory effect of paired associative stimulation in cervical dystonia\textsuperscript{18}, partial modulation of sensorimotor hyperactivity in laryngeal, orofacial and cervical dystonias\textsuperscript{9, 10, 19-21}, and even normalization of white matter hemispheric asymmetry in cervical dystonia\textsuperscript{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\textsuperscript{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. non-motor vs. resting conditions, and non-standardized data acquisition and signal processing protocols. Notably, the major focus of previous studies has been on BoNT central effects at 1-1.5 months post-injection, which captured its immediate neuromodulatory action during peak efficacy but limited our understanding of its short- and long-term action on disorder pathophysiology (Fig. 1).
Short-term central effects of BoNT treatment

In this study of LD patients 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 to patients who received treatment without clinically apparent benefits and to patients who never received injections. Among BoNT-naïve 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 as 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, cervical and musician’s dystonias. Evidence from these studies suggests that precuneus alterations may play a role in subtle deficits of cognitive function in dystonia patients, including impaired visuospatial attention, temporal discrimination, motor imagery or spatially guided behaviors. It is plausible that restoration of precuneus activity with BoNT treatment, as shown in the current 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. 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-naïve 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. 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) via thalamo-parietal connections. Furthermore, the negative relationship between thalamic activity and symptom severity in BoNT-treated ADLD patients suggests greater modulation of this region in more severe patients.

Conversely, ABLD BoNT-treated patients had decreased inferior frontal activity compared to ABLD BoNT-naïve patients. Alterations in this region have been previously linked to task-specificity of speech impairment in LD 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.

**Long-term central effects of BoNT treatment**

LD patients with intermediate BoNT treatment duration of 6-12 years had reduced activity in right cerebellum compared to patients with either shorter (1-5 years) or longer (13-28 years) treatment duration, as well as reduced activity in middle frontal gyrus compared to patients with...
shorter (1-5 years) treatment duration. These data suggest that LD patients 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 non-
response\textsuperscript{12}.

BoNT modulation of cerebellar activity in LD patients with intermediate treatment duration
aligns well with the involvement of this structure in dystonia pathophysiology\textsuperscript{40}. Cerebellar
changes are thought to contribute to abnormal sensorimotor integration and maladaptive
plasticity in dystonic patients, partly due to deficient cerebellar output to the basal ganglia,
leading to abnormal motocortical excitability\textsuperscript{41}. BoNT effects on the cerebellar lobule VI are
especially relevant to LD symptomatology given its well-documented role in speech
production\textsuperscript{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 LD
patients 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\textsuperscript{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\textsuperscript{46,47}, its reduced structural connectivity with the basal ganglia
and increased activity following positive feedback learning found in writer’s cramp\textsuperscript{48,49}, and pre-
treatment homogeneity related to varied response to BoNT injections identified in cervical
dystonia\textsuperscript{50}. By modulating middle frontal activity, BoNT treatment in these patients may have cascading effects on 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.

\textit{Potential methodological limitations}

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

Another study limitation is pertinent to clinically low efficacy of BoNT injections in phenotypically rare ABLD patients\textsuperscript{3}. Reduced availability of BoNT-benefiting ABLD patients might have impacted 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 LD patients, while other unified outcome measures of dystonic voice symptoms are not yet developed\textsuperscript{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 improvements following 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.

Conclusions

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

References


# Tables

## Table 1. Patient demographics and clinical characteristics

<table>
<thead>
<tr>
<th>LD patient cohorts for assessment of short-term BoNT central effects</th>
<th>BoNT naïve (n = 29)</th>
<th>BoNT treated (n = 28)</th>
<th>BoNT benefit (n = 14)</th>
<th>BoNT no-benefit (n = 14)</th>
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<tbody>
<tr>
<td><strong>Age (yrs; mean ± SD)</strong></td>
<td>54.1±14.2</td>
<td>55.4±12.8</td>
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<td>55.9 ± 12.0</td>
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<tr>
<td><strong>Sex (Female:Male)</strong></td>
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<td>20:8</td>
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<td>10:4</td>
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<td><strong>Handedness</strong></td>
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<td>Right (Edinburgh Inventory)</td>
<td>Right (Edinburgh Inventory)</td>
<td>Right (Edinburgh Inventory)</td>
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<td>Monolingual Native English</td>
<td>Monolingual Native English</td>
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<tr>
<td><strong>Cognitive status</strong></td>
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<td>MMSE ≥ 27 points or MoCA ≥ 26 points</td>
<td>MMSE ≥ 27 points or MoCA ≥ 26 points</td>
<td>MMSE ≥ 27 points or MoCA ≥ 26 points</td>
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</table>

<table>
<thead>
<tr>
<th>LD patient cohorts for assessment of long-term BoNT central effects</th>
<th>BoNT benefit 1-5 yrs (n = 18)</th>
<th>BoNT benefit 6-12 yrs (n = 19)</th>
<th>BoNT benefit 13-28 yrs (n = 17)</th>
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<tbody>
<tr>
<td><strong>Age (yrs; mean ± SD)</strong></td>
<td>51.5±12.4</td>
<td>59.2±11.3</td>
<td>60.9±11.9</td>
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<td><strong>Sex (Female:Male)</strong></td>
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<td>16:3</td>
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<td>Right (Edinburgh Inventory)</td>
<td>Right (Edinburgh Inventory)</td>
<td>Right (Edinburgh Inventory)</td>
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<td>Monolingual Native English</td>
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<tr>
<td><strong>Cognitive status</strong></td>
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<p>| Patient stratification by LD phenotype for assessment of BoNT central effects |</p>
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<thead>
<tr>
<th>BoNT-naïve ADLD (n = 13)</th>
<th>BoNT-naïve ABLD (n = 13)</th>
<th>BoNT-treated ADLD (n = 13)</th>
<th>BoNT-treated ABLD (n = 13)</th>
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<tbody>
<tr>
<td><strong>Age (yrs; mean ± SD)</strong></td>
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<td></td>
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<td><strong>Sex (Female:Male)</strong></td>
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<tr>
<td><strong>Handedness</strong></td>
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<tr>
<td><strong>Language</strong></td>
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<tr>
<td><strong>Cognitive status</strong></td>
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<table>
<thead>
<tr>
<th>Abbreviations: ABLD – abductor LD; ADLD – adductor LD; BoNT – botulinum toxin; HC – healthy controls; LD – laryngeal dystonia; DTv – dystonic tremor of voice; MMSE – Mini-Mental State Examination; MoCA – Montreal Cognitive Assessment; n/a – non-applicable; SD – standard deviation; yrs – years, BFMDRS – Burke-Fahn-Marsden Dystonia Rating Scale. MMSE and MoCA were adjusted by age and education.</th>
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<tbody>
<tr>
<td><strong>Age</strong> (yrs; mean ± SD)</td>
<td>53.9±15.0</td>
<td>54.3±13.5</td>
<td>58.1±14.5</td>
<td>52.4±10.1</td>
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<tr>
<td><strong>Sex (Female:Male)</strong></td>
<td>15:3</td>
<td>7:4</td>
<td>12:3</td>
<td>8:5</td>
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<tr>
<td><strong>Handedness</strong></td>
<td>Right (Edinburgh Inventory)</td>
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<td></td>
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</tr>
<tr>
<td><strong>Language</strong></td>
<td>Monolingual Native English</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Cognitive status</strong></td>
<td>MMSE ≥ 27 points or MoCA ≥ 26 points</td>
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<tr>
<td><strong>Dystonia duration</strong> (yrs; mean ± SD)</td>
<td>11.6±12.7</td>
<td>15.8±13.6</td>
<td>15.4±12.2</td>
<td>14.2±9.3</td>
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<tr>
<td><strong>Symptom severity (BFMDRS)</strong></td>
<td>4.9±3.6</td>
<td>4.0±2.0</td>
<td>5.2±3.5</td>
<td>5.8±3.7</td>
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<tr>
<td><strong>Dystonia onset</strong> (yrs; mean ± SD)</td>
<td>42.3±17.0</td>
<td>38.5±17.4</td>
<td>42.0±15.4</td>
<td>38.3±11.7</td>
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<tr>
<td><strong>Number of BoNT injections</strong> (mean ± SD)</td>
<td>n/a</td>
<td>n/a</td>
<td>25.5±27.7</td>
<td>7.2±8.6</td>
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<tr>
<td><strong>Duration of BoNT treatment</strong> (yrs; mean ± SD)</td>
<td>n/a</td>
<td>n/a</td>
<td>7.2±7.5</td>
<td>2.2±2.4</td>
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<tr>
<td><strong>Duration of BoNT treatment cycle</strong> (months; mean ± SD)</td>
<td>n/a</td>
<td>n/a</td>
<td>3.9±2.6</td>
<td>3.3±0.8</td>
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Table 2. Differences in brain activity during symptomatic speech production

<table>
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<tr>
<th>Anatomical Regions</th>
<th>Cluster peak coordinates (x,y,z)</th>
<th>Cluster size (mm³)</th>
<th>Cluster peak t-level</th>
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<tr>
<td><strong>Laryngeal dystonia &gt; Healthy controls</strong></td>
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<tr>
<td>R primary sensorimotor cortex, extending to auditory cortex</td>
<td>47, -11, 34</td>
<td>9,818</td>
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<td>L primary sensorimotor cortex</td>
<td>-51, -11, 38</td>
<td>4,244</td>
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<tr>
<td>L cerebellum (lobule VI)</td>
<td>-16, -57, -18</td>
<td>2,443</td>
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<tr>
<td>R cerebellum (lobule VI)</td>
<td>12, -57, -18</td>
<td>2,229</td>
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<tr>
<td>L inferior frontal gyrus</td>
<td>-47, -1, 6</td>
<td>1,501</td>
<td>5.6</td>
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<tr>
<td>L auditory cortex</td>
<td>-33, -32, 13</td>
<td>1,157</td>
<td>6.9</td>
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<tr>
<td>L thalamus</td>
<td>-12, -18, 6</td>
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<td>6.9</td>
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<tr>
<td>L insula</td>
<td>-33, 10, 10</td>
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<td>6.4</td>
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<td>L lentiform nucleus</td>
<td>-23, -8, -1</td>
<td>385</td>
<td>6.5</td>
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<tr>
<td><strong>Laryngeal dystonia &lt; Healthy controls</strong></td>
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<tr>
<td>R inferior parietal cortex</td>
<td>47, -60, 20</td>
<td>343</td>
<td>6.5</td>
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</tbody>
</table>

*Short-term central effects of BoNT treatment*

| LD BoNT-naïve > LD BoNT-treated | | | |
| L superior parietal lobule (precuneus) | 5, -46, 41 | 1,757 | 3.9 |
| **LD BoNT benefit < LD BoNT no-benefit** | | | |
| L superior parietal lobule (precuneus) | -9, -60, 20 | 1,029 | 3.9 |
| **BoNT-naïve ADLD < BoNT-naïve 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-naïve ADLD > BoNT-treated ADLD** | | | |
| R thalamus | 16, -22, -1 | 1,200 | 4.1 |
| **BoNT-naïve 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 to 5 yrs > LD BoNT benefit 6 to 12 yrs | | | |
| 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 to 28 yrs > LD BoNT benefit 6 to 12 yrs** | | | |
| 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; yrs – years.
Figure Legends

Fig. 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). The majority of studies have recruited patients around the peak efficacy of BoNT injections at 1-1.5 months post-treatment, whereas 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 LD patients 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. **Abbreviations:** BoNT – botulinum neurotoxin; LD – laryngeal dystonia.
Fig. 2. Overview of patient stratification depicts (A) primary experimental groups and (B) secondary experimental groups used for statistical comparisons. Abbreviations: ABLD – abductor LD; ADLD – adductor LD; BoNT – botulinum neurotoxin; LD – laryngeal dystonia; yrs - years.

Fig. 3. Short- and long-term central effects of BoNT treatment in LD patients.

(A) Statistically significant differences in brain activity during speech production are shown between (a) an aggregate group of LD patients and healthy controls [FWE-corrected $p \leq 0.05$ with minimum voxelwise threshold $p \leq 0.00001$ and cluster size $\geq 343 \text{ mm}^3$]; (b) BoNT-naïve and BoNT-treated LD patients [FWE-corrected $p \leq 0.05$ with minimum voxelwise threshold $p \leq 0.01$ and cluster size $\geq 1,715 \text{ mm}^3$]; (c) LD patients with and without benefits from BoNT treatment [whole-brain voxelwise-corrected $p \leq 0.01$ with a minimum cluster size of $\geq 858 \text{ mm}^3$], 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 \( \geq 858 \text{ mm}^3 \)]. (B) In phenotypically stratified groups, statistically significant differences in brain activity during speech production are shown between (I) BoNT-naïve ADLD and ABLD patients; (II) BoNT-treated ADLD and ABLD patients; (III) BoNT-naïve and treated ADLD patients, and (IV) BoNT-naïve and treated ABLD patients. Statistical significance is set at whole-brain voxelwise-corrected \( p \leq 0.01 \) with minimum cluster size \( \geq 858 \text{ mm}^3 \). Brain slices are shown in the AFNI standard Talairach-Tournoux space. Color bars represent the \( t \)-scores. Abbreviations: ABLD – abductor type LD; ADLD – adductor type LD; BFMDRS – Burke-Fahn-Marsden Dystonia Rating Scale; BoNT – botulinum neurotoxin; LD – laryngeal dystonia.

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Fig. 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 LD patients; (b) duration and age of disorder onset in BoNT-naïve LD patients, (c) symptom severity in BoNT-naïve LD patients, and (d) symptom severity in BoNT-treated LD patients with 13-28 year of treatment duration. (B) Scatter plots depict statistically significant correlations between regional mean percent BOLD signal change and (a) disorder duration in BoNT-naïve ADLD patients and BoNT-treated ABLD; (b) age of disorder onset in BoNT-treated ADLD patients, (c) symptom severity in BoNT-treated ADLD patients, and (d) symptom severity in BoNT-naive ABLD patients and BoNT-treated ABLD patients. Statistical significance is set at whole-brain voxelwise-corrected \( p \leq 0.05 \) with minimum cluster size \( \geq 214 \text{ mm}^3 \) and \( R_s \geq \pm 0.40 \). Abbreviations: ABLD – abductor type LD; ADLD – adductor type LD; BFMDRS – Burke-Fahn-Marsden Dystonia Rating Scale; BoNT – botulinum neurotoxin; LD – laryngeal dystonia.
Short and Long-Term Central Action of Botulinum Neurotoxin Treatment in Laryngeal Dystonia

Lena Carolyn O'Flynn and Kristina Simonyan

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