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White matter abnormalities in dystonia normalize after botulinum toxin treatment

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Abstract

The pathophysiology of dystonia is still poorly understood. We used diffusion tensor imaging to screen for white matter abnormalities in regions between the basal ganglia and the thalamus in cervical and hand dystonia patients. All patients exhibited an abnormal hemispheric asymmetry in a focal region between the pallidum and the thalamus. This asymmetry was absent 4 weeks after the same patients were treated with intramuscular botulinum toxin injections. These findings represent a new systems-level abnormality in dystonia, which may lead to new insights about the pathophysiology of movement disorders. More generally, these findings demonstrate central nervous system changes following peripheral reductions in muscle activity. This raises the possibility that we have observed activity-dependent white matter plasticity in the adult human brain.

Keywords

basal ganglia; botulinum toxin; diffusion imaging; dystonia; plasticity; white matter

Introduction

It is often challenging to identify disease mechanisms in neurological and psychiatric disorders with no known structural brain pathology. Diffusion tensor imaging (DTI) can now be used to detect abnormalities in brain microstructural properties, such as neural connectivity and axon morphology [1], in such disorders.

Primary focal dystonias are not associated with any known structural brain pathology. Although abnormal pallidal output to the thalamus has long been hypothesized as a putative

mechanism for primary dystonia [2,3], we still know very little about the functional and / or structural characteristics of this abnormality. We hypothesized that if such output were abnormal, we might observe abnormal white matter microstructure in one or more of these pathways. To address this, we used DTI in patients with either primary cervical or primary hand dystonias to screen for microstructural abnormalities in basal ganglia input and output fiber pathways. Two previous studies have used DTI to evaluate brain microstructure in primary dystonias [4,5] but neither applied analysis methods that were sensitive to abnormalities in the small fiber bundles we wished to evaluate. In the present study, we used a region-of-interest (ROI) analysis designed to detect abnormalities in the small fiber bundles that project to and from the putamen and pallidum.

In order to determine whether white matter abnormalities in patients reflected hard-wired abnormalities versus activity-dependent alterations in these pathways, we acquired DTI images before and after the cervical dystonia patients were treated with botulinum toxin (BTX). BTX blocks release of acetylcholine at the motor neuron terminal, and thus acts peripherally to reduce symptoms of dystonia without crossing the blood–brain barrier [6]. BTX has been hypothesized to exert additional therapeutic effects by indirectly altering motor afferent feedback to the brain and thereby altering brain activity [7,8]. Empirical evidence for this has been shown in a study in which cortical (gray matter) functional mapping abnormalities known to exist in focal dystonias [9,10] reversed during BTX treatment [11]. In the present study, we sought to determine whether BTX exerts beneficial effects in subcortical motor circuitry, and whether such effects manifest in the white matter of this circuitry. More generally, this approach allowed us to test whether white matter exhibits the capacity for ‘experience-dependent’ plasticity, analogous to well-established gray matter plasticity observed after peripheral deafferentation [12–14].

Methods

Subjects

Six patients diagnosed with primary focal dystonias (Table 1) and six age, gender, and handedness-matched healthy control participants took part in this study after giving informed consent. Four patients presented with cervical dystonia and two with hand dystonia. Cervical patients were selected as patients for whom BTX was known to be effective; all four had experienced symptom improvement with previous sets of injections, with symptoms returning at the end of each treatment. Hand dystonia patient 1 had never received BTX injections, and hand dystonia patient 2 had received only one, unsuccessful set of injections 2 years prior to this study. No patients were on oral medications for their dystonia, with the exception of one of the hand patients (hand 2) who was taking pergolide. Handedness was assessed by self-report, including hand used for writing. Potential participants were excluded if reported handedness was not decisive. This study was conducted in accordance with the Institutional Review Board of the Massachusetts General Hospital.

Scanning protocol

In each scanning session, a high-resolution (2 mm isotropic) whole head DTI scan was acquired on a Siemens 3.0 Tesla Allegra Magnet System (Siemens AG, Medical Solutions, Erlangen, Germany); repetition time (TR)=24 s; echo time (TE)=81 ms; slice thickness=2 mm, 60 slices total, 128×128 matrix, 256×256 mm field of view (FOV), six averages, six noncolinear directions with b -value=700 s / mm², and one image with b -value=0 s / mm². DTI scans were acquired in each participant using auto-align software [15] to normalize brain image slice orientation between participants and scanning sessions.

Hand dystonia patients were scanned once. Cervical dystonia patients were scanned twice, once just before a set of BTX injections ('pre-BTX') and once 4 weeks after these injections ('post-BTX'). All pre-BTX scans took place at least 3 months after any previous injections. In the current study, the pre-BTX scan took place first for three patients, whereas the post-BTX scan took place first for the fourth patient (cervical 4). Dystonic symptoms improved in all four patients in response to the BTX injections (Table 1).

Four of the control subjects returned for a second scanning session 7–19 months after their first scanning session, as a test–retest control for normal fluctuations in white matter microstructure over time. In the second scanning session, the control subjects were placed in the scanner with head positions matching the head positions of the four cervical dystonia patients in pre-BTX scans.

Data analysis

DTI images were first preprocessed as previously described [16]. We then used a region of interest (ROI) analysis to evaluate white matter microstructure, as measured by fractional anisotropy, incrementally along the region where white matter fibers run between the putamen / pallidum and the thalamus [17] (Fig. 2d). ROIs were drawn on each participant's individual fractional anisotropy map, in native space. ROIs were drawn in each hemisphere as single voxel-wide lines down the center of the posterior limb of the internal capsule and just rostroventral to this region, at two millimeter slice intervals (20 ROIs per hemisphere) in coronal orientation. We calculated average anisotropy within each ROI for each participant. We hypothesized that we would see abnormal anisotropy in one or more of these ROIs in dystonia patients.

Because we observed a large left/right hemispheric asymmetry in individual patients, we took this into account in our analyses. We first screened for patient / control differences in individual asymmetry values (left minus right fractional anisotropy) in each of the 20 ROIs in all participants, using Mann–Whitney tests owing to the small sample size and a Bonferroni correction for multiple comparisons. We then used a three-factor repeated-measures analysis of variance to evaluate the effects of hemisphere (left versus right), treatment condition (pre-BTX versus post-BTX), and ROI number (for the ROIs that exhibited patient / control differences) on fractional anisotropy in cervical dystonia patients. This same analysis was conducted for the test–retest control participants using scanning session (first versus second) as the repeated measures factor.

Results

We observed a white matter hemispheric asymmetry in patients pre-BTX, which was not observed in patients post-BTX or in controls in either scanning session.

Specifically, we observed significantly different hemispheric asymmetries in patients pre-BTX versus controls in the three most rostral ROIs (ROI 18: $P < 0.05$, corrected; ROIs 19 and 20: $P < 0.01$, corrected). The average left / right anisotropy difference across the three rostral ROIs was -0.155 ($SE=0.0500$) for patients, and $+0.0631$ ($SE=0.0223$) for controls (Fig. 1a). All individual patients exhibited the abnormal asymmetry (Fig. 2a and c; Table 1).

The analysis of variance showed a significant interaction of the effects of hemisphere and treatment condition on fractional anisotropy in the three most rostral ROIs in cervical dystonia patients ($F=20.854$; $P=0.0000657$; Fig. 1b). Fractional anisotropy normalized toward control values in all cervical patients post-BTX (Fig. 2b; Table 1). Control subjects showed no effect of hemisphere ($F=0.0060$; $P=0.9385$), scanning session ($F=0.9872$;

$P=0.3277$), or interaction of hemisphere and scanning session ($F=0.3323$; $P=0.5682$) on fractional anisotropy in these ROIs (Fig. 1b and c).

Finally, we verified that there were no significant correlations between hemispheric asymmetry values and head position (magnitude and direction of head tilt or rotation, and position change pre / post-BTX) during scanning (data not shown). There were also no significant differences in head motion between groups or conditions.

Discussion

We have detected a white matter microstructural abnormality in dystonia patients, which was observed before, but not after, BTX treatment. More generally, the rapid changes in white matter microstructure observed in patients provide preliminary evidence for activity-dependent brain white matter plasticity in adult humans.

Why might BTX influence basal ganglia output? BTX has been hypothesized to exert some of its effects centrally through its indirect effect on motor afferent feedback to brain motor regions, including the thalamus and sensorimotor cortex [7,8]. Since the thalamus and sensorimotor cortex project back to the basal ganglia [17], it would not be surprising if BTX-induced changes in motor afferent feedback alter activity in the basal ganglia as well.

Why might abnormalities and changes in neural activity be associated with abnormalities and changes in white matter microstructure? Little is known about whether white matter exhibits activity-dependent plasticity in adults, in contrast with well-established gray matter plasticity [12–14]. Our findings suggest that the white matter asymmetry observed in dystonia patients before treatment may have reflected activity-dependent microstructural changes in the projection fibers of neurons exhibiting abnormal activity. The microstructural abnormalities in these projections may have then decreased after abnormal activity was tempered by treatment. Because the asymmetries here were observed in patients after earlier BTX injections had lost clinical effectiveness, it is unlikely that the white matter normalization observed after treatment was permanent.

DTI is sensitive to several microstructural characteristics of white matter [1] that could potentially be modulated by neural activity. These include fiber density changes owing to collateral sprouting [18], myelination changes, activity-dependent changes in fast axonal transport [19], and activity-dependent changes in axonal microstructure, such as cell swelling or beading [20]. Because white matter asymmetries normalized after treatment, it is unlikely that the etiology is degenerative.

We hypothesized in this study that there would be abnormalities in the fibers of basal ganglia input or output pathways in dystonia patients, since abnormal basal ganglia activity has been observed in both primary [21] and secondary [22] dystonias. The basal ganglia pathway which would best spatially localize with the white matter asymmetries we detected would be the ansa lenticularis, which is a distinct bundle that projects from the pallidum to the thalamus just rostromedial to the posterior limb of the internal capsule [17,23]. Because there are several other fiber tracts that run near this region, however, it is also possible that the asymmetry reflects abnormalities in tracts such as the anterior commissure, the inferior thalamic peduncle, the medial forebrain bundle, or the amygdalofugal tract.

What is the relevance of hemispheric asymmetries to dystonia and why was the asymmetry observed in the same direction in all patients? A recent study of dopamine receptor pharmacology in adult rats has raised the possibility that the left and right striata upregulate D2-like receptors asymmetrically (right more than left) in response to reduced dopaminergic input [24]. Since dopamine function is known to be altered in several forms of dystonia [25],

similar hemispheric asymmetries in receptor density / function might develop in the putamen in some or many forms of dystonia. Given that putamen receptor function influences pallidal activity and that neural activity may influence white matter microstructure, it is conceivable that receptor asymmetries in the putamen could promote development of microstructural asymmetries in pallidal output fibers. If BTX treatment reduces the level of abnormal activity in these fibers, there may be a concomitant reduction in microstructural asymmetries.

Conclusion

We have used DTI to detect subcortical white matter abnormalities in patients with primary dystonias. These abnormalities were not observed 4 weeks after peripheral treatment of dystonic symptoms. These findings provide evidence for a new systems-level abnormality in primary dystonia and indicate new directions to pursue to advance our understanding of this disorder. More generally, these findings raise the possibility that white matter in the adult brain exhibits the capacity for activity-dependent plasticity. Further studies are required to investigate the etiology of the asymmetry and the mechanism for its attenuation with treatment.

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Fig. 1.

Group and condition averages of hemispheric asymmetry values [left minus right fractional anisotropy (FA)] for patients versus controls. Asymmetry values were averaged across the three most rostral ROIs in each participant and then averaged for each group/condition of interest. (a) Average asymmetry values for all patients pre-botulinum toxin (BTX) (left red bar; CD=cervical dystonia; HdD=hand dystonia) versus average asymmetry values for all controls in their first scanning session (right blue bar). (b) Average asymmetry values for cervical dystonia patients pre-BTX (left red bar), cervical dystonia patients post-BTX (middle red bar), and matched control subjects (right blue bar). (c) Average asymmetry values for the four test–retest controls in their first scanning session (left blue bar) and second scanning session (right blue bar). Error bars indicate standard error of the mean for each group/condition average.

Fig. 2.

Graphs depicting left hemisphere (red) and right hemisphere (orange) fractional anisotropy (FA) for each of the six individual patients. FA is shown for each patient across each of the 20 ROIs (plotted by ROI number/slice position, caudal to rostral) and is presented as the difference from average control fractional anisotropy for the same ROI/hemisphere. Differences were calculated using values from the first control scanning session in all graphs to accurately represent patient changes between sessions. (a) Left versus right fractional anisotropy for patients pre-BTX. (b) Left versus right fractional anisotropy for cervical dystonia patients post-BTX. (c) Axial slice through a T1-weighted structural magnetic resonance image, with yellow lines marking the caudal (ROI 1) to rostral (ROI 20) range of ROIs for which fractional anisotropy measurements are graphed in (a) and (b) (L, left hemisphere; R, right hemisphere). Red arrows indicate the path of the ansa lenticularis. The green box on the smaller image on the left indicates the field-of-view for the image on the right.

Table 1

Dystonia patient information and fractional anisotropy asymmetry values

Patient	Affected regions	Side affected	Age/handedness/gender	Severity pre-BTX/ post-BTX ^b	Asymmetry values ^c (avg for ROIs 18–20) pre-BTX/post-BTX
Cervical 1	Neck only, with dystonic tremor	Both ^a , with left head tilt	55/left/F (ctrl:52/left/F)	BFM: 6/4; Tsui: 8/4	−0.0484/+0.0383 (ctrl: +0.0381 ^d)
Cervical 2	Neck only	Both ^a , with right head tilt	54/right/M (ctrl:56/right/M)	BFM: 6/4; Tsui: 10/5	−0.0746/+0.0686 (ctrl: +0.0108 ^d)
Cervical 3	Neck, trunk, craniofacial	Both ^a , with left head tilt, right craniofacial	35/right/F (ctrl: 34/right/F)	BFM: 18/8; Tsui: 4/2	−0.162/−0.0442 (ctrl: +0.0830 ^d)
Cervical 4	Neck only, with dystonic tremor	Both ^a , with left head tilt	57/right/F (ctrl: 59/right/F)	BFM: 4/2; Tsui: 7/2	−0.228/+ 0.0504 (ctrl: + 0.00695 ^d)
Hand 1	Hand only	Right hand	34/right/M (ctrl: 31/right/M)	BFM: 3	−0.0554 (ctrl: +0.148 ^d)
Hand 2	Hand, leg	Right hand, left leg	59/right/M (ctrl: 57/right/M)	BFM: 20	−0.360 (ctrl: +0.0912 ^d)

^a Cervical dystonia typically involves muscles on both sides of the body. There is usually some degree of head tilt and head rotation; head tilt is usually caused by dystonic activity in the levator scapulae and/or splenius muscles ipsilateral to the direction of tilt, while head rotation is usually caused by dystonic activity in the sternocleidomastoid muscle contralateral to the direction of rotation and the splenius muscle ipsilateral to the direction of rotation.

^b We have included scores from the Burke Fahn Marsden (BFM) and Tsui torticollis dystonia severity scales (for each scanning session) since the BFM scale, which assesses dystonia across the body, allows relative comparisons of hand and cervical dystonia patients, whereas the Tsui scale gives more precise information regarding cervical dystonia symptoms specifically.

^c Left minus right fractional anisotropy.

^d Asymmetry value for matched control, first scanning session.