Neurobiology of Disease 73 (2015) 327–333

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

# Dynamic cortical gray matter volume changes after botulinum toxin in cervical dystonia

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ARTICLE INFO

*Article history:*
Received 15 May 2014
Revised 15 September 2014
Accepted 20 October 2014
Available online 28 October 2014

*Keywords:*
Cervical dystonia
Motor planning
Botulinum toxin
Structural plasticity
Spatial computation

ABSTRACT

Previous electrophysiological and functional imaging studies in focal dystonia have reported on cerebral reorganization after botulinum toxin (BoNT) injections. With the exception of microstructural changes, alterations in gray matter volume after BoNT have not been explored. In this study, we sought to determine whether BoNT influences gray matter volume in a group of cervical dystonia (CD) patients.

We analyzed whole brain gray matter volume in a sample of CD patients with VBM analysis. In patients, scans were repeated immediately before and some weeks after BoNT injections; controls were only scanned once. We analyzed 1) BoNT-related gray matter volume changes within patients; 2) gray matter volume differences between patients and controls; and 3) correlations between gray matter volume and disease duration and disease severity.

The pre- and post-BoNT treatment analysis revealed an *increase* of gray matter volume within the right precentral sulcus, at the lateral border of the premotor cortex. In comparison to healthy controls, CD patients had *reduced* gray matter volume in area 45 functionally corresponding to the left ventral premotor cortex. No gray matter volume increase was found for CD patients in comparison to controls. Gray matter volume of the left supramarginal gyrus and left premotor cortex correlated positively with disease duration, and that of the right inferior parietal lobule correlated negatively with disease severity.

We have identified structural, yet dynamic gray matter volume changes in CD. There were specific gray matter volume changes related to BoNT injections, illustrating indirect central consequences of modified peripheral sensory input. As differences were exclusively seen in higher order motor areas relevant to motor planning and spatial cognition, these observations support the hypothesis that deficits in these cognitive processes are crucial in the pathophysiology of CD.



## Introduction

Primary cervical dystonia (CD) is characterized by involuntary abnormal movements and postures of the head and neck due to sustained contractions of the cervical musculature. Injecting the involved cervical muscles with botulinum toxin (BoNT) is an effective and evidence-based treatment of CD. In contrast to an identifiable cause in secondary dystonia, e.g. infarction of the basal ganglia, primary dystonia – including primary CD – has no evidence of an underlying cause, except for a possible genetic background as for example ANO3, GNAL and CIZ1. Although CD patients have several abnormalities in motor system physiology (Cakmur et al., 2004), its precise pathophysiology still remains to be clarified. In recent years, several functional cerebral abnormalities have been found in CD by means of functional magnetic resonance imaging (fMRI) (Naumann et al., 2000; Delnooz et al., 2013a; de Vries et al., 2012), which may originate from underlying structural abnormalities.

Structural differences have been shown using diffusion tensor imaging, demonstrating altered structural integrity in the striatum, corpus callosum and the cortical motor circuitry (Blood et al., 2006; Fabbrini et al., 2008). Studies using voxel-based morphometry (VBM) reported alterations in gray matter (GM) volume in the basal ganglia (Draganski et al., 2003; Pantano et al., 2011; Prell et al., 2013), the cortical motor circuitry (Draganski et al., 2003; Pantano et al., 2011; Prell et al., 2013), and the cerebellum in CD (Draganski et al., 2003; Prell et al., 2013). Despite several reports on structural differences between dystonia patients and healthy controls *at baseline*, few studies exist on *longitudinal* structural changes. Interestingly, several electrophysiological and functional imaging studies in focal dystonia have reported on cerebral reorganization after BoNT treatment (Delnooz et al., 2013a; Kojovic et al., 2011; Thickbroom et al., 2003). Even more, Blood et al. reported on structural *white* matter alterations after BoNT injections

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**Available online on ScienceDirect (www.sciencedirect.com).**

http://dx.doi.org/10.1016/j.nbd.2014.10.013
0969-9961/

(Blood et al., 2006). Following these previous reports, we sought to investigate the possible influence of BoNT on structural *gray* matter volume in CD patients. Since baseline differences in GM volume have not been consistent across studies, probably due to small sample sizes, clinical heterogeneity of the patients studied, and different methodological approaches (e.g. *region of interest analysis* versus *whole brain* analysis, and with or without correction for non-stationarity), it remains unclear which GM abnormalities in CD are sufficiently consistent to be regarded as the structural fingerprint of CD.

VBM of T1-weighted structural MRI images provides information about the regional differences in volumetric organization of the brain by measurement of the quantity of tissue within a voxel (Ashburner and Friston, 2000). Regional GM alterations have been demonstrated in several movement disorders. VBM has also been of value in the identification of longitudinal GM alterations in neurological diseases, reflecting for example disease progression or treatment response (Pantano et al., 2011). In this study, we tested the hypothesis that BoNT treatment modifies the 'dystonic brain', by evaluating *whole-brain* GM volume (GMV) by means of VBM methodology in a longitudinal pre- and post-treatment design within CD patients. Also, we investigated GMV in a sample of CD patients and a group of matched, healthy controls, and the relationships between GMV and disease severity, disease duration and duration of BoNT treatment.

## Methods

### Subjects

Twenty-three primary CD patients (14 women; mean age 57.2 years, 21 right-handed) and 22 healthy controls (12 women; mean age 54.5 years, 22 right-handed) participated after giving informed consent. The genetic status was not evaluated. The study was approved by the Ethics Committee of the Radboud University Nijmegen Medical Centre. The exclusion criteria included age under 18 years, severe head tremor or dystonia outside the cervical region, and absence of regular BoNT treatment. In two thirds of the patients, torticollis was the dominant feature, of which 9 patients presented with torticollis to the left. In the other patients, laterocollis was the predominant symptom (Table 1). Patients were all being treated with BoNT type A (Dysport®) every 2 to 4 months (mean duration of BoNT treatment: 7.6 years). No other neurotropic medication was used. Dystonia severity was measured with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) severity subscore. The results of TWSTRS scores ranged from 6 to 22 (median 19) before injection ($t = 0$) and from 1 to 17 (median 11) after injection ($t = 1$), demonstrating a significant improvement due to BoNT (related-samples Wilcoxon signed rank test TWSTRS $t = 0$ vs. $t = 1$: $Z = -4.11$, $p = .00$).

### Image acquisition

To investigate treatment-related changes, patients were scanned three times: before BoNT injection ($t = 0$), after 4–5 weeks ($t = 1$), and just before the next BoNT treatment ($t = 2$). Healthy controls were only scanned at baseline. Time point $t = 2$, which is comparable to $t = 0$ for the following cycle of BoNT treatment, was added in order to verify that possible BoNT-related effects were consistent over time. The timing of $t = 1$ was chosen because a maximal effect of the BoNT injections could be expected at this point. The mean delay between BoNT treatment and the MRI scan was 93.2 ± 13.9 days for the $t = 0$ scan and 93.2 ± 17.9 days for the $t = 2$ scan (related-samples Wilcoxon signed rank test interval scan-BoNT $t = 0$ vs. $t = 2$: $Z = -.21$, $p = .83$). Subjects laid supine with their eyes closed. The necessity of head immobility was emphasized to each subject, while head movements were minimized by an adjustable padded head holder. None of the subjects had dystonic symptoms during scanning, as confirmed in a post-scanning briefing. Images were acquired on a 3-T Siemens Magnetom Allegra Scanner (Erlangen, Germany) equipped with a 32-channel head coil. All scans were made with the same scanner; no scanner updates were

**Table 1**
Patient details.

| Patient no. | Sex | Age (years) | Handedness | Duration symptoms (years) | Duration BoNT treatment (years) | TWSTRS | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $t = 0$ | $t = 1$ | $t = 2$ |
| 1 | M | 46 | R | 2 | 2 | 6 | 1 | 8 |
| 2 | M | 45 | R | 25 | 15 | 21 | 8 | 20 |
| 3 | F | 53 | R | 15 | 1 | 15 | 12 | 20 |
| 4 | F | 58 | R | 2 | 5 | 14 | 8 | 20 |
| 5 | F | 60 | R | 20 | 12 | 19 | 13 | 20 |
| 6 | F | 58 | R | 10 | 10 | 17 | 9 | 20 |
| 7 | F | 48 | R | 15 | 2 | 20 | 14 | 19 |
| 8 | M | 40 | R | 20 | 10 | 22 | 17 | 22 |
| 9 | F | 82 | R | 22 | 21 | 17 | 12 | 19 |
| 10 | F | 63 | R | 9 | 8 | 19 | 15 | 20 |
| 11 | F | 60 | R | 3 | 0.25 | 17 | 7 | 17 |
| 12 | F | 50 | R | 21 | 11 | 19 | 9 | 17 |
| 13 | M | 57 | R | 18 | 15 | 17 | 3 | 20 |
| 14 | F | 71 | R | 5 | 5 | 20 | 3 | 17 |
| 15 | F | 61 | R | 9 | 8 | 20 | 9 | 19 |
| 16 | M | 50 | L | 3 | 2 | 21 | 14 | 21 |
| 17 | M | 60 | L | 4 | 0.25 | 19 | 10 | 18 |
| 18 | F | 69 | R | 10 | 2 | 19 | 15 | 20 |
| 19 | M | 47 | R | 8 | 3 | 22 | 14 | 22 |
| 20 | F | 49 | R | 12 | 20 | 18 | X | X |
| 21 | M | 67 | R | 16 | 15 | 20 | 12 | 20 |
| 22 | M | 59 | R | 20 | 17 | 20 | 16 | 21 |
| 23 | F | 64 | R | 16 | 1 | 20 | 8 | 19 |
| Mean (SD) or median (IQR)[a] | F | 60.4 (9.3) | | 12.8 (6.9) | 7.6 (6.7) | 19 (17–19.8) | 9 (8–13) | 19 (19–20) |
| | M | 52.3 (8.8) | | 12.6 (8.1) | 9.0 (6.9) | 20 (19–21) | 12 (8–14) | 20 (20–21) |
| | Total | 57.3 (9.8) | | 12.7 (7.2) | 8.0 (6.7) | 19 (17–20) | 11 (8–14) | 20 (19–20) |

F = female; IQR = interquartile range (first quartile–third quartile); L = left; M = male; no. = number; R = right; TWSTRS = Toronto Western Spasmodic Torticollis Rating Scale; X = only MRI scan at $t = 0$.

[a] Mean used for age and duration of symptoms/BoNT treatment; median used for TWSTRS scores.