Multimodal investigations into the pathophysiology of myoclonus-dystonia

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White matter abnormalities in gene positive Myoclonus-Dystonia

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Abstract

Background: Myoclonus-Dystonia is an autosomal dominantly inherited movement disorder clinically characterized by myoclonic jerks and dystonic movements of the upper body. Functional imaging and structural gray matter imaging studies in M-D suggest defective sensorimotor integration and an association between putaminal volume and severity of dystonia, possibly due to neuronal plasticity. As we expect changes in the connections between the cortical and subcortical regions we performed a combination of white matter voxel based morphometry (wVBM) and diffusion tensor imaging (DTI) to detect respectively macro- and microstructural white matter changes in DYT-11 mutations carriers (M-D).

Patients and methods: Sixteen clinically affected DYT-11 mutation carriers and 18 control subjects were scanned with 3 Tesla MRI to compare white matter volume, FA and mean diffusivity between groups.

Results: In DYT11 mutation carriers, an increase in white matter volume and FA and a decreased mean diffusivity was found in the sub-thalamic area of the brainstem, including the red nucleus. Furthermore, decreased mean diffusivity was found in the subgyral cortical sensorimotor areas.

Conclusions: The white matter changes found in the sub-thalamic area of the brainstem, connecting the cerebellum with the thalamus, are compatible with the hypothesis that abnormal function in M-D involves a network that includes the cerebellum, brainstem and basal ganglia. Whether these changes are causative or an effect of M-D requires further study.
5.1 Introduction

Myoclonus-Dystonia is a movement disorder clinically characterized by myoclonic jerks and dystonic postures or movements of the upper body. It usually becomes clinically manifest within the first two decades and is often responsive to alcohol. M-D is autosomal dominantly inherited and is caused by mutations in the epsilon-sarcoglycan gene (SGCE) on chromosome 7q21 (DYT11) in about one-third of all patients. M-D is considered a dystonia-plus syndrome and the pathophysiology of M-D is still not well understood. In dystonia the basal ganglia are hypothesized to play a major role and neuronal models of dystonia have postulated hyperactivity of the direct putamen-pallidal pathway with reduced inhibitory output of the internal segment of the globus pallidus (GPI).

Intensive use of the motor system or abnormalities in neural plasticity are associated with subtle changes in the volume of brain structures. Voxel-based morphometry (VBM) is an imaging technique that can quantify such subtle changes. In several hyperkinetic movement disorders, VBM studies have revealed either changes in grey matter volume relative to healthy controls, or a link between grey matter volume and disease severity. In M-D, such a link has also been reported; the severity of dystonia was correlated with the bilateral putaminal volume.

Changes in the brain due to plasticity not only affect grey matter, but can affect white matter connecting grey matter areas of the brain as well. Changes in white matter due to plasticity can be measured with VBM (wVBM), in which case regional white matter volumes are examined. In addition, white matter can be examined with diffusion tensor imaging (DTI).

Diffusion Tensor Imaging (DTI) is a technique specifically for investigating microstructural properties of white matter, such as structure and integrity. DTI allows for the quantification of diffusivity of water molecules and produces two measures: mean diffusivity (abbreviated as MeanD to avoid confusion with the affliction investigated) for the magnitude of diffusivity and fractional anisotropy (FA) for the directionality of diffusivity. Abnormal plasticity between grey matter regions would show up as an increased white matter volume that is detected with wVBM. With DTI, increased number of axons or myelin would lower mean diffusion (MeanD) as water is more contained. If the increased number of axons would all be in the same direction, higher FA is expected. However, axonal growth could also potentially reduce FA if axons are not increased consistently in the same direction.

Several DTI and wVBM studies investigating structural white matter abnormalities in different groups of dystonia patient have been published; all studies report abnormal connectivity between basal ganglia, cerebellum and sensorimotor and (pre)frontal cortices. However, in dystonia, there are no studies as of yet that use combined DTI and wVBM in the study of white matter changes. We investigated white-matter changes in M-D with the combination of the two techniques wVBM and DTI. Based on the sub-cortical pathophysiology of M-D, we hypothesized to find white matter abnormalities in tracts between sensorimotor and prefrontal cerebral cortex, basal ganglia and cerebellum. Based on previous plasticity studies, due to the constant hyperkinetic state of M-D patients we hypothesized to find increased white-matter volume, decreased MeanD, and increased fractional anisotropy (FA) in the aforementioned white matter tracts.
CHAPTER 5. WHITE MATTER FINDINGS IN M-D WITH VBM-DTI

Table 5.1: Patient characteristics. UMRS: Unified Myoclonus Rating Scale, BFMDRS: Burke-Fahn-Marsden Dystonia Rating Scale.

<table>
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<th>subject</th>
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<th>age</th>
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<th>BFMDRS</th>
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<td>clonazepam, phenobarbital propanolol</td>
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5.2 Patients and Methods

5.2.1 Subjects

We recruited 16 genetically confirmed M-D patients (mean age 43.5 years, range 18-66 years, 8 males) and 18 healthy age and sex matched control subjects (mean age 43.6 years, range 21-71 years, 9 males). The study was approved by the local Medical Ethics Committee; all participants were capable of giving written informed consent and did so, after receiving full information on the study. Patients were clinically examined and recorded on video on the day of scanning and clinically scored from the video by a blinded observer using the Burke-Fahn-Marsden dystonia rating scale (BFMDRS)\cite{33} and the Unified Myoclonus rating scale (UMRS)\cite{73}. The clinical characteristics of the M-D patients are summarized in Table 5.1.

5.2.2 Mask for the sensorimotor system

In order to constrain our analysis to the sensorimotor system, we created a white-matter mask for use in normalized space that contained all white-matter tracts between the grey matter regions associated with the motor system. In order to do so, fiber-tracking was performed in the International Consortium for Brain Mapping (ICBM) Tensor atlas\cite{131}. To select the grey-matter regions for the fiber tracking analysis, the Wake Forest University (WFU) PickAtlas (www.fmri.wfubmc.edu/cms/software) was used to create predefined regions of interest (ROIs). Cortical regions, basal ganglia, brainstem and cerebellum were bilaterally selected. The cortical regions included Brodmann areas 1 through 7, 9, 16, 24 and 40. The basal ganglia regions included putamen, caudate, globus pallidus, sub-thalamic nucleus and the thalamus. The brainstem region included the entire brainstem and pons. The cerebellar region contained the entire cerebellum. Fibers were tracked between these regions and converted into masks. All masks created by the fiber tracking were combined into a single
5.2. PATIENTS AND METHODS

Figure 5.1: A) Definition of regions of interest (ROIs) for subsequent fiber-tracking. Cortical regions comprised Brodmann areas 1 through 7, 9, 16, 24 and 40. The basal ganglia comprised the Putamen, Caudate, Globus Pallidus, Sub-thalamic Nucleus and Thalamus. B) Fibers were tracked between the ROI’s mentioned in (A). Each line in (B) represents one tracking calculation. The 9 fibers trackings were converted into 9 ROI images, which were combined to make one ROI image containing all connections. C) The ROI image containing all connections that is used as a ‘white-matter’ mask for the motor system.

white-matter mask for further analysis (see Figures 5.1 and 5.2).

5.2.3 MRI data acquisition

For the voxel based morphometry analyses, T1-weighted 3D anatomical images of the entire brain were obtained with a 3.0 Tesla MRI system (Philips Intera, Best, the Netherlands) using the following sequence parameters: field of view 256x256 mm²; scanning matrix 256x256; 170 slices; slice thickness 1 mm; sagittal slice orientation; TE/TR = 4.6/25 ms; SENSE-factor 2.5.

DTI data were acquired using multi-slice spin echo single shot echo-planar imaging using the following parameters: TE/TR = 94/4834 ms; diffusion sensitivities of b=0 and b=1000 s/mm²; 32 diffusion gradient directions; 45 continuous (no inter-slice gap) slices, slice thickness 3 mm, field of view 229x229 mm²; acquisition matrix 128x128;
Figure 5.2: Average (across all participants) warped FA, MeanD and WM images, and the WM mask projected to a standard brain. FA and WM image intensities correspond to values between 0 (black) and 0.9 (white). MeanD image intensity is between 0 (black) and 0.0002 (white).
acquisition voxel size 1.79x1.79x3 mm.

5.2.4 Data processing

All acquired images were converted to Analyze format and processed with SPM8 (Wellcome Department of Cognitive Neurology, London, UK), in MATLAB version 7.3.0 (2006b) (The Mathworks, MA, USA). The Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL)\(^{[10]}\) tools, included in SPM8, were used for the inter-subject spatial normalization to an existing DARTEL template in MNI space. This template was derived from 550 healthy European subjects of average age in the IXI-database (http://www.brain-development.org). The normalization with DARTEL improves the inter-subject analyses as it is more accurate in warping both cortical and subcortical regions than standardized normalization included in SPM\(^{[104]}\).

5.2.5 VBM: White Matter volume (WM)

T1-weighed images were checked for scanning artifacts, gross anatomical abnormalities and rigidly aligned to the pre-existing template. The images underwent an initial segmentation process using VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) to extract WM probability images. Using DARTEL, flow fields were applied to the WM images to warp them to the common MNI template, and modulated using the non-linear part of the Jacobian determinants to account for local volume changes. By using the non-linear part, the normalized WM images are intrinsically corrected for differences in head size between subjects. Covariance across the sample was checked prior to smoothing to exclude images with artifacts, and finally the images were smoothed with a Gaussian kernel of 8 mm full width at half-maximum (FWHM).

5.2.6 DTI: Fractional Anisotropy (FA) and Mean Diffusivity (MeanD)

Image distortions in DTI data induced by eddy currents and head motion were corrected for by applying a full affine alignment of each diffusion image to the mean no-diffusion-weighted image\(^{[123]}\). A single tensor model was fit to the DTI data, from which the fractional anisotropy (FA) and mean diffusion (MeanD) were derived\(^{[152]}\). The FA images were rigidly co-registered to the unwarped WM image of the corresponding subject. Subsequently, the FA images were warped into MNI space by applying the corresponding DARTEL flow fields. Both transformations were also applied to the MeanD images. Covariance across the sample was checked for both FA and MeanD, and the images were smoothed with an 8 mm FWHM Gaussian filter.

5.2.7 Voxel-Based Analysis of FA, MeanD and WM Volume

To test for FA, MeanD and WM volume differences between M-D patients and controls, voxel-wise statistics were performed using independent-sample t-tests implemented in the general linear model approach of SPM8. Global normalization was omitted, since in this study the regional imaging parameters (FA, MeanD and WM) were not dependent on global measures. The white-matter mask was used as a
Figure 5.3: Areas of abnormalities found in brainstem/lower thalamic area. Left panel: increase of white matter volume, Right panel: decrease of mean diffusivity.

mask to constrain the analysis to areas related to the sensorimotor system for all three measures Ũ WM, FA and MeanD. WM was used as an imaging covariate for FA and MeanD group differences, and FA and MeanD were both used as imaging covariates for WM group differences. Consequently, differences found wVBM are not driven by changes found with DWI and vice versa. Age and gender were also used as covariates. To use imaging covariates, the Biological Parametric Mapping (BPM) toolbox implemented in SPM[38] was used. To control for multiple comparisons, statistic images were assessed for cluster-wise significance using a cluster-defining threshold of P=0.005; the 0.05 family wise error-corrected critical cluster size was 619[155].

5.3 Results

The ROIs to create a white-matter mask are depicted in figure 5.1 (a). Figure 5.1 (b) and (c) illustrate the creation of the WM-mask using tractography in the tensor atlas. To illustrate the inter-subject normalization, the average WM-, MeanD and FA-volumes after registration, as well as the WM mask, are depicted in Figure 5.2.

Voxel based group comparisons of M-D patients with healthy control subjects (see Figure 5.3 and Table 5.2) showed an increase in white matter volume bilaterally in the brainstem lower thalamus area (cluster size 738). There were no significant clusters for the FA analysis when comparing M-D patients with controls in either direction. Decreased MeanD was found in the brainstem, lower thalamus area (cluster size 2804), in white matter near the dorsolateral prefrontal cortex bilaterally (left cluster size 2152, right cluster size 4478), in white matter near the sensorimotor cortex bilaterally (left cluster size 2008, right cluster size 1287), in white matter near the cerebellum bilaterally (a single cluster with size 7432) and in white matter near the left insula (cluster size 619).
### 5.4 Discussion

In the present study, we clearly demonstrate abnormalities in white matter tracts in M-D patients. The genetically homogenous study population and the relatively large number of participants add to the reliability of this study. Differences were mainly localized in the brainstem in the area under the thalamus, a region that contains connections between the cerebellum and the basal ganglia. We also showed decreased mean diffusivity in the white matter close to cortical sensorimotor areas, cerebellum and more frontal areas.

We detected abnormalities in the brainstem (lower thalamus area). In M-D the most prominent and characteristic feature is the multifocal myoclonus. Little is known about the pathophysiology of M-D and its generation of myoclonic jerks. Hypotheses in the literature regarding associated brain regions for subcortical myoclonus in general point towards several brainstem areas. One reason is the resemblance of myoclonus to startle-like jerky movements, whose ‘generator’ is thought to be located in the lower brainstem. Furthermore, the lower thalamus area of the brainstem is part of the cerebello-thalamo-cortical pathway that is hypothesized to be involved in dystonia. Abnormal connectivity in this region was also seen in a small group of primary torsion dystonia (DYT-1 and DYT-6) patients using DTI; however, in contrast to our study, a reduction in FA was reported. Possibly, the relatively more prominent myoclonus in M-D could account for this. Another reason might be that increased plasticity in M-D causes more directional axonal growth, resulting in an increase of FA, whereas axonal growth in DYT-1 and DYT-6 might have been more divergent. In the study in DYT-1/DYT-6 patients, no wVBM was performed and no mean diffusion data was reported.

Our results show increased WM and reduced MeanD in the lower thalamus region of the brainstem. Nuclei within this region act as a relay station between cerebellum and basal ganglia. Cerebellar involvement in M-D is suggested as a brain specific isoform of SGCE has been found to be notably highly expressed in the cerebellum in M-D. Furthermore, cerebellar BOLD hyperactivation has been detected with fMRI in M-D patients during a motor task. This fits well with a recently proposed model for dystonia in which malfunctions in a network of brain regions including the...
cerebellum are associated with dystonia\textsuperscript{139}.

We detected decreased MeanD, but no changes in FA or WM, in the sub-gyral cortical sensorimotor areas in M-D. In these areas, a reduction in FA was reported for primary torsion dystonia (DYT-1 and DYT-6), suggesting abnormal connectivity with other brain regions\textsuperscript{36,37}. The reduction in MeanD without changes in FA or WM might indicate a more subtle change in cortical neural plasticity for M-D. This is in accordance with electrophysiological findings; while cortical reduced inhibition has been reported for several types of dystonia\textsuperscript{89}, in M-D reduced inhibition is absent\textsuperscript{117,21}.

DTI studies in other types of dystonia do not show a consistent pattern of affected brain areas. In cervical dystonia and blepharospasm, an increase in FA in the putamen, corpus callosum, prefrontal cortex and supplementary motor area (SMA) has been reported, consistent with increased connectivity in the basal ganglia and loss of neurons in the prefrontal cortex SMA and the corpus callosum\textsuperscript{44,64}. Using DTI probabilistic tractography, in idiopathic cervical dystonia disrupted thalamic prefrontal pathways have been found\textsuperscript{25}. In patients with writer's cramp, increased FA was detected bilaterally in the white matter of the posterior limb of the internal capsule and adjacent structures, involving fiber tracts connecting the primary sensorimotor areas with subcortical structures. Taken together, findings in different types of dystonia suggest the most common brain regions involved are cortical sensorimotor regions. However, vastly different findings are generally reported and could indicate different pathophysiological mechanisms underlying the different dystonias. The importance of the underlying anatomy, particularly of crossing fibers, is crucial for the correct interpretation of FA\textsuperscript{55,56}. This aspect is often overlooked in literature on dystonia. Furthermore, Mean Diffusion (MeanD) is often not reported at all. The decreased mean diffusivity in the white matter close to cortical sensorimotor areas in M-D is consistent with increased/decreased FA findings in the same regions in other forms of dystonia, but might indicate a more subtle alteration.

One limitation of this study is the use of DTI scans with anisotropic resolution, which makes our FA measures more prone to partial volume effects\textsuperscript{148}. This could have affected the sensitivity of our FA analysis. The diffusivity measure MeanD is not affected by anisotropic voxels dimensions.

In conclusion, the most prominent changes in connectivity in our study are located in the brainstem in the area under the thalamus. How these changes relate to the involuntary movements in M-D is unclear, but our findings are consistent with brainstem involvement in the pathophysiology of dystonia, and a malfunction in the network for hyperkinetic movement disorders involving the cerebellum, brainstem and basal ganglia\textsuperscript{140}. Whether these changes are specific for M-D remains unclear and would be an interesting hypothesis to test in a larger group of dystonic and myoclonic patients.