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Forebrain knock-out of torsinA reduces striatal free-water and impairs whole-brain functional connectivity in a symptomatic mouse model of DYT1 dystonia

Jesse C. DeSimone [a], Samuel S. Pappas [b], Marcelo Febo [c], Roxana G. Burciu [a], Priyank Shukla [a], Luis M. Colon-Perez [c], William T. Dauer [b,d,e], David E. Vaillancourt [a,f,g,*]

[a] Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, USA
[b] Department of Neurology, University of Michigan, Ann Arbor, MI 48109, USA
[c] Department of Psychiatry, College of Medicine, University of Florida, Gainesville, FL 32611, USA
[d] Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA
[e] Veteran Affairs Ann Arbor Healthcare System, University of Michigan, Ann Arbor, MI 48105, USA
[f] Department of Neurology, College of Medicine, University of Florida, Gainesville, FL 32610, USA
[g] Department of Biomedical Engineering, University of Florida, Gainesville, FL 32611, USA

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ABSTRACT

Multiple lines of evidence implicate striatal dysfunction in the pathogenesis of dystonia, including in DYT1, a common inherited form of the disease. The impact of striatal dysfunction on connected motor circuits and their interaction with other brain regions is poorly understood. Conditional knock-out (cKO) of the DYT1 protein torsinA from forebrain cholinergic and GABAergic neurons creates a symptomatic model that recapitulates many characteristics of DYT1 dystonia, including the developmental onset of overt twisting movements that are responsive to antimuscarinic drugs. We performed diffusion MRI and resting-state functional MRI on cKO mice of either sex to define abnormalities of diffusivity and functional connectivity in cortical, subcortical, and cerebellar networks. The striatum was the only region to exhibit an abnormality of diffusivity, indicating a selective microstructural deficit in cKO mice. The striatum of cKO mice exhibited widespread increases in functional connectivity with somatosensory cortex, thalamus, vermis, cerebellar cortex and nuclei, and brainstem. The current study provides the first in vivo support that direct pathological insult to forebrain torsinA in a symptomatic mouse model of DYT1 dystonia can engage genetically normal hindbrain regions into an aberrant connectivity network. These findings have important implications for the assignment of a causative region in CNS disease.

1. Introduction

Dystonia musculorum deformans ("Oppenheim's dystonia") is an inherited neurodevelopmental movement disorder (DYT1 dystonia) characterized by sustained, involuntary twisting movements and disabling postures (Breakefield et al., 2008; Fahn, 1988; Fahn et al., 1998; Ozelius and Lubarr, 1993). DYT1 dystonia is caused by a dominant three base-pair deletion (ΔGAG) in the *TOR1A* gene that eliminates a single glutamic acid residue (ΔE) in the C-terminus of the AAA+ protein torsinA (Ozelius et al., 1997).

Convergent evidence from human and animal studies establishes the striatum as a key region in dystonia pathophysiology. Lesion studies point to an association between disturbed putaminal integrity and clinical symptoms in patients with secondary dystonia (Bhatia and Marsden, 1994; Burton et al., 1984; Fross et al., 1987; Marsden et al., 1985). Deep brain stimulation of major striatal output targets such as the globus pallidus internus and subthalamic nucleus is an effective therapy for DYT1 dystonia (Kupsch et al., 2006; Ostrem et al., 2014; Vidailhet et al., 2005). Moreover, studies in multiple mouse models of DYT1 dystonia have implicated abnormal function of striatal cholinergic interneurons (SCI) to be involved in disruption of cortico- and thalamo-striatal synaptic integration and plasticity (Dang et al., 2012; Martella et al., 2009; Maltese et al., 2014; Pisani et al., 2006; Sciamanna et al., 2012a, 2012b). It remains unclear how striatal dysfunction affects network-level changes in functional connectivity (FC) (Biswal et al., 1995; Fox et al., 2005) across

Abbreviations: cKO, conditional knock-out; dMRI, diffusion magnetic resonance imaging; FC, functional connectivity; FW, free-water; KI, knock-in; KO, knock-out; MD_T, free-water corrected mean diffusivity; rsfMRI, resting-state functional magnetic resonance imaging; SCI, striatal cholinergic interneuron.

* Corresponding author at: Department of Applied Physiology and Kinesiology, University of Florida, 1864 Stadium Road, 100 FL-Gym, Gainesville, FL 32611-8205, USA.
E-mail address: vcourt@ufl.edu (D.E. Vaillancourt).

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cortical, subcortical, and cerebellar networks. Understanding how striatal pathology affects network-level FC in pre-clinical models is important for dissecting pathophysiology and providing readouts for disease modifying interventions. Further, understanding network-level connectivity in a translational model of generalized dystonia is opportune because motor impairment in human subjects with focal dystonia points to disturbances in FC (Battistella et al., 2015, 2016).

To explore these questions, we acquired in vivo diffusion MRI (dMRI) and resting-state functional MRI (rsfMRI) in a mouse model characterized by Cre-recombinase expression and conditional knockout (cKO) of torsinA from forebrain (i.e., striatum, cortex, globus pallidus, basal forebrain, and reticular thalamic nucleus) cholinergic and GABAergic neurons (Pappas et al., 2015). In contrast to human DYT1 dystonia, which does not exhibit overt structural lesions, this mouse model exhibits selective neurodegeneration specific to SCIs. As well, surviving SCIs exhibit altered morphology (i.e., hypertrophy) and dysfunctional electrophysiological properties, implicating striatal connectivity abnormalities. In turn, the structural and functional integrity of forebrain GABAergic neurons is preserved despite the lack of torsinA. We tested two hypotheses. First, we used diffusion MRI and a bi-tensor model to test the hypothesis that torsinA loss-of-function in cKO mice causes abnormal microstructural changes in free-water (FW) and tissue compartment diffusivity (free-water corrected mean diffusivity: MD_T) within the striatum. This computational approach fits a bi-tensor model to dMRI data, separating the diffusion properties of water in brain tissue from that of water in the extracellular space (Metzler-Baddeley et al., 2012; Pasternak et al., 2009). Since prior work in the cKO model demonstrated that surviving SCIs are associated with significant cellular soma hypertrophy (Pappas et al., 2015), we predicted that the extracellular FW compartment of striatal regions would be reduced, whereas the MD_T would be increased. Second, we used rsfMRI to test the hypothesis that FC is impaired between the pathologically abnormal striatum and other key cortical, subcortical, and cerebellar motor regions.

2. Materials and methods

2.1. Animals and housing

Mice originating in the Dauer Laboratory at the University of Michigan were housed and imaged at the University of Florida McKnight Brain Institute. Eighteen (10 male, 8 female; age 6.9 ± 0.8 mo) Dlx5/6-Cre^{+} Tor1a$^{flx/-}$ (cKO) and 18 (7 male, 11 female; age 6.7 ± 1.3 mo) Cre^{-} Tor1a$^{flx/+}$ littermate wild-type controls were used in this experiment. Mice were prepared and genotyped for Tor1a and Cre using the PCR protocol described by Liang et al. (2014) and cKO and control mice were bred as previously described (Pappas et al., 2015). Prior to animal transport, motor abnormalities (i.e., forelimb and hindlimb clasping, abnormal posturing) were confirmed in all cKO mice by postnatal days 49–56 via the tail suspension assessment (Pappas et al., 2015). Mice were housed in groups of one to three in a temperature and humidity controlled environment, maintained on an alternating 12 h. light-dark cycle (i.e., lights off at 19:00 h), and were provided ad libitum food and water access. All experimental protocols and procedures were approved and monitored by both the University of Michigan Committee on the Use and Care of Animals (UCUCA) and the University of Florida Institutional Animal Care and Use Committee (IACUC). Animals were acquired and cared for in accordance with the ethical standards set forth by the Guide for the Care and Use of Laboratory Animals (8th Edition, 2011) and the American Association for Laboratory Animal Science guidelines.

2.2. MRI preparation and data acquisition

Experimenters involved in data collection had no a priori knowledge of the genotype of each animal during MRI acquisition, and the blind was not broken until the final between-group statistics were performed. Mice were anesthetized for the duration of the experiment. Isoflurane anesthesia was delivered using compressed air through a Surgivet vaporizer (Dublin, OH, USA) connected to a charcoal trap. Mice were initially induced at 3–4% isoflurane for 1–2 min in an enclosed knock-in chamber. Anesthesia was reduced to 2% for animal setup and 1.0–1.5% for MRI acquisition. Notably, this level of anesthesia was considered appropriate based on previous evidence that concentrations within this bandwidth preserve FC patterns in rodent models (Ferron et al., 2009; Liu et al., 2013). Animals were placed in a prone position on a custom design plastic mouse bed equipped with a bite bar that served to immobilize the head and deliver anesthesia during scanning. An in-house 2.5 × 3.5 cm quadrature surface transmit/receive coil (Advanced Magnetic Resonance Imaging and Spectroscopy Facility, University of Florida, Gainesville, FL, USA) was affixed to the top of the skull and tuned to 470.7 MHz (^{1}H resonance) for B^{1} excitation and signal detection. Respiratory vitals and core body temperature (37–38 °C) were monitored and maintained using a respiration pad and a recirculating waterbed heating system (SA Instruments, Stony Brook, NY, USA).

MRI data were acquired using an 11.1 Tesla Magnex Scientific horizontal magnet (Agilent, Inc., Santa Clara, CA, USA, 205/120HD gradient set with 120 mm inner gradient bore size; maximum gradient strength 600 mT/m and rise time of 130 μs) at the University of Florida McKnight Brain Institute. MRI sequences were prepared and acquired using VNMRJ software (Agilent Technologies, Version 3.1) and included acquisition of anatomical scout sequences for real-time visual depiction of brain positioning, as well as whole-brain voxel shimming for magnetic field homogeneity. The MRI acquisition sequences were ordered as follows: one dMRI scan, two rsfMRI scans, and one T_2-weighted anatomical scan.

Diffusion weighted images were acquired using an 8-shot echo planar imaging (EPI) sequence with the following parameters: repetition time (TR) = 2500 ms; echo time (TE) = 25.88 ms; flip angle = 90°; max b-value = 900 s/mm^2; averages = 3; dummy scans = 2; directions = 42; slices = 12; coronal orientation; thickness = 0.75 mm; gap = 0 mm; field of view (FOV) = 19.2 × 19.2 mm; data acquisition matrix = 128 × 128 in-plane.

Resting-state fMRI was performed using a 2-shot EPI sequence with the following parameters: TR = 1000 ms; TE = 20 ms; repetitions = 210; flip angle = 90°; dummy scans = 4; slices = 12; coronal orientation; thickness = 0.75 mm; gap = 0 mm; FOV = 19.2 × 19.2 mm; data acquisition matrix = 64 × 64 in-plane.

Anatomical images were acquired using a fast-spin echo T_2-weighted imaging sequence with the following parameters: TR = 2000 ms; effective TE = 31.6 ms; echo spacing = 8.04; echo train length = 8 ms; slices = 12; coronal orientation; thickness = 0.75 mm; gap = 0 mm; FOV = 19.2 × 19.2 mm; data acquisition matrix = 192 × 192 in-plane.

2.3. Diffusion MRI pre-processing and statistical analysis

Diffusion MRI pre-processing and analysis was performed using previously described methods and a rodent-modified bi-tensor diffusion analysis pipeline (DeSimone et al., 2016; Pasternak et al., 2009). We used the FMRIB Software Library (FSL: Oxford, UK) and custom designed UNIX shell scripts in Analysis of Functional NeuroImages software (AFNI: Cox, 1996; Version 16.0.19, https://afni.nimh.nih.gov/afni/download/afni/psc_project_view) to correct for eddy current and head motion artifacts, compensation of diffusion gradient rotations in response to these corrections, and manual skull stripping for the removal of non-brain tissue. FW and FW-corrected diffusion tensor imaging (DTI) maps were calculated from the pre-processed motion and eddy current corrected volumes using custom code written in MATLAB (Version R2013a; The Mathworks, Natick, MA, USA). To create the FW map, a minimization procedure was used such that a bi-tensor model (Pasternak et al., 2009) was fit to each voxel in order to quantify FW fractional volume. The FW component is then eliminated from each