DYSTONIA

Samuel Belzberg 6th International Dystonia Symposium Abstract Book

Dublin, Ireland, 01–03 June 2023

frontiers

Introduction

The abstracts submitted to the Samuel Belzberg 6th International Dystonia Symposium provide a comprehensive overview of the latest research on dystonia, presenting crucial insights into diagnosis, treatment, and understanding of the disorder. Researchers from around the globe contribute their expertise, offering advancements that hold significant importance for the dystonia community.

Ebook ISBN: 978-2-8325-4404-4
ISBN no dashes: 9782832544044
DOI: 10.3389/978-2-8325-4404-4

Citation - Dystonia Medical Research Foundation. (2023). Samuel Belzberg 6th International Dystonia Symposium Abstract Book.

Table of contents

Motor Cortex Activation During Writing In Focal Upper-Limb Dystonia: An fNIRS Study

Renata Prôa[1,2], Joana Balardin[1], Artur Marques Paulo[1], Danilo Donizete de Faria[3,4], João Ricardo Sato[5], Vanderci Borges[3], Sonia Azevedo Silva[3,4], Henrique Ballalai Ferraz[3], Patricia de Carvalho Aguiar[1,3]

[1]Hospital Israelita Albert Einstein, Sao Paulo, Brazil
[2]Columbia University's Zuckerman Institute, New York, USA
[3]Universidade Federal de Sao Paulo, Sao Paulo, Brazil
[4]Hospital do Servidor Público Estadual de Sao Paulo, Sao Paulo, Brazil
[5]Universidade Federal do ABC, Santo Andre, Brazil

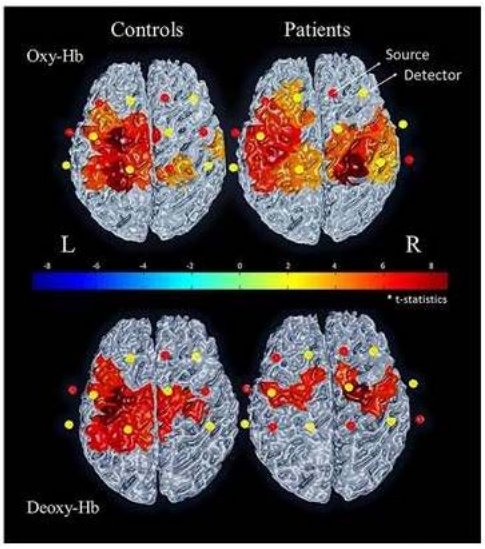


Figure 1 | Oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) activation maps for the contrast Writing > Resting, Activation maps from the channel-wise analysis showing the mean activation of controls (left) and patients (right) for oxy-Hb (above) and deoxy-Hb (below).Patients' map Shows a more bilateral activation pattern. Red and yellow dots represent sources and detectors, respectively, Results for each channel were Bonferroni corrected for multiple comparisons (P value< .002)

Introduction

Functional near-infrared spectroscopy (fNIRS) offers a new noninvasive possibility for investigating cortical areas and the neural correlates of complex motor behaviors in unconstrained settings.

Materials and Methods

We compared the cortical brain activation of patients with idiopathic focal upper-limb dystonia and controls during the writing task under natural conditions using fNIRS. The total number of written letters, as well as legibility, assessed by two blinded investigators, were analyzed. The primary motor cortex (M1), the primary somatosensory cortex (S1), and the supplementary motor area were chosen as regions of interest (ROIs).

Results

Regarding the number of written letters, controls had a statistically significant better performance (p =0.003). We observed no association between group and legibility. Group average activation maps revealed an expected pattern of contralateral recruitment of motor and somatosensory cortices in the control group and a more bilateral activation pattern in the dystonia group (figure 1). Between-group comparisons focused on specific ROIs revealed an increased activation of the contralateral M1 and S1 cortices and also of the ipsilateral M1 cortex in patients.

Conclusions

Overactivity of contralateral M1 and S1 in dystonia suggests a reduced specificity of the task-related cortical areas, in agreement with the pathological mechanism of cortical inhibition failure known to happen in dystonia. Ipsilateral cortical activation has been observed in healthy subjects during complex tasks which demand high accuracy. In this study, where the task was not highly demanding, patients' ipsilateral activation was significantly higher than controls. This could indicate a primary disorder of the motor cortex or an endophenotypic pattern. To our knowledge, this is the first study using fNIRS to assess cortical activity in dystonia during the writing task under natural settings, outlining the potential of this highly portable and low-cost technique for monitoring sensory and motor retraining in dystonia rehabilitation.

Funding source(s)
FAPESP grant #2021/14108-4

Cutaneous Silent Period In Patients With Idiopathic Craniocervical Dystonia

Talyta Grippe,[1,2,5] Natalia Spinola da Cunha,[3] Renata Vargas,[4] Gustavo Bachtold,[4] Rubens Fernandez,[4] Francisco Cardoso,[2] Robert Chen[1,5]

[1]Edmond J. Safra Program in Parkinson's Disease, Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, UHN, Toronto, Ontario, Canada. Division of Neurology, University of Toronto, Toronto, Ontario, Canada
[2]Neuroscience Graduate Program, Federal University of Minas Gerais, Belo Horizonte, Brazil
[3]Department of Child Neurology, Hospital da Criança de Brasília José de Alencar Brasília, Brazil
[4]Department of Neurophysiology, Instituto Hospital de Base do Distrito Federal Brasília, Brazil
[5]Krembil Brain Institute, Toronto, Ontario, Canada

Background
The cutaneous silent period (CSP) is a robust cutaneomuscular reflex and evaluates sensorimotor integration involved in the regulation of spinal cord excitability. The main CSP parameters include onset latency, duration, and end latency. Previous studies reported conflicting results regarding the differences between patients with dystonia and controls.

Methods
We tested 17 patients with idiopathic craniocervical dystonia (age, 53.7 ± 10.3 years; mean ± standard deviation; 9 women and 8 men) and 10 age-matched healthy subjects (age, 48.9 ± 8.5 years; 5 women and 5 men). Eleven patients had isolated cervical dystonia, three patients had blepharospasm, one patient had cervical and limb dystonia, one patient had facial dystonia, and one patient had facial and laryngeal dystonia. All patients had received botulinumtoxin treatment, with a minimum of 3 months since their last injections. We evaluated the CSP ith electrical stimulus in digit 5 (D5) and recording from the abductor digiti minimi (ADM) muscles bilaterally.

Results
31 arms were evaluated in the dystonia group. The median, the 2nd and 98th percentile for CSP duration was 22.7 (11.3 – 48.1) ms, for onset latency was 84 (68.6 –115) ms, and for end latency was 107 (88.6 – 140) ms. Twenty-one arms were evaluated in the control group. The median for CSP duration was 28.9 (18 – 51) ms, onset latency was 86.2 (69.4 – 108) ms, and median end latency was 120 (93.4 – 143) ms. The CSP duration (Mann Whitney test p=0.022) and the end latency (p=0.036) were significantly shorter in the idiopathic craniocervical dystonia group compared to the control group. The linear regression for CSP duration (R=0.45; p=0.014) and end latency (R=0.38; p=0.04) showed that the ulnar F wave latency was a significant predictor.

Conclusions
The patients with craniocervical dystonia had a reduced CSP duration compared to controls. This may indicate reduction of inhibitory function during sensory motor integration involving the spinal cord in the craniocervical dystonic patients. The F wave values correlate with the reduced duration and end latency in CSP of the dystonic patients and reinforces the role of the spinal cord in this alteration. Further studies are needed to confirm these findings.

Funding source(s), including NIH support, if any:
None

Microstructural Asymmetry Of The Dentato-Rubro-Thalamic Tract In Cervical Dystonia

Rachel Sondergaard[1,2], Conrad Rockel[2,3,4], Fil Cortese[2,5], Bruce Pike[1,2,3,4], Zelma H T Kiss[1,2], Davide Martino[1,2]

[1]Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada
[2]Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada
[3]Department of Radiology, University of Calgary, Calgary, Alberta, Canada
[4]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[5]Neuroimaging Research Unit, Seaman Family MR Research Centre, Foothills Medical Centre, Calgary, Alberta, Canada

The dentato-rubro-thalamic tracts (DRTT) are underexplored in cervical dystonia (CD), despite the relevance of the cerebellum in both CD and cerebellum-mediated inhibition of movement. A proposed model of idiopathic cervical dystonia (CD) suggests that feedback asymmetry arising within the motor network drives dysfunction in a graded manner. Substantiation of this so-called faulty head neural integrator (HNi) model of CD presently converges at the globus pallidus internus (GPi) as physiological and structural evidence originating elsewhere in the motor network is currently lacking. Advancements in neuroimaging have made it possible to explore microstructural aspects of the DRTT with tractography. In fact, we have previously reported bilateral microstructural abnormalities of the DRTT. We were interested to see if there was evidence of microstructural asymmetry along these tracts in CD, and if they scaled with CD severity.

Diffusion and T1 weighted magnetic resonance imaging scans were acquired in n=35 healthy controls and n=32 subjects with CD at least 12 weeks after botulinum toxin injections in the CD patients. Diffusion images were pre-processed using FSL *topup* and *eddy* programs to register the images and correct for susceptibility, eddy current and motion distortion. A template of the DRTT was created and probabilistic tractography was completed; diffusion tractography imaging (dti) metrics were calculated for all subjects (fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD)). Asymmetry index (AI) for these measures (i.e., (left-right metric)/(left+right metric)) was calculated.

A GLM comparing asymmetry index of MD, AD, RD and FA between healthy controls and CD subjects and controlling for age, sex and handedness revealed a significant effect of group (Wilks' Lambda =0.840, F=2.811, p=0.03). Asymmetry index of mean diffusivity was significantly impacted by group with CD subjects having reduced AI of MD relative to controls (F=10.727, p=0.002, estimated means CD: -0.012, HC: 0.005). There were no significant correlations between CD severity and AI measures, however a sub-analysis of 'left' affected CD subjects (n=13) revealed a negative correlation between torticollis severity and AI of MD (PCC:-0.552, p=0.031). It appears that asymmetry index of MD is significantly different between HC and CD patients. Interestingly a relationship between asymmetry, sidedness of torticollis and severity emerged in a sub-analysis, requiring a larger sample size to confirm but providing potential for support of the faulty HNi model derived from outside of the basal ganglia.

The peer reviewed abstract, as submitted in March 2023 above, may be published in the dystonia journal.

No specific funding was received for the completion of this work, however RS was the recipient of an NSERC doctoral scholarship.

Finely-Tuned Gamma Oscillations In Patients With Isolated Dystonia Implanted With Sensing-Enabled Pulse Generators

Stephanie Cernera, Maria Shcherbakova, Carina Oehrn, Simon Little, Philip Starr

University of California San Francisco

Finely-tuned gamma (FTG) has been implicated as a "prokinetic" neural oscillation in dystonia[1] and Parkinson's disease[2]. Intraoperative recordings in isolated dystonia show that native cortical FTG emerged during dystonic posturing[1]. The entrainment of FTG to the harmonics of stimulation frequency by deep brain stimulation (DBS) is hypothesized to be a mechanism of therapeutic effect[2]. However, the functional relevance of both native (pre-stimulation) and DBS-entrained FTG in naturalistic settings has never been studied.

We recorded local field potentials from basal ganglia and cortical leads (covering precentral and postcentral gyri) in two patients with isolated dystonia at-home, using a sensing-enabled DBS device (Summit® RC+S, Medtronic). We assessed head tremor with a head-worn accelerometer. Patients reported activities through a patient-facing graphical user interface[3]. We divided time series into 20-second epochs and estimated power spectral density using Welch's method. We determined the degree of native and entrained gamma using the fooof algorithm[4]. The severity of head acceleration was measured as the 20th, 40th, 60th, and 80th percentiles derived from the envelope across an individual's at-home recording. Additionally, we collected in-clinic data to assess changes in entrained FTG as a function of stimulation amplitude and frequency while patients performed standardized tasks and rated symptom severity.

We recorded >500 hours of at-home neural data. We observed spatially specific native and entrained FTG. In one patient, native FTG was of highest amplitude on postcentral gyrus and increased with more severe head movements. Across patients, entrained FTG was more prominent on precentral gyrus, modulated with stimulation amplitude, and activities. Further, gamma entrainment amplitude depended on stimulation frequency and amplitude in a non-linear manner. In one patient, the highest entrained FTG amplitude on precentral gyrus was at 3 mA, 110.6 Hz and decreased with relatively higher stimulation amplitudes. Entrained FTG on precentral gyrus was negatively correlated with subjective symptom severity when controlling for stimulation amplitude (partial Pearson correlation, $R = -0.436$, $p = 0.023$).

Chronic sensorimotor recordings in patients with isolated dystonia reveal that native and entrained FTG are spatially specific and are related to symptom severity. Further, there are amplitude-frequency combination sweet spots for entraining FTG. These signals may potentially be used to optimize programming or in adaptive DBS protocols.

References

[1] Miocinovic et al., 2018.

[2] Muthuraman et al., 2020.

[3] Gilron et al., 2021.

[4] Donoghue et al., 2020.

Functional MRI-Guided Personalized TMS Identifies Motor Network Reorganization Associated With Behavioral Improvement In Writer's Cramp Dystonia

Noreen Bukhari-Parlakturk*[1,2], Patrick J. Mulcahey[1], Michael W. Lutz[1], Rabia Ghazi[1], Ziping Huang[1], Moritz Dannhauer[3], Zeynep B. Simsek[1], Skylar Groves[1], Mikaela Lipp[1], Michael Fei[1], Tiffany K. Tran[1], Eleanor Wood[4], Lysianne Beynel[3], Chris Petty[5], James T. Voyvodic[2,5], Lawrence G. Appelbaum[7], Hussein R. Al-Khalidi[6], Simon W. Davis[1,2], Andrew M. Michael[2], Angel V. Peterchev[2,8,9,10,11], Nicole Calakos[1,2,12]

[1]Department of Neurology, Duke University School of Medicine
[2]Duke Institute for Brain Sciences, Duke University
[3]Non Invasive Neuromodulation Unit, Experimental Therapeutics and Pathophysiology Branch, National Institute of Mental Health, National Institute of Health, Bethesda, Maryland, USA
[4]Jefferson Medical College, Philadelphia, Pennsylvania
[5]Brain Imaging & Analysis Center, Duke University School of Medicine
[6]Department of Biostatistics and Bioinformatics, Duke University School of Medicine
[7]Department of Psychiatry, University of California, San Diego, California, USA
[8]Department of Psychiatry, and Behavioral Sciences, Duke University School of Medicine
[9]Department of Electrical and Computer Engineering, Duke University
[10]Department of Neurosurgery, Duke University
[11]Department of Biomedical Engineering, Duke University
[12]Department of Neurobiology, Duke University School of Medicine, Durham, North Carolina, USA

Background

Writer's cramp (WC) dystonia is an involuntary movement disorder with broadly distributed abnormalities in the brain's motor network. Prior studies established the potential for repetitive transcranial magnetic stimulation (rTMS) at either premotor cortex (PMC) or primary somatosensory cortex (PSC) to modify symptoms. Clinical effects, however, have been modest with limited understanding of the neural mechanisms limiting improvements of this promising approach.

Objective
This study aims to reveal the motor network effects of rTMS in WC subjects that correspond to behavioral efficacy. We hypothesized that clinical efficacy is associated with modulation of cortical and subcortical motor network areas.

Methods
In a double-blind, cross-over design, twelve WC subjects underwent weekly 10 Hz rTMS in one of three conditions (Sham-TMS, PSC-TMS, PMC-TMS) while engaged in a writing task to activate dystonic movements and measure writing fluency. Brain connectivity was evaluated using task-based functional magnetic resonance imaging (fMRI) after each TMS session.

Results
PSC-TMS, but not PMC-TMS, significantly improved writing dysfluency. Mechanistically, PSC-TMS significantly weakened functional connectivity (FC) between cortex and basal ganglia and strengthened nigral-cerebellar connectivity, relative to Sham, and in distinction from PMC-TMS. FC relationships strongly correlated with writing dysfluency and differed between Sham-TMS and PSC-TMS. Writing dysfluency was most strongly predicted by basal ganglia-cerebellar and cortico-subcortical region connectivity in Sham-TMS and cortico-cortical and cortico-cerebellar connectivity in PSC-TMS.

Conclusions
10 Hz rTMS to PSC improves writing dysfluency in WC, causing broad redistribution of connectivity in the motor network. These findings offer mechanistic hypotheses to further improve therapeutic benefits of TMS for dystonia.

Funding sources for study
This work was supported by grants to NBP from Dystonia Medical Research Foundation (Clinical Fellowship Training Program), Doris Duke Charitable Foundation (Fund to Retain Clinician Scientists), American Academy of Neurology (career development award) and NIH NCATS (1KL2TR002554). NBP was also supported by a career development award from the Dystonia Coalition (NS065701, TR001456, NS116025) which is part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), supported by the Office of Rare Diseases Research (ORDR) at the National Center for Advancing Translational Science (NCATS), and the National Institute of Neurological Diseases and Stroke (NINDS). AVP's contributions were supported in part by the National Institutes of Health (R01MH128422, R01NS117405). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

When Deep Brain Stimulation In Childhood-Onset Dystonias Is Not Enough. Post-DBS Outcomes And The Need For Rehabilitation To Improve Everyday Activities

Hortensia Gimeno[1,2,3], Daniel Lumsden[3,4], Richard Selway[4], Harutomo Hasegawa[4,3], Jean-Pierre Lin[3,4]

[1]Barts Health NHS Trust, London, United Kingdom
[2]uCentre for Preventive Neurology, Wolfson Institute of Population Health, Queen Mary University of London, London, United Kingdom
[3]Paediatric Neurosciences, Evelina London Children's Hospital, London, United Kingdom
[4]King's College London, London, United Kingdom

Introduction
Childhood-onset dystonias are a heterogeneous group of disorders. Whilst patient-reported outcomes have been described, objective outcome measures of self-care and daily life activities post-DBS are lacking.

The assessment of motor and process skills (AMPS), a standardised observational evaluation, measures a person's observed quality of activities of daily living performance.

We hypothesised that:

(i) individuals with childhood-onset dystonia would display motor and process skills difficulties,
(ii) motor skills would change post-DBS but not process skills.

Materials and Methods
Blind-rated open-label case series. AMPS was administered by an AMPS-accredited occupational therapist at baseline, 1- and 2-years post-DBS. Burke-Fahn-Marsden Dystonia Rating scale (BFMDRS) was also used. Ordinal scores were transformed into motor (AMPS-m) and process (AMPS-p) skills logits, using a Rasch measurement model (age, task challenge, skill difficulty and rater severity considered). At baseline, gross motor function and manual classification systems (GMFCS/MACS) were used.

Independent Samples Kruskal-Walls Test was used (significance level <0.05) to explore distribution of logits across aetiology, and GMFCS/MACS groups. Baseline and follow-up differences were examined using the Wilcoxon Signed Rank Test.

Motor logits <2 and process logits <1 indicate the person is below the threshold of what would be expected for their age.

Results
All patients with idiopathic (n=11) or inherited monogenetic dystonias (n=33) and post-DBS data were included.

The distribution of baseline AMPS-m was significantly different across GMFCS (p=0.012) and MACS (p<0.001) levels. For AMPS-p only MACS levels showed a significant difference (p=0.010) but not for GMFCS levels (p=0.495).

The distribution of AMPS-m was the same across both aetiology groups (p=0.267) but significantly different for AMPS-p skills (p=0.005). There were no differences across BFMDRS (p=0.726)

A significant change in all measures (AMPS-m-, AMPS-p, and BFMDRS) was seen at all timepoints (p<0.001).

When thresholds were applied, most individuals remained below normative data (<2 for AMPS-m and <1 for AMPS-p).

Discussion
Both motor and process skills are affected in childhood-onset dystonias and below normative data post-DBS. Whilst there is a focus on motor symptoms in these disorders, additional non-motor difficulties (i.e., process skills) play an important role in our understanding of DBS effect.

Despite statistical improvement in dystonia severity, and motor and process skills, augmentation of DBS outcomes might be needed via complementary interventions such as occupational therapy to support further skill acquisition.

Differential Body Part Response To Pallidal Deep Brain Stimulation In Patients With Isolated Non-Acquired Dystonia

Margi Patel[1], Stewart Factor[2], Svjetlana Miocinovic[3]

Department of Neurology, Baylor University Medical Center, TX, USA
Department of Neurology, Emory University, GA, USA
Department of Neurology, Emory University, GA, USA

Objective and Background
To evaluate response of dystonia in distinct body regions to pallidal deep brain stimulation (DBS) using the Global Dystonia Rating Scale (GDRS) scores in patients with isolated non-acquired dystonia. Limited evidence suggests that blepharospasm, cervical dystonia, dystonia affecting speech and swallowing do not respond as well as limb dystonia, but contrary findings have also been reported. Establishing which regions are most impacted by DBS would guide appropriate patient selection and counseling.

Methodology
In a retrospective examination of subjects who underwent pallidal DBS between 2008-2020 at a university hospital, GDRS scores were obtained through blinded, randomized assessment of standardized video exams, rated by two movement trained neurologists, separately and then averaged. For patients with individual body part score difference >=2 and/or total score difference of >=3, a third rater independently reviewed the video and the score was then included in the average. The primary outcome measure was the change in GDRS score in each body region from baseline to follow-up.

Results
20 patients were identified, 10 were women, average age of dystonia onset was 35 $\pm$ 20 (range 5-70) years with

Table 1 | GDRS subscores before and after DBS surgery (median (interquartile range^))

	Baseline GDRS	*Follow-up GDRS*	*p-value**	*# patients with dystonia (score >=1)*	*Point change if dystonia present*
Eyes	0.00 (0.00)	0.00 (0.00)	0.313	4 → 1	2.9±4.3
Lower face	0.00 (0.75)	0.00 (0.83)	0.578	5 → 5	1.1±2.5
Jaw and tongue	0.00 (2.25)	0.00 (2.25)	0.754	6 → 7	-0.2±3.2
Larynx	0.00 (0.00)	0.00 (0.50)	0.688	3 → 5	-0.5±2.0
Neck	6.17 (5.50)	2.50 (3.75)	**<0.001**	17 → 14	3.5±2.5
Shoulder and proximal arm	2.00 (5.67)	0.00 (1.50)	**<0.001**	14 → 8	3.3±3.7
Distal arm and hand including elbow	2.00 (6.50)	1.75 (5.67)	0.531	12 → 11	0.3±4.8
Pelvis and proximal leg	0.00 (1.25)	0.00 (0.00)	0.125	5 → 2	7.0±7.8
Distal leg and foot including knee	0.00 (2.00)	0.00 (0.67)	**0.031**	6 → 5	3.3±2.5
Trunk	0.00 (2.58)	0.00 (0.00)	**0.016**	6 → 1	5.0±2.4
TOTAL	16.91 (8.66)	10.00 (9.75)	**<0.001**	20 → 18	10.5±9.3

^ difference between the 75th and the 25th percentiles

* Paired, two-sided Wilcoxon signed rank test since data not normally distributed

disease duration of 15 ± 11 (range 2-34) years at the time of surgery. Among these, 8 (40%) had generalized dystonia (3 DYT-1 and 1 DYT-6), 9 (45%) segmental dystonia, 2 (10%) hemidystonia and 1 (5%) had focal dystonia. Average follow-up postoperative interval was 14 ± 7 months (range 6-30). Table 1 summarizes the GDRS score change across different body parts, as well as the total score. It shows a significant response in neck, shoulder and proximal arm, distal leg and foot including knee as well as trunk in a differential pattern.

Conclusion

Pallidal DBS is an effective treatment for isolated non-acquired dystonia. There is likely a differential response to DBS among the various body regions.

Funding source(s), including NIH support, if any:
The study was supported by Dystonia Medical Research Foundation Clinical Fellowship grant to Margi Patel, MD

Probing the Inhibitory Motor Circuits in Adductor Laryngeal Dystonia During A Dystonia-Unrelated Finger-Tapping Task

Baothy Huynh[1], Mo Chen[2,3], Teresa J. Kimberley[1,4], Yi-Ling Kuo[5]

[1]Department of Rehabilitation Sciences, MGH Institute of Health Professions, Boston, MA
[2]Neuroscience Research Program, Gillette Children's Specialty Healthcare, St. Paul, MN
[3]Department of Psychiatry and Behavioral Sciences, University of Minnesota, Minneapolis, MN
[4]Department of Physical Therapy, MGH Institute of Health Professions, Boston, MA
[5]Department of Physical Therapy Education, SUNY Upstate Medical University, Syracuse, NY

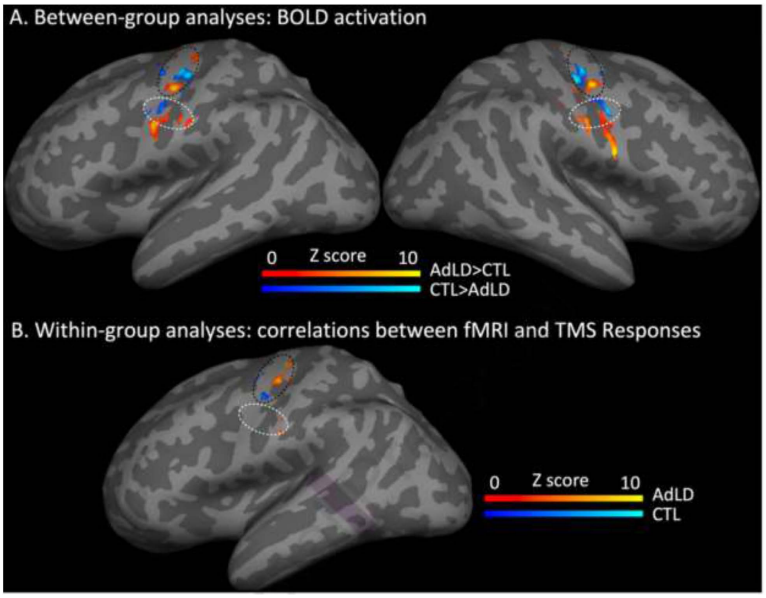


Figure 1 | Activated voxels were superimposed on the inflated brain surface. (A) Between-group comparisons of fMRI BOLD activation in M1 during either left or right finger-tapping. Warm colors represent AdLD > CTL contrast, and cool colors represent CTL > AdLD contrast. CTL showed greater activation within the hand motor cortex whereas AdLD showed dispersed activation to the inferior portion of the primary motor cortex, close to the laryngeal motor cortex indicated by the white dotted line. (B) Correlations between BOLD activation and cortical silent period (cSP) in the left hemisphere during right finger-tapping. There was no significant correlation in the right hemisphere (data not shown). AdLD demonstrated strong positive correlations between BOLD activation and intracortical inhibition within the hand motor cortex, while CTL showed no correlations in the hand motor cortex. AdLD: adductor laryngeal dystonia; CTL: controls; BOLD activation: blood-oxygen-level dependent activation; fMRI: functional magnetic resonance imaging; TMS: transcranial magnetic stimulation. Black dashed lines: estimated hand motor cortex projected on the inflated brain surface. White dashed lines: estimated laryngeal motor cortex projected on the inflated brain surface

Adductor laryngeal dystonia (AdLD) is a focal dystonia [FD] that impairs verbal communication due to excessive contraction of the intrinsic muscles in the larynx. This results in a strained, harsh, or tremulous voice impacting effective communication. The pathophysiology of AdLD is currently not known. However, growing evidence suggests that FD is associated with abnormalities in intracortical inhibition within the primary motor cortex (M1), as measured using transcranial magnetic stimulation (TMS). Previous work using task-based functional magnetic resonance imaging (fMRI) has also indicated abnormalities in the sensorimotor networks during phonation tasks. While neuroimaging and brain stimulation provide specific insights into the FD pathophysiology, it is unclear if TMS-derived inhibition relates to the response measured by fMRI. This study investigated the relationship between M1 cortical responses obtained by TMS and fMRI, two complementary techniques, in a dystonia-unrelated (finger-tapping) task in AdLD and healthy controls (HC). We hypothesized that, due to widespread abnormalities of inhibitory motor networks, AdLD would demonstrate greater neural activation in the M1 and increased intracortical inhibition during the dystonia-unrelated task. Sixteen AdLD (63.9±4.8 years) and 16 HC (51.5±7.9 years) completed fMRI and TMS assessments. Intracortical inhibition was assessed using TMS-evoked cortical silent periods (cSP) in the left hemisphere with responses measured from the right first dorsal interosseous. Neural activation was measured using blood-oxygen-level-dependent (BOLD) responses to a finger-tapping task. Results indicated cSP duration was significantly shorter in AdLD (88.41±22.55 ms) compared to HC (111.16±31.30 ms) (p=0.03, d=-0.83, 95% CI [-42.45, -3.05]). AdLD also demonstrated more dispersed BOLD activation responses not localized to the M1 hand region. Greater positive correlations were also found between BOLD and cSP in AdLD, as compared to HC (Figure 1). Overall, abnormalities in neural networks related to dystonia may not be limited to the representation of the dystonic musculature alone but may indicate more global inhibitory dysfunction.

Funding Sources

This work was partly supported by the National Institute of Deafness and Other Communication Disorders, National Institutes of Health (R21DC012344, R01DC015216).

Impaired Modulation Of Sensorimotor Cortex Mu Activity During Active And Passive Movement In Children With Dystonia And Dystonic Cerebral Palsy

Verity McClelland[1], Fischer Petra[2], Eleonora Foddai[1], Sofia Dall'Orso[3], Elena Cioffi[1], Jemima Tsang[1], Aaron Yurkewich[3], Etienne Burdet[3], Peter Brown[4], Jean-Pierre Lin[5]

[1]King's College London, London, United Kingdom
[2]Bristol Univeristy, Bristol, United Kingdom
[3]Imperial College London, London, United Kingdom
[4]University of Oxford, Oxford, United Kingdom
[5]Guys and St Thomas' NHS Foundation Trust, London, United Kingdom

Introduction
Sensorimotor processing is abnormal in adult genetic/idiopathic dystonia, but has rarely been explored in acquired or childhood dystonia. This study investigates cortical sensorimotor processing by measuring event-related desynchronization (ERD) and synchronisation (ERS) in response to passive and active movement tasks in children/young people with dystonia/dystonic cerebral palsy (CP).

Materials and Methods
The study received ethical approval. Informed consent was obtained from each participant or, if <16 years, the parent/guardian. 30 young people with dystonia (20 genetic/idiopathic; 10 dystonic CP) and 22 controls age 5-21years, participated in a passive movement task in which a robotic wrist interface delivered passive wrist extension movements, producing a brief stretch stimulus of the wrist flexors (10 degrees from neutral). Each hand was tested separately except for 5 patients, in whom only the dominant hand was tested. 23 participants (9 dystonia, 14 controls) also performed an equivalent active wrist extension task. Scalp EEG was recorded with a BrainVision amplifier using the 10-20 international system. Impedances were maintained below 10kOhm. EEG was amplified, filtered (DC-500Hz) and sampled at 2500Hz. Wrist position was monitored and movement onset synchronised with EEG recordings. Data were segmented into 4.5 second epochs (1 second pre- and 3.5 seconds post-stimulus). Epochs with inadequate wrist movement profile or contaminated by excessive movement or eye blink artefacts were rejected. Up to 160 data epochs were collected per subject. Time-frequency analyses were performed using continuous Morlet wavelet.

For the passive task, controls showed a prominent early alpha/mu ERD (0.5-1s post-stimulus) and later alpha/mu ERS (1.5–2.5s post-stimulus) over contralateral sensorimotor cortex. The dystonia group showed significantly smaller alpha/mu ERD compared with controls for the dominant (ANCOVA $F(2,47)=4.45$ $p=0.017$) and non-dominant hand (ANCOVA $F(2,42)=9.397$ $p<0.001$). Alpha ERS was also significantly smaller in dystonia than in controls for the dominant hand (ANCOVA $F(2,47)=7.786$ $p=0.001$). Findings were comparable for genetic/idiopathic dystonia and dystonic CP. For the active task, a similar pattern of reduced mu modulation was observed in dystonia compared with controls.

The impaired alpha/mu modulation indicates an abnormality of sensorimotor processing of proprioceptive information during both active and passive movement, which is common to many genetic/idiopathic dystonias and dystonic CP.

Using Ultrasound In The Assessment Of Dystonic Tremor

A.H. Snijders[1], J.L.M. van Doorn[2], R.C.G. Helmich[3], N. van Alfen[2]

[1]Department of Neurology and Clinical Neurophysiology, Donders Institute for Brain, Cognition and Behaviour, Center of Expertise for Parkinson and Movement Disorders, Radboud University Medical Center, Nijmegen, Netherlands
[2]Department of Neurology and Clinical Neurophysiology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, Netherlands
[3]Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Center of Expertise for Parkinson and Movement Disorders, Radboud University Medical Center, Nijmegen, Netherland

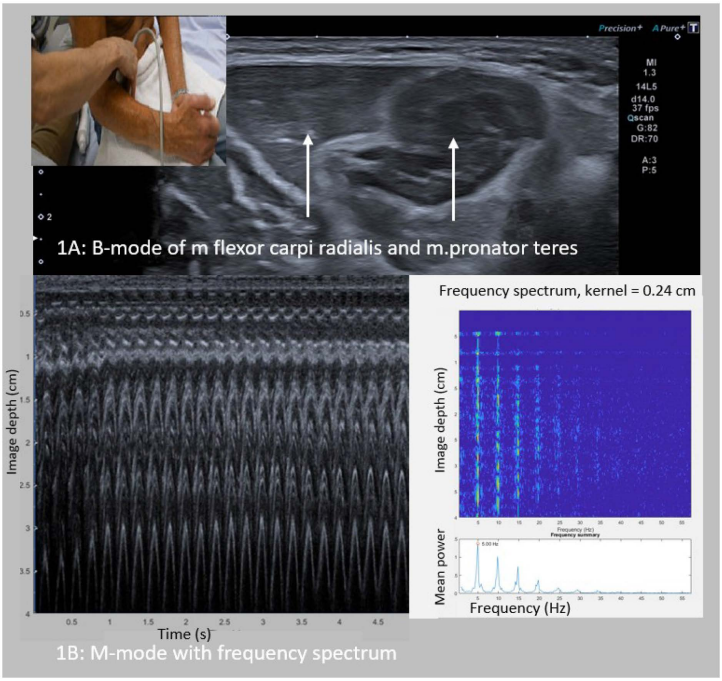


Figure 1 | use of ultrasound in dystonic tremor:
1A: B-mode still of video, showing anatomical 'yin yang' configuration of right pronator teres and flexor carpi, where in the video the pronator teres is very active.
1B: M-mode with frequency spectrum

Traditionally polymyography is used to select muscles for treatment with botulinum toxin in complex cases of dystonic tremor. Surface polymyography has a high temporal resolution and allows to investigate multiple muscles at the same time under different test conditions. In overlapping muscles, intramuscular (needle) polymyography is used. Needle polymyography of multiple muscles in a tremulous arm or neck can challenging for the examiner and painful for patients. Ultrasound may offer a solution.

We present a case of dystonic tremor to show the add-on value of ultrasound in the assessment of arm tremor.

A 78-year-old man presented with tremulous movements of the arms for 11 years, with insufficient effect of medical treatment. Based on jerky bilateral postural tremor (right > left) without resetting, dystonic posturing of the head and subtle dystonia of the right arm, he was diagnosed with dystonic tremor. To assess which muscles to select for botulinum toxin treatment, polymyography was performed, followed by ultrasound in B-mode (two dimensional) and M-mode (observing a single image line over time). Both surface polymyography and M-mode ultrasound showed a posture-dependent 5 Hz tremor. Polymyography showed most activity in biceps brachii, brachioradialis, supinator, flexor carpi radialis, and extensor digitorum communis muscles. With visual inspection of B-mode ultrasound video, tremulous activity could be specifically localized to the pronator teres, and not the flexor carpi radialis muscle (Figure 1). In addition, tremor in m. biceps brachii, m. triceps, and m. extensor digitorum communis was confirmed. Botulinum toxin injections in his biceps brachii, extensor digitorum communis and pronator teres gave the patient a satisfactory reduction of the tremor.

Ultrasound has a high spatial resolution, which is more accurate than polymyography when overlapping muscles are involved. B-mode ultrasound may help to select muscles for treatment with botulinum toxin. M-mode ultrasound can be used to quantify tremor frequency.

Generalized Dystonia, Neurodevelopmental Regression, And Premature Ovarian Insufficiency Due To An *IRF2BPL* Pathogenic De Novo Nonsense Variant

Laura Armengou-Garcia[1], Grace Yoon[2], Katja Lohmann[3], Marta Ruiz-Lopez[4], Anthony Lang[1]

[1]Edmond J. Safra Program in Parkinson's Disease, Rossy Progressive Supranuclear Palsy Centre and the Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada
[2]Division of Clinical and Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, M5G 1X8 and Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Canada
[3]Institute of Neurogenetics University of Lübeck Lübeck Germany
[4]University Hospital Cruces. Neurodegenerative Diseases Group, Biocruces Bizkaia Health Research Institute, Barakaldo, Bizkaia, Spain

Introduction

Interferon regulatory factor 2 binding protein-like (IRF2BPL) de novo variants have been linked to a Neurodevelopmental Disorder with Regression, Abnormal Movements, Loss of Speech, and Seizures (NEDAMSS). Dystonia may be a prominent manifestation.

Materials and Methods

A 19-year-old female, whose delivery and pregnancy were uneventful, achieved normal motor developmental milestones until the age of 7. She then developed progressive gait dysfunction, learning disability, generalized dystonia, dysarthria, and oculomotor abnormalities. By age 19 she was wheelchair-bound and communicated with a device. On examination, she was almost mute, had preserved simple-command comprehension, disconjugate gaze, absence of optokinetic nystagmus, spasticity with bilateral pyramidal tract signs, and generalized dystonia. Trials of levodopa and baclofen were unsuccessful. Botulinum toxin has been effective for generalized dystonia and spasticity for 5 years. Short-lasting episodes of upward eye deviation and dystonic posturing of the extremities without EEG correlate have been treated with lamotrigine.

Results

3T brain MR is shown in figure 1. Initial MR spectroscopy showed decreased N-acetyl-aspartate not evident on a later study. Further investigations were negative for lysosomal and metal storage diseases, organic aciduria, and autoimmune disorders. CSF 5-hydroxyindoleacetic acid was borderline low and homovanillic acid was decreased. Repeated EEGs were within normal limits. Initial diagnostic exome sequencing was negative but later re-analysis and novel sequencing revealed a de novo, nonsense pathogenic variant in the IRF2BPL gene (NM_024496.3:c.499C>T, p.Gln167*). Amenorrhea resulted in a diagnosis of premature ovarian insufficiency due to hypergonadotropic hypogonadism.

Discussion and Conclusion

NEDAMSS due to IRF2BPL mutations should be considered in these patients with prominent dystonia. IRF2BPL transcripts are expressed in multiple tissues with functions related to neuronal development, cell homeostasis, and regulation of gonadotropin-releasing hormone. To our knowledge, this is the first case reported to have premature ovarian insufficiency and supports the evaluation of gonadal function, both for management and to advance our understanding of disease mechanisms.

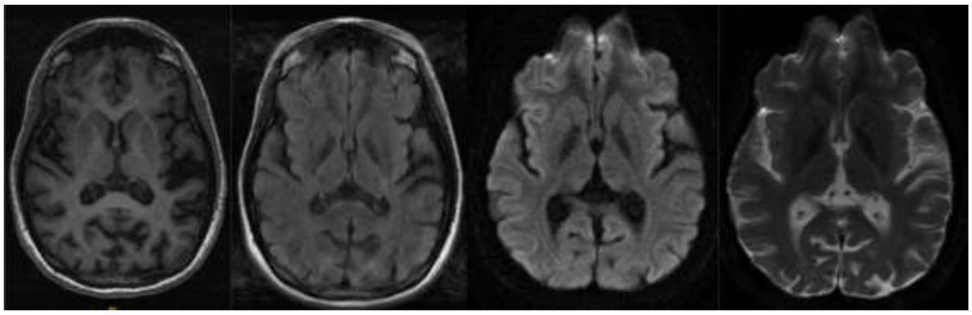


Figure 1 | Brain MR T1, T2 FLAIR, and diffusion weighted imaging sequences showing bilateral globus pallidus iron deposition and posterior atrophy in the parietal and occipital lobes

Funding source(s), including NIH support, if any
No funding sources.

Form vs Function: Inappropriate Behaviors In Cervical Dystonia Beyond Deficits Predicted By Social Cognition Testing

Matthew Woodward[1], Brian D. Berman[2], Jeanne Feuerstein[1]

[1]University of Colorado School of Medicine, Department of Neurology, Aurora, CO
[2]Virginia Commonwealth University School of Medicine, Department of Neurology, Richmond VA

Objective
Describe the discordance of social cognition testing and observed socially inappropriate behaviors in adult-onset focal cervical dystonia (CD).

Background
Impairment in social cognition has been identified in CD. Though impairment on formal social cognition testing is assumed to be reflected in real world behavior, the validity of this relationship is seldom studied. We present a case series of study participants with CD who participated in a social cognition study and were observed to exhibit socially inappropriate behavior during the study visit.

Methods
We reviewed data from our cross-sectional study evaluating social cognition in CD using the Social Norms Questionnaire (SNQ22).1 Socially inappropriate behavior exhibited during study visits was documented and categorized.2 If a participant repeated multiple acts in the same category the category was recorded only once for that participant.

Results
32 CD participants were included in this descriptive analysis. 31% (n=10) of the participants exhibited at least one socially inappropriate behavior during their study visit. Out of 20 behaviors recorded, 45% were categorized as tactlessness (poor manners), 10% as inappropriate emotional responses, 25% as social awkwardness, 5% as improper verbal acts, 5% as disagreeableness, 5% as improper physical acts, and 5% as inappropriate physical contact (see Table 1).

Discussion
We observed a variety of inappropriate social behaviors during a study of social cognition in CD that were not reflected in their SNQ22 testing results. For example,

Table 1 | SNQ-22 scores, answers on questions missed, breaking norms (B) vs over adhere (OA) and descriptions of behaviors

Patient	SNQ22 Score	Questions Missed	Socially Unacceptable Behaviors
1	0		Bit nails and spit them out, spoke at length regarding family wealth and salaries of family members
2	0		Put feet on table, picked nose
3	0		Hugged researcher (never met before), touched researcher's pregnant stomach after asked not to (3 times)
4	1	Tell coworker you think they lost weight (N/OA)	Discussed urination and left used tissues on the table
5	1	Cut in line (Y/B)	Told researcher to "freshen up", asked researcher their age, said they seemed young to be having a baby and that they looked pretty.
6	2	Pick up money on the sidewalk (N/OA) Ask coworker their age (Y/B)	Discussed personal relationship with husband and details about sex life.

(*Continued*)

(*Continued*)

7	2	Tell coworker your age (N/OA) Tell coworker you think they lost weight (N/OA)	Said room looked like a dungeon, discussed husband's health problems, cried twice, was defensive about performance
8	2	Tell stranger you like their hair (N/OA) Tell opinion of movie they haven't seen (N/OA)	Said room was a "nasty place", inappropriate jokes, inappropriate laughter, ate lunch during cognitive testing
9	3	Wear same shirt every day (Y/B) Laugh when someone else trips and falls (Y/B) Ask coworker their age (Y/B)	Took off shirt during visit because was inside out, brought burger and fries (and condiments) and ate them during study visit
10	4	Tell coworker your age (N/OA) Cry at movies (N/OA) Blow nose public (N/OA) Tell opinion of movie they haven't seen (N/OA)	Brought a note to explain how the researcher should interact including speaking directly and letting the participant know when she makes off handed comments, commented the room was dingy, that being pregnant looked uncomfortable and that she didn't know why she decided to do the study

SNQ 22= Social Norms Questionnaire 22, Questions missed identifies which question was missed and the answer provided by the subject is in parentheses

two participants directly answered questions incongruent with observed behaviors (one hugged the researcher but answered that it was not socially acceptable to hug a stranger, and another asked the researcher their age but answered it was not socially acceptable to ask a coworker their age). Our findings suggest self-report scales such as the SNQ22 may fail to detect some social cognition deficits in CD which may be better identified with alternative observation-based assessments.

Funding
The Movement Disorders Center (MDC) of the University of Colorado School of Medicine provided a pilot grant for this project

Development Of A Patient-Centered Outcome (PCO) Measure For Dystonia

Arlann Erskine[1], Paul Reyes[1], Fares Qeadan[2], Brian Berman[3], Sarah L. Schneider[4], Janet Hieshetter[5], Charlene Hudgins[6], Kimberly Kuman[7], Cynthia Comella[8], David Peterson[9], Mark Hallett[10], Gamze Kilic-Berkmen[11], Laura Wright[12], Samantha Pentecost[1], Joel S. Perlmutter[12], H. A. Jinnah[11], Sarah Pirio-Richardson[1]

[1]Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA
[2]Parkinson School of Health Sciences and Public Health, Loyola University, Chicago, IL, USA
[3]Department of Neurology, Virginia Commonwealth University, Richmond, VA, USA
[4]Department of Otolaryngology Head and Neck Surgery, University of California San Francisco, San Francisco, CA, USA
[5]Dystonia Medical Research Foundation, Chicago, IL, USA
[6]Benign Essential Blepharospasm Research Foundation, Beaumont, TX, USA
[7]Dysphonia International, Itasca, IL, USA
[8]Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA
[9]Computational Neurobiology Laboratory, Salk Institute for Biological Studies, and Institute for Neural Computation, University of California San Diego, La Jolla, CA, USA
[10]Human Motor Control Section, NINDS, NIH, Bethesda, MD, USA
[11]Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA
[12]Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

Background
The purpose of the Patient-Centered Outcome (PCO) Project is to establish clinical trial readiness for novel treatments of dystonia. Botulinum neurotoxin (BoNT) is a first-line therapy for focal dystonia including cervical dystonia (CD), blepharospasm (BSP), and laryngeal dystonia (LD). Although BoNT provides significant improvement, approximately one-third of patients discontinue use suggesting that BoNT therapy may not meet patient expectations.

Methods
We set out to develop a PCO that accurately captures the patient experience during therapy. After a modified Delphi process to identify candidate PCO items and based on FDA guidance, we surveyed a large number of patients with dystonia to explore the following: 1) PCO item relevance to the patient's disease; 2) PCO item importance to improve or change with therapy; and 3) minimal change in the PCO item that would be meaningful to the patient.

Results
We surveyed approximately 500 CD patients, 300 BSP patients, and 600 LD patients. All PCO items surveyed were rated as highly relevant to the patient experience and would be important to treat. A minimal meaningful change in the PCO items overall was reported by the majority of patients as 25% (CD), 20% (BSP), and 27% (LD).

Conclusion
We used robust patient engagement and verification to identify PCO items for CD, BSP, and LD with relevance to their disease and importance to reflect response to therapy. In addition, we have prospective data on what the minimal meaningful change will be in each PCO item that we can compare with the live data we are collecting from the 300 patients participating in this project.

Funding
DMRF

Development of a Smartphone Application Able to Capture Patient-Centered Outcome (PCO) Measures for Dystonia

Paul Reyes[1], Arlann Erskine[1], Brian Berman[2], Sarah L. Schneider[3], Janet Hieshetter[4], Kimberly Kuman[5], Cynthia Comella[6], David Peterson[7], Gamze Kilic-Berkmen[8], Laura Wright[9], Fares Qeadan[10], Samantha Pentecost[1], Joel S. Perlmutter[9], Sarah Pirio-Richardson[1], H. A. Jinnah[8]

[1]Department of Neurology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA
[2]Department of Neurology, Virginia Commonwealth University, Richmond, VA, USA
[3]Department of Otolaryngology Head and Neck Surgery, University of California San Francisco, San Francisco, CA, USA
[4]Dystonia Medical Research Foundation, Chicago, IL, USA
[5]Dysphonia International, Itasca, IL, USA
[6]Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA
[7]Computational Neurobiology Laboratory, Salk Institute for Biological Studies, and Institute for Neural Computation, University of California San Diego, La Jolla, CA, USA
[8]Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA
[9]Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA
[10]Parkinson School of Health Sciences and Public Health, Loyola University, Chicago, IL, USA

Background

Botulinum neurotoxin (BoNT) is a first line therapy for many types of dystonia and results in significant improvement, yet approximately one-third of patients discontinue use of BoNT suggesting that BoNT therapy may not fully address patient expectations. Symptom Snap was developed to capture Patient-Centered Outcome (PCO) measures across motor, disability, and psychosocial domains, enabling clinicians and researchers to characterize the therapeutic response to BoNT therapy over time on a frequent basis.

Methods
In collaboration with TekSynap, we developed a smartphone application able to capture PCO measures tailored for three major dystonia subtypes: cervical dystonia (CD), blepharospasm (BSP), and laryngeal dystonia (LD). The app is accessible to users on both Android and iOS operating systems.

Results
Symptom Snap features a user-friendly interface with easy-to-read text, making data entry a very straightforward process (Figure 1). Within the app, each major dystonia subtype has its own set of questions (16 questions for CD, 18 questions for BSP, and 15 questions for LD). All questions are formatted in a numerical rating scale, and some have an additional answer box to choose when appropriate. There are additional tools integrated into the app including a "Contact Us" menu option, which serves as a platform where users can submit troubleshooting inquiries, and a notification switch, which allows the app to send push notifications when a weekly questionnaire is due for submission.

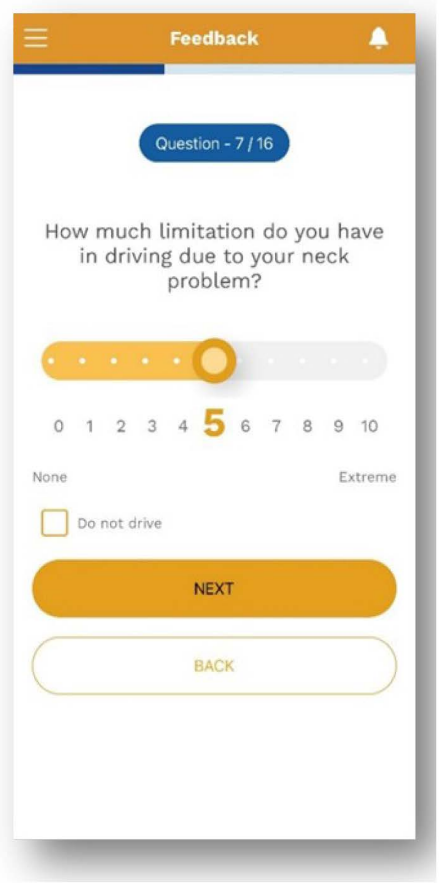


Figure 1 | Example of cervical dystonia questionnaire in Symptom Snap app

Conclusion
Symptom Snap will be tested as a primary outcome measure and, in the future, may be used as a journal for users with dystonia to document the impact that their symptoms have over various lifestyle domains. Not only will their data help to improve the care they receive, but also allow researchers to assess which domain(s) BoNT therapy does not fully address, helping provide direction in the development of novel treatments for dystonia.

Funding
DMRF

Treatment Of Task Specific Dystonia In Sports, A Systematic Review

B. Nijenhuis[1,2,3] E. van Wensen[3*], M. Smit[1,2], T. van Zutphen[5], J. Zwerver[4,6], M.A.J. Tijssen[1,2]

[1]Department of Neurology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands
[2]Expertise Center Movement Disorders Groningen, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands
[3]Department of Neurology, Gelre Ziekenhuizen Apeldoorn and Sports Dystonia Centre, Apeldoorn, The Netherlands
[4]Sports Valley, Sports Medicine, Gelderse Vallei Hospital, Ede, The Netherlands
[5]University of Groningen/Faculty Campus Fryslân, Leeuwarden, The Netherlands
[6]Center for Human Movement Sciences, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
*Presenting author at the 6th International Dystonia Symposium.

Introduction
Task specific dystonia is a movement disorder only affecting a highly practiced skill, and is found in a broad set of expert movements including in sports. Despite affecting many sports, there is no comprehensive review of treatment options, which is in contrast to better studied forms of task specific dystonia in musicians and writers. Studies involving an intervention to treat task specific dystonia in sports were systematically reviewed, with special attention for the quality of outcome measures.

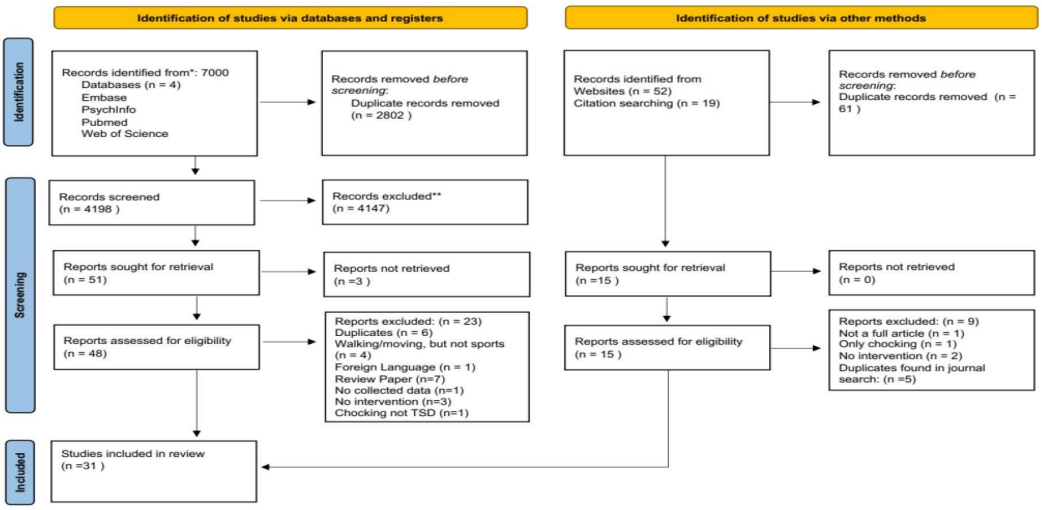


Figure 1 | Articles identified for inclusion in review

Table 1 | Interventions associated with task specific dystonia in sports

Non Invasive						
TSD-S	**N**	**Is**	**Ps/♂/♀**	**Intervention**	**Effect**	**MCRF (out of 16)**
Golf	9	7	27 ♂	Looking at the hole SFGI EMDR EFT Acupuncture PPR SMR	+ +/+/+ + + + + +	13 7/9/9 11 6 10 7 7
Running	3	4	15 (9♂/6♀)	Physical Therapy Weighted Pack SMR Sensory Trick	+ + + +	10 10 11 7
Rowing	1	1	1♂	CBT	+	8
Total	13	12	43			
Invasive/Pharmacological						
TSD-S	**N**	**Is**	**Ps/♂/♀**	**Intervention**	**Effect**	**MCRF (out of 16)**
Golf	3	7	20♂	BTX-A (2) DBS PPL THP/CLZ/BCF/TZD	+/- ++ + -/-/-/-	8/7 7 13 7
Running	6	9	26 (12♂/14♀)	BTX-A (5) VoTM CLZ (3) L-Dopa (4) CBZ (2) THP (4) DZP BCF	4 +/ 1 - CR +/+/- +/ 3- +/- 2 +/ 2 - + -	10/5/13/7/7 7 10/13/7 10/5/7/7 10/7 10/7/7/7 7 7
Rowing	1	1	1♂	L-Dopa	-	3
Table Tennis	2	3	2 (1♂/1♀)	VoTM (2) THP/CLZ	CR/+ -/-	7/10 7/7
Dancing	1	2	1 ♀	L-Dopa/ BZD	-/-	3
Tennis	2	2	3 ♂	VoTM THP	+ +	10 4
Juggling	3	3	3♂	BTX-A VoTM MMT	+ + +	3 10 4
Baseball	2	4	3♂	BTX-A LDCi THP/TBZ	- + -/-	6 6 3/3
Billiards	2	4	2♂	BTX-A (2) PPL/BZD/ACs	+/- -/-/-	5/4 4/4
Pistol shooting	1	1	1♂	BTX-A	-	4
Total*	21	17	62			

N = Number of articles, Is = Number of interventions, Ps = Number of participants, MCRF = McMaster Critical Review Form, SFGI = Solution Focused Guided-Imagery,
EMDR = Eye Movement Desensitization Reprocessing, EFT = Emotional Freedom Technique, PPR = Pre-Performance Routine, SMR = Sensory Motor Retraining,
CBT = Cognitive Behavioral Therapy, BTX-A = Botulin-Toxin type A injections, DBS = Deep Brain Stimulation, PPL = Propranolol, THP = Trihexyphenidyl, CLZ = Clonazepam,
BCF = Baclofen, TZD = Tizanidine, VoTM = Ventro-oral Thalamotomy, CBZ = Carbamazepine, DZP = Diazepam,
BZP =Benzodiazepine, MMT = Memenatine, LDCi = Lidocaine-injections,
TBZ = Tetrabenazine, ACs = Anticholinergics, - = no effect, + = some effect, ++ good effect,
CR = Complete Remission, * = doubles included

Methods
Guidelines for the Preferred Reporting Items for Systematic Reviews and Meta Analysis were followed.

Results
In April 2022 Pubmed, Embase, Web of Science, and Psychinfo were searched.

Of the 7000 articles identified, (Figure 1), 31 were included that described non-invasive psychological and invasive and/or pharmacological interventions. There was a lack of formal standardized outcome measures in studies resulting in low quality evidence for the effectiveness of treatment options.

A descriptive synthesis showed non-invasive emotional regulation was effective, but was exclusively tried in golfers. Invasive interventions like botulinum toxin or pharmacology had a similar effectiveness compared to studies in musicians dystonia, however there was almost no formal evidence for these treatments (Table 1).

Conclusion
1. The quality of studies was low with a lack of standardized outcome measures.
2. Future studies with larger cohorts and quantitative outcome measures are needed to improve understanding of treatments for task specific dystonia in athletes.

Funding source(s), including NIH support, if any:
None

Dystonia In A PFBC Cohort And Description Of 3 Peculiar Cases

Giulia Bonato, Miryam Carecchio

Movement Disorders Unit, Neurology Clinic, Neuroscience Department, University of Padova, Padova, Italy

Introduction
Primary familial brain calcification (PFBC) is a rare neurodegenerative disorder of adulthood characterized by calcium deposition in basal ganglia. Parkinsonism is the main manifestation. Dystonia is reported in up to 20% of cases in large series, with limited characterization.

Materials and Methods
Clinical, genetic (NGS) and radiological examination of a cohort of 65 PFBC patients.

Results
Dystonic features were observed in 15/65 patients (23% of the cohort, 60% females); mean age at onset was 47 years. Dystonic tremor was found in 73% of cases; upper limbs were the most affected body part (73%), followed by neck-face (3 subjects); 10 patients had concomitant parkinsonism and pyramidal signs, 3 cerebellar signs and 1 chorea. Psychiatric or cognitive symptoms were reported 50%.

Genetic analysis resulted negative in 6 patients (40%), whereas pathogenic mutations were found in 7 subjects (46.6%; 3 SLC20A2, 1 PDGFB, 1 PDGFRB and 2 MYORG), 2 are ongoing.

Dystonia was the most prominent feature in 3 patients.

The first is a 40-year-old female with a 2-year history of severe dystonia of the larynx, oromandibular district and blepharospasm (Meige syndrome); she also had brisk reflexes, mild upper limb tremor and anxiety; CT scan showed pontine involvement.

The second is a 70-year-old woman presenting at age 54 with cervical and laryngeal dystonia with associated tremor of upper limbs, parkinsonism and brisk reflexes; genetic testing detected a pathogenic mutation in PDGFB gene (p.Arg100Cys).

The third is a 48-year-old female with paroxysmal dystonic spasms in the right hand triggered by physical exercise or emotional stress lasting up to one minute, occurring several times a day from age 42, with good response to carbamazepine. She carried a mutation in SLC20A2 gene (p.Leu127Argfs44*).

Discussion
Dystonia was previously reported in 20% of PFBC symptomatic patients, often without details on anatomical distribution and disease course. We found dystonic manifestations in 23% of our cohort, being the prominent clinical feature in 4.6% of cases. Dystonia occurred in association with additional neurological and psychiatric manifestations in most cases and had a progressive

course in all but one case, that presented paroxysmal attacks and good response to carbamazepine.

Funding source(s), including NIH support, if any
No funding or support was received for this work.

Suitability Of Automated Writing Measures For Clinical Trial Outcome In Writer's Cramp

Noreen Bukhari-Parlakturk[1,2*], Michael W. Lutz[1], Hussein R. Al-Khalidi[3], Shakthi Unnithan[3], Joyce En-Hua Wang[4], Burton Scott[1], Pichet Termsarasab[5], Lawrence G. Appelbaum[6], Nicole Calakos[1,2,7]

[1]Department of Neurology, Duke University School of Medicine, Durham, North Carolina, USA
[2]Duke Institute for Brain Sciences, Duke University School ofMedicine, Durham, North Carolina, USA
[3]Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Durham, North Carolina, USA
[4]Georgetown University Medical School, Washington, District of Columbia, USA
[5]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[6]Department of Psychiatry, University of California, San Diego, California, USA
[7]Department of Neurobiology, Duke University School of Medicine, Durham, North Carolina, USA

Introduction
Writer's cramp (WC) dystonia is a rare disease that causes abnormal postures during the writing task. Successful research studies for WC and other forms of dystonia are contingent on identifying sensitive and specific measures that relate to the clinical syndrome and achieve a realistic sample size to power research studies for a rare disease. Although prior studies have used writing kinematics, their diagnostic performance remains unclear. This study aimed to evaluate the diagnostic performance of automated measures that distinguish subjects with WC from healthy volunteers.

Methods
A total of 21 subjects with WC and 22 healthy volunteers performed a sentence-copying assessment on a digital tablet using kinematic and hand recognition softwares. The sensitivity and specificity of automated measures were calculated using a logistic regression model. Power analysis was performed for two clinical research designs using these measures. The test and retest reliability of select automated measures was compared across repeat sentence-copying assessments. Lastly, a correlational analysis with subject- and clinician-rated outcomes was performed to understand the clinical meaning of automated measures.

Results
Of the 23 measures analyzed, the measures of word legibility and peak accelerations distinguished WC from healthy volunteers with high sensitivity and specificity. The measures of word legibility and peak accelerations also demonstrated smaller sample sizes suitable for rare disease studies. The kinematic measure of peak accelerations showed high reliability across repeat visits, while both word legibility and peak accelerations measures showed significant correlations with the subject- and clinician-rated outcomes.

Discussion
Novel automated measures that capture key aspects of the disease and are suitable for use in clinical research studies of WC dystonia were identified.

Funding sources for study
This work was supported by grants to NBP from Dystonia Medical Research Foundation (Clinical Fellowship Training Program), Doris Duke Charitable Foundation (Fund to Retain Clinician Scientists), American Academy of Neurology (career development award) and NIH NCATS (1KL2TR002554). NBP was also supported by a career development award from the Dystonia Coalition (NS065701, TR001456, NS116025) which is part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), supported by the Office of Rare Diseases Research (ORDR) at the National Center for Advancing Translational Science (NCATS), and the National Institute of Neurological Diseases and Stroke (NINDS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Variant Frequencies In Dystonia And Parkinson's Disease Genes Cross Phenotypic Boundaries

Lara M. Lange[1,2], Anastasia Illarionova[3], Karen Grütz[1], Eva-Juliane Vollstedt[1], Björn-Hergen Laabs[4], Sebastian Löns[5], Gamze Kilic-Berkmen[6], Frauke Hinrichs[1], Heike Pawlak[1], Laurel Screven[7], Tobias Bäumer[5], H. A. Jinnah[6], Norbert Brüggemann[1,2], Zih-Hua Fang[3], Katja Lohmann[1], Christine Klein[1,2], and *the Global Parkinson's Genetic Program (GP2)* and *the ProtectMove Research Unit*

[1]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany
[2]Department of Neurology, University Clinic Schleswig-Holstein, Lübeck, Germany
[3]German Center for Rare Neurodegenerative Diseases (DZNE), Tübingen, Germany
[4]Institute of Medical Biometry and Statistics, University of Lübeck, Lübeck, Germany
[5]Institute of Systems Motor Science (ISMS), University of Lübeck, Lübeck, Germany
[6]Departments of Neurology and Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USA
[7]Center for Alzheimer's and Related Dementias (CARD), National Institute on Aging and National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

Introduction

The impact of genetic variants on phenotypic expression is difficult to predict and ranges from high disease-related effects for pathogenic variants via low effects of risk variants to no (yet known) effect for benign variants. Comparative studies of variant frequencies between affected and unaffected carriers help in variant interpretation. However, the situation is complicated by phenomena like reduced penetrance and variable expressivity (e.g., dystonia-causing variants being present in patients with Parkinson´s disease (PD)).

Methods

The ProMoveGene sample, including individuals with PD, dystonia, and controls, was genotyped using the Global Screening Array (GSA) containing a custom content covering ~1000 rare variants in PD- or dystonia-related genes. Another ~1,000 individuals with early-onset or familial PD were analyzed using short-read genome sequencing as part of the Global Parkinson's Genetics Program (GP2) [1] and screened for rare variants in known dystonia (*TOR1A, THAP1, GNAL, ANO3, KMT2B, PRKRA, GCH1, SGCE)* or PD (*GBA1, LRRK2, SNCA, VPS35, PINK1, PRKN, PARK7)* genes. Only (likely) pathogenic variants and variants of uncertain significance (VUS) were considered.

Results

The ProMoveGene sample includes ~2,600 individuals with PD, ~4,400 with dystonia, and ~5,800 controls. Variants in dystonia genes were most frequent in individuals with dystonia (2.3%) and rare in PD patients and controls (0.5% each). While variants in PD genes were most frequent in PD patients (8.6%), a considerable number was also detected in dystonia patients (5.1%), and controls (4.4%) (Figure 1A).

Of the ~1,000 patients with early-onset/familial PD from GP2, 7.6% carried (likely) pathogenic variants or VUS in dystonia and 11.5% in PD genes (Figure 1B).

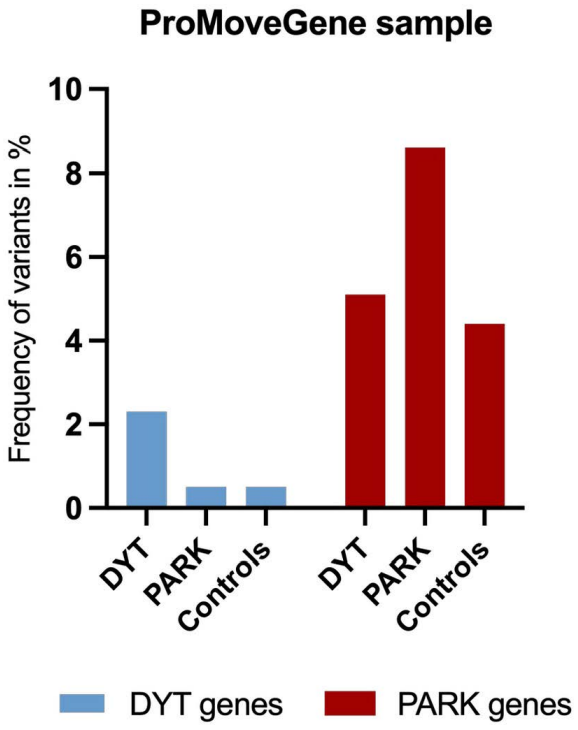


Figure 1 | **A. Detected variants in the ProMoveGene sample.** This chart reflects the percentage of patients with dystonia (DYT) or parkinsonism (PARK), and healthy controls with detected variants in dystonia (DYT; blue bars) or parkinsonism (PARK, red bars) genes via hotspot screening through GSA genotyping

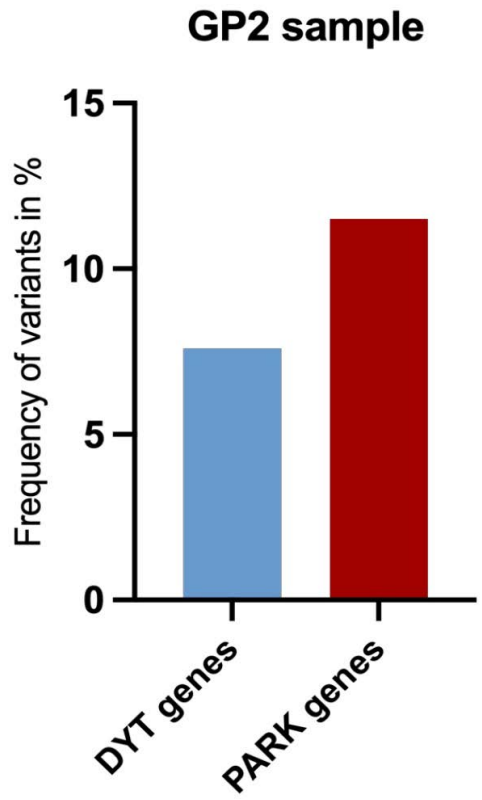


Figure 1 | **B. Detected variants in the GP2 sample.** This chart shows the percentage of individuals with detected variants in dystonia (DYT, blue) and parkinsonism (PARK, red) genes in the investigated GP2 sample, including patients with early-onset and/or familial PD, via whole-genome sequencing

Conclusion

Variants in dystonia genes can be found in patients with dystonia but also with PD and vice versa, and in controls, the latter may correspond to reduced penetrance or in case of VUS, are not disease-causing. The impact of these variants needs to be further investigated in larger sample sizes.

Funding sources

Lara M. Lange was supported by the Bachmann Strauss Dystonia Foundation.

Reference

1. Global Parkinson's Genetics, P., *GP2: The Global Parkinson's Genetics Program.* Mov Disord, 2021. **36**(4): p. 842-851.

Atypical Nuclear Envelope Condensates Linked To Dystonia Are Proteotoxic And Reveal Nucleoporin-Directed Chaperone Activities

Sarah Prophet[1], Anthony Rampello[1], Robert Niescier[1], Anthony Koleske[1,2], Sunanda Mallik[1], Christian Schlieker[1,3]

[1]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, USA
[2]Department of Neuroscience, Yale School of Medicine, New Haven, USA
[3]Department of Cell Biology, Yale School of Medicine, New Haven, USA

DYT1 dystonia is a debilitating neurological movement disorder arising from mutation in the AAA+ ATPase TorsinA. The hallmark of Torsin dysfunction across disease models is nuclear envelope (NE) blebbing resulting from defects in nuclear pore complex biogenesis. However, whether blebs actively contribute to disease manifestation is unknown, as we lack molecular markers that can be harnessed for functional investigations and probing possible implications for disease etiology. We developed a series of novel proteomic tools and model substrates, allowing us for the first time to define the proteome of NE blebs. We further report that FG-nucleoporins (FG-Nups) in the bleb lumen form aberrant condensates and contribute to DYT1 dystonia by provoking two proteotoxic insults. First, short-lived ubiquitylated proteins that are normally rapidly degraded partition into the bleb lumen and become stabilized. Additionally, our proteome analysis reveals that blebs selectively sequester a specific HSP40/HSP70 chaperone network in dependence of the bleb component MLF2, a poorly understood yet highly abundant component of these NE lesions. Using in vitro reconstitution, we show that MLF2 suppresses the ectopic accumulation of FG-Nups and modulates the properties and size of condensates in vitro. Importantly, MLF2 also potently suppresses amyloid formation of asparagine-rich FG-NUPs, and regulates the subcellular localization of DNAJB6, a key player for neuronal protein

homeostasis. These data lead us to propose a "two-hit" hypothesis for disease etiology of DYT1 dystonia. We posit that the combined cellular defects stemming from perturbed nuclear transport and a severe misbalance of neuronal protein homeostasis (proteostasis) are major contributing factors. Since NE blebs in animal models form transiently around the time of symptom onset, it is tempting to speculate that this proteostatic imbalance poses a transient threat for neurons that could be manipulated pharmacologically to reduce the risk of dystonia onset. Ongoing efforts in this direction exploiting MLF2 as biomarker for high-throughput screening will be discussed.

Funding
This work is supported by DOD PR200788 (CS), NIH 5T32GM007223-44 (SMP), NIH F31NS120528 (SMP), NIH R56MH122449 (AJK), NIH R01MH115939 (AJK), NIH NS105640 (AJK), NIH F31MH116571 (JEG) and the Dystonia Medical Research Foundation (CS and AJR).

Specific Cerebellar Spike Train Signatures Predict The Behavioral Presentation Of Cerebellar Pathophysiology

Meike E van der Heijden[1], Amanda M Brown[1], Dominic J. Kizek[1], Roy V Sillitoe[1]

Department of Neuroscience, Baylor College of Medicine, Houston, TX

Background and Objective
Dystonia can arise from dysfunctional cerebellar circuits. Yet, cerebellar dysfunction is also known to produce other movement disorders that can be comorbid with dystonia, namely ataxia and tremor. However, how altering the same cerebellar circuit can produce distinct movement defects remains unknown. We therefore set out to examine whether signals generated in the cerebellum can be used to distinguish unique predictive signatures that induce abnormal movements.

Methods
We performed *in vivo* awake head-fixed recordings of cerebellar output neurons, known as the nuclei neurons, in healthy control mice and mouse models of dystonia, ataxia, and tremor. We comprehensively defined the spiking activity of each neuron using twelve measurements. We trained an unsupervised classifier model on the spike activity measurements to differentiate neural signatures between dystonia, ataxia, tremor, and control mice. We tested whether different mouse models, but with similar phenotypes, displayed similar neural activity. We then used optogenetics to mimic the neural activity signatures associated with each disease phenotype.

Results
The classifier network found differences in spiking activity between dystonic, ataxic, and tremoring mice. More than half the neurons in mice with abnormal phenotypes had a spiking signature corresponding to the phenotypic presentation (dystonia, ataxia, tremor), irrespective of the mouse model used. Optogenetic stimulation of Purkinje cell terminals in the interposed cerebellar nucleus mimicked distinct neural activity signatures suggested by the classifier: a constant pattern (ataxia), a regularly oscillating pattern (tremor), or an irregularly bursting pattern (dystonia). Optogenetic stimulation caused abnormal motor phenotypes in freely moving mice.

Discussion and Conclusions
We show that alterations in cerebellar nuclei spiking activity predict the presentation of cerebellar movement disorders. We find that cerebellar models have distinct spiking signatures that are shared across mouse models with different etiologies and are sufficient to induce motor impairments in otherwise healthy mice.

Funding
This work was supported by Baylor College of Medicine (BCM), Texas Children's Hospital, The Hamill Foundation, and the National Institutes of Neurological Disorders and Stroke (NINDS) R01NS100874, R01NS119301, and R01NS127435 to RVS. Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number P50HD103555 for use of the Cell and Tissue Pathogenesis Core and *In Situ* Hybridization Core (the BCM IDDRC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the

National Institutes of Health. MEvdH was supported by a postdoctoral award from the Dystonia Medical Research Foundation (DMRF) and by the K99NS130463 (NINDS). A portion of the data were collected when AMB was supported by F31NS101891 (NINDS).

Dystonia Treatment With Injections Supplemented by TMS: The D-TWIST Study

Jessica Frey[1], John Yu[2], Janine Lobo Lopes[2], Lauren Fanty[2], Manahil Wajid[2], Adolfo Ramirez-Zamora[2], Irene A. Malaty[2], Jackson Cagle[2], Coralie de Hemptinne[2], Aparna Wagle Shukla[2]

[1]Department of Neurology, West Virginia University, Morgantown, WV
[2]Department of Neurology, University of Florida, Gainesville, FL

Introduction
Repetitive transcranial magnetic stimulation (rTMS) may prolong the benefits of BoNT for patients with cervical dystonia.

Materials and Methods
This was a double-blind, sham-controlled, crossover study for adult patients with cervical dystonia who report BoNT benefit lasting ≤ 9 weeks. At 9 weeks following BoNT, patients were randomized to active or sham neurostimulation: 1-Hz rTMS over the dorsal premotor cortex (dPMC) for 30 minutes (1800 pulses) at 90% resting motor threshold. Patients underwent 4 sessions per day for 4 consecutive days. Primary outcome was blinded video ratings of the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). Secondary outcomes included mood symptoms measured by Beck Depression Inventory (BDI) and safety and tolerability measured via patient-reported outcomes. Two-way ANOVA was used for statistical analysis. Outcome measures were collected at three time points: baseline, immediately after rTMS, and 2 weeks after rTMS.

Results
Demographically, the patient population (N=5) included: average age 68.7 years (range 52-79), 4/5 female, 1/5 had blepharospasm, 4/5 had isolated cervical dystonia, and 2/5 had sensory tricks. There was no statistically significant difference in TWSTRS scores, but there was a trend toward improvement in the active group compared to the sham group (Figure 1). There was a significant difference in BDI scores between groups (p =0.0495) although Bonferroni correction was unrevealing. All patients tolerated the stimulation sessions well, with 3/5 reporting transient headache/neck discomfort, which is typical for standard TMS sessions. In terms of blinding, 3/5 reported "no idea" which coil was the sham, and 1/5 correctly identified the sham coil suggesting effective blinding.

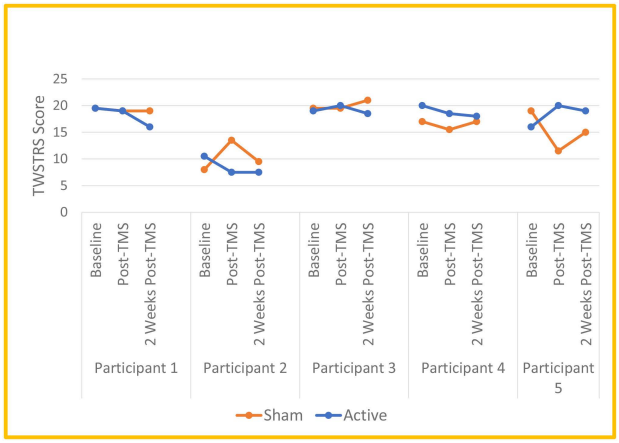


Figure 1 | Response to Active vs. Sham rTMS

Discussion
This novel, accelerated rTMS protocol is safe and well-tolerated for cervical dystonia. Synergism between rTMS and BoNT can potentially be used for dystonia patients experiencing suboptimal benefits with BoNT, and further work is needed to explore the non-motor and physiological implications of this intervention.

Funding source(s), including NIH support, if any
Dystonia Medical Research Foundation

Dystonia- History Of A Movement Disorder

P. Tacik

University of Bonn Medical Center, Department of Neurodegenerative Diseases and Geriatric Psychiatry, Bonn, Germany

Background
1911 is considered to be a crucial year in the history of dystonia due to a number of memorable publications. The German psychiatrist Theodor Ziehen coined in 1911 the term "tonic torsion neurosis" in relation to dystonia indicating its psychogenic origin. In 1911 the two Polish neurologists Edward Flatau and Władysław Sterling spoke of it as a "progressive torsion spasm" drawing attention to its genetic nature and stressing that it was not a muscular disorder. Finally, in the same year the German neurologist Hermann Oppenheim introduced the term "dystonia musculorum deformans" and postulated its organic cause. However, dystonia seems to have been known to the artists and physicians a long time before its first description in 1911.

Methods
Paintings and sculptures of famous artists such as Sandro Botticelli, Amedeo Modigliani, Constantin Brâncuși, Egon Schiele, Josep Ribera y Cucó, Stanisław Wyspiański, Pieter Bruegel the Elder, Pieter Brueghel the Younger, Matthias Grünewald, Franz Xaver Messerschmidt, Peter Anton von Verschaffelt, Francisco de Zurbarán were analysed for medical signs that could indicate the presence of dystonia in their models. In addition, excerpts from the 1983 film "Spring Symphony" by Peter Schamoni, handwriting samples and biographical data of Robert Schumann, Albert Schweitzer and Demosthenes regarding dystonia were studied.

Results
It seems plausible that Robert Schumann, Albert Schweitzer and Demosthenes suffered from different types of dystonia. Dystonic movement disorders due to ergotism can be suspected in works of Matthias Grünewald. Also works of the remaining artists are highly suggestive of different dystonic conditions.

Conclusions
Dystonia had been known to the artists and physicians a long time before it was first described in 1911.

Globus Pallidus internus Power Spectral Densities Progressively Change With Increasing Dystonia or Parkinsonism Severity

Angela Hewitt[1], Manuel Gomez-Ramirez[1], Karlo Lizarraga[1], Jonathan Mink[2]

[1]University of Rochester, Rochester, NY, USA
[2]Consultant, Pittsford, NY, USA

Introduction
Abnormal globus pallidus internus (GPi) neural activity likely contributes to both Parkinsonism and dystonia symptoms, but human investigations have been limited to recordings collected from patients shortly after deep brain stimulation (DBS) electrode surgery. Findings suggest that GPi local field potential (LFP) activity differs between dystonia and Parkinsonism. However, post-surgical changes (e.g. inflammation, microlesion effect) could affect neural firing and dystonia symptoms typically take weeks to respond to DBS therapy. The new Percept PC neurostimulator allows us to evaluate how GPi LFP activity correlates with symptoms over longer time periods.

Materials and Methods
We recorded 1-minute samples of LFP activity while subjects performed 3 trials each of finger taps with their right hand, left hand, or rest. Recordings were collected at multiple time points (minimum of 3 recording sessions) ranging from 3 weeks to 3 months post DBS surgery. Peak power spectral densities were determined for each subject and task. The area under the curve was calculated for different frequency bins. Ratios compared delta, theta, alpha, and beta activity with the severity of dystonia or Parkinsonism symptoms.

Results
Recordings were collected from 4 subjects (3 M, 1F) with different combinations of dystonia and Parkinsonism. Individuals with moderate to severe dystonia symptoms all had peaks in the delta-theta range (2-4 Hz). Both delta (3 Hz) and alpha (9 Hz) peaks were identified when moderate dystonia and Parkinsonism were both present. Conversely, a patient with Parkinsonism and no dystonia had relatively low delta-theta power and a prominent

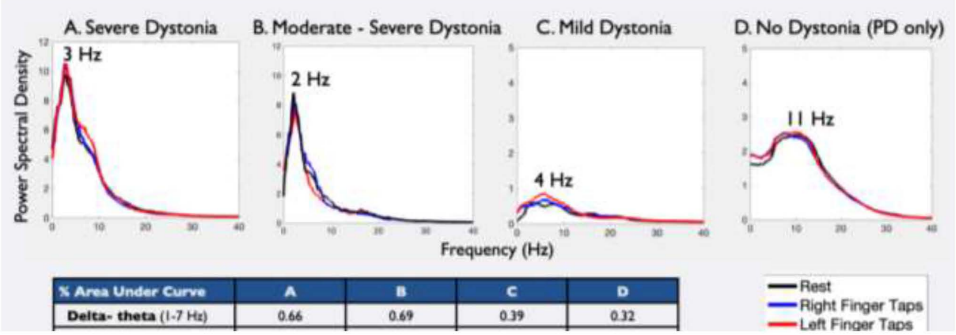


Figure 1 | Power spectral density (PSD) plots are progressively ordered from severe dystonia symptoms (left side) to mild/no dystonia (right). Note that the y-axis (PSD amplitude) changes across plots. (A) Parkinson disease (PD) + dystonia, with severe hand and neck dystonia during recording; (B) Dystonia secondary to cerebral palsy/stroke with moderate-severe bilateral dystonic hand posturing; (C) Idiopathic childhood onset truncal and facial dystonia with mild symptoms status post successful DBS therapy (>10 years); (D) PD only (no dystonia). Black text specifies the maximal peak frequency during rest, while line color denotes task (rest versus finger taps, see legend). Peak frequencies were consistent regardless of task. The table compares area under the curve (AUC) by frequency bin (i.e. delta-theta, alpha-beta, or gamma) for the above plots. Delta-theta frequencies were more prominent with moderate to severe dystonia, while alpha-beta activity decreases with increasing dystonia severity

alpha-beta peak. Peak frequencies for all subjects were consistent regardless of the task (Figure 1). Ratios of delta to theta power tended to increase with worsening dystonia severity, while alpha and beta ratios decreased.

Discussion

Preliminary analyses suggest GPi LFP activity correlates with dystonia and Parkinsonism severity, and may be a promising biomarker. Future analyses will evaluate response to DBS therapy.

Clinical Characterization and Treatment Outcomes Of VPS16 Dystonia

Mariel Pullman[1], Deborah Raymond[1], Walter Molofsky[1], Naomi Lubarr[1], Katherine Leaver[1], Roberto Ortega[1], Maya Rawal[1], Steffany Bennett[2], Evan Bushnik[2], Azita Khorsandi[3], Fedor Panov[4], Jean Paul Vonsattel[5], Laurie Ozelius[6], Rachel Saunders-Pullman[1], Susan Bressman[1]

[1]Neurology, Icahn School of Medicine at Mount Sinai, Mount Sinai Beth Israel
[2]Biochemistry, Microbiology and Immunology, and Chemistry, Neural Regeneration Laboratory, Ottawa Institute of Systems Biology, University of Ottawa
[3]Radiology, Icahn School of Medicine, Mount Sinai, New York
[4]Neurosurgery, Icahn School of Medicine at Mount Sinai
[5]Neuropathology, Columbia University Medical Center,
[6]Neurology, Massachusetts General Hospital and Harvard Medical School

Objective

To expand the clinical, radiologic and pathologic understanding of VPS16 related dystonia.

Background

Variants in VPS16 have been associated with isolated dystonia in 35 cases from Chinese and European cohorts, including report of brainstem signal change on MRI.

Design/Methods

We describe clinical features in 10 additional individuals with isolated dystonia and VPS16 variants, pathology in one, and perform pooled analysis with published cases.

Results

80% were men, mean age onset was 14.1 years (range 5-30), and mean age at exam was 35.89 years (range 11-73). Four began with arm, 3 with leg, and 3 with neck dystonia. Only one remained focal (arm); others became generalized (7), multifocal (1) or segmental (1). Brachial involvement was present in 90%, with 80% crural, 70% cranial/bulbar, and 50% cervical involvement. Neuropathologic evaluation in one case (post bilateral GPi DBS) demonstrated asymmetric severe gliosis and marked neuronal loss of the left subthalamic nucleus with optically empty vacuoles and much less right-sided involvement.

Four individuals underwent bilateral GPi DBS; two with significant improvement in all symptoms except speech, which was maintained for 8+ years; one with surgery at age 64 had approximately 50% response in limb and truncal dystonia but persistent dysarthria; a fourth with surgery in adulthood did not report significant benefit. A fifth had bilateral thalamotomy, and speech deficits were reported post-surgery.

Of the 10 participants, seven were known to have levodopa trials, two with significant early benefit, however this was not fully sustained, and three without improvement. In addition the following medications were utilized without dramatic improvement: trihexyphenidyl (7 participants), baclofen (4), benzodiazepines (5), carbamazepine (2), benztropine, haloperidol, thioridazine, reserpine, tetrabenazine (1 each).

Conclusions
Expansion of known cases of VPS16 dystonia further support that it is a childhood- or adolescent-onset disorder, typically with limb or cervical onset that often spreads to the arm and leg. While deep brain stimulation and oral medications may improve symptoms, additional therapies are needed as these lead to incomplete response. Additional histopathologic and metabolomic evaluation is underway; additional cases and pathologic samples will ultimately help elucidate the contribution of lysosomal and endolysosomal pathophysiology to VPS16 dystonia.

Funding source(s), including NIH support, if any:
Bachmann-Strauss Dystonia and Parkinson's Foundation, Bachmann-Strauss Professorship, Empire Clinical Research Investigator Program (MP, RSP), Dystonia Medical Research Foundation (MP, RSP, SBB, LJO, DR), National Institute of Neurological Disorders and Stroke (NINDS) (U01NS107016-01A1) (RSP, LO).

Canadian Institute of Health Research PJT 183604 (SB).

Validation Of A Clinical Rating Scale For Embouchure Dystonia

André Lee[1,2*], Tobias Mantel[2*], Eckart Altenmüller[1], Bernhard Haslinger[2]

[1] Institute of Music-Physiology and Musician's Medicine, Hannover, Germany
[2] Department of Neurology, Technical University Munich, Germany
*Co-first authors

Background
Embouchure dystonia (ED) is a task-specific movement disorder that leads to loss of fine motor control of the embouchure in wind-instrumentalists. Identification of involved muscles is challenging due to the variety of involved muscles (embouchure, laryngeal muscles or the tongue). This poses a major disadvantage for clinical and scientific assessment, especially since no validated clinical rating scale for ED exists. We aimed at validating an ED severity rating scale (EDSRS) allowing for a standardized estimation of symptom severity.

Methods
The EDSRS is composed of six items: three standardized tasks (sustained notes, scales, fourths) played at three pitch-registers assessed from a right and left lateral view to account for symptom asymmetries (three taskstwo sides=six items).

We video-recorded the six items in 17 musicians with ED. Recordings were assessed by two experts in ED on a 5-point Likert scale (0-4) with regard to playing ability/quality, giving an EDSRS range between 0-24 points.

We assessed internal consistency, inter- and intra-rater reliability (re-assessment by the raters after 30 months), reliability and construct validity with the fluctuation of the fundamental frequency (F0) of the sustained notes as an objective measure.

Results
The EDSRS showed high internal consistency (Cronbach's α=0.975–0.983, corrected item-total correlations r=0.90–0.96), inter-rater reliability (intraclass correlation coefficient [ICC] for agreement/consistency=0.90/0.96), intra-rater reliability (ICC per rater=0.93/0.87), good precision (standard error of measurement=2.19/2.65) and correlated significantly with F0 variability (r=0.55-0.60, p=0.011-0.023) (Figure 1).

Conclusion
The EDSRS is a valid and reliable clinical assessment tool of ED severity when applied by expert raters, with a high internal consistency as well as inter-rater and intra-rater reproducibility, fulfilling three of four proposed criteria for scores in MD: 1) reliable and valid, 2) specifically designed for MD and 3) practical in clinical setting. We consider it to be suited in everyday clinical routine and in clinical studies at clinics specialized in musicians' medicine.

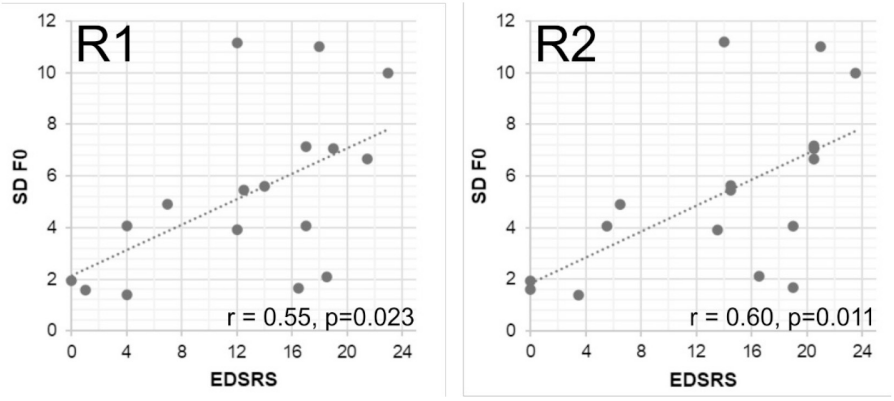


Figure 1 | Correlation of the EDSRS of both expert raters (R1 and R2) with the fluctuation of the fundamental frequency of the sustained notes (F0) as an objective measure of ED-severity. EDSRS=Embouchure Dystonia Severity Rating Scale; SD=standard deviation; R1/R2=Rater1/Rater2

Funding sources
University of Music, Drama and Media Hannover, Germany

Proprioceptive Stimuli Trigger Abnormal Micro-Scale Neuronal Connectivity In Children With Dystonia

Dimitris Sakellariou[1], Sofia Dall'Orso[2], Etienne Burdet[2], Jean-Pierre Lin[3], Mark Richardson[1], Verity McClelland[1]

[1] King's College London, London, United Kingdom
[2] Imperial College London, London, United Kingdom
[3] Guys and St Thomas' NHS Foundation Trust, London, United Kingdom

Introduction

Dystonia is a network disorder resulting from dysfunction of, or abnormal connectivity between, sites within the cortico-basal ganglia-cerebellar network. Despite the dynamic nature of the disorder, stimulus-related changes in neuronal connectivity have not previously been explored. This study investigates modulation of functional neuronal connectivity by a proprioceptive stimulus in young people with dystonia.

Materials and Methods

Sixteen young people with dystonia and eight controls participated (age 6-20years, mean 12.5). The study received ethical approval and informed consent was obtained from each participant or, if <16 years, the parent/guardian. A robotic wrist interface delivered passive wrist extension movements of the right upper limb, producing a brief controlled stretch of the wrist flexors (10 degrees from neutral position, rise-time 240ms). Scalp EEG was recorded with a BrainVision amplifier using the 10-20 international system, with impedances below 10kOhm. EEG was amplified, filtered (DC-500Hz) and sampled at 2500Hz. Wrist position was monitored and movement onset was synchronised with EEG recordings. Data were segmented into 4.5 second epochs (1 second pre- and 3.5 seconds post-stimulus). Manual artefact rejection was performed to remove epochs with inadequate wrist movement profile and those contaminated by excessive movement or eye blink artefacts. Up to 160 epochs were averaged to produce a Stretch Evoked Potential (Stretch EP) in each individual lived networks related to the Stretch EP. Global connectedness (GC) was calculated to estimate the spatial extent of the StretchEP networks.

Results

Clear Stretch EPs were evoked over contralateral sensorimotor cortex, with similar amplitudes between groups. Individual dynamic connectivity maps revealed a strik-

ing difference between dystonia and controls, with particularly strong event-related connectivity in the theta (4-8Hz) band in dystonia, across multiple brain regions. At group level, theta band connectivity (GMC) was significantly higher in dystonia than controls (p=0.045). GC was also stronger in dystonia than controls (non-significant trend).

Discussion/Conclusion
Young people with dystonia show an exaggerated dynamic network response to proprioceptive stimuli, displaying excessive, widespread theta-band synchronisation and over-recruitment across the sensorimotor network.

In vivo Assessment Of Striatal Compartments In Patients With Upper Limb Dystonia

Artur Marques Paulo[1], Jeffrey Waugh[2], Joselisa Péres Queiros de Paiva[1], Danilo Donizete de Faria[3,4], João Ricardo Sato[5], Vanderci Borges[3], Sonia Azevedo Silva[3,4], Henrique Ballalai Ferraz[3], Patricia de Carvalho Aguiar[1,3]

[1]Hospital Israelita Albert Einstein, Sao Paulo, Brazil
[2]The University of Texas Southwestern, Dallas, USA
[3]Universidade Federal de Sao Paulo, Sao Paulo, Brazil
[4]Hospital do Servidor Público Estadual de Sao Paulo, Sao Paulo, Brazil
[5]Universidade Federal do ABC, Santo Andre, Brazil

Introduction
The striatum is an essential hub in the motor system associated with dystonia and other movement disorders. The function of the striosomes and matrix in motor control is not clear. Anatomopathological findings in some types of dystonia indicate that striosomes are affected. A recently developed method using diffusion tensor imaging (DTI) enables us to distinguish compartments of the striatum, namely matrixes-like and striosomes-like voxels, *in vivo.* This study aims to assess the volume of striatal matrix and striosome compartments in patients with idiopathic upper limb dystonia using DTI.

Methods
We analyzed 3T MRI images from 26 patients with idiopathic upper limb dystonia aged 43.88 ± 11.32 years (SD; range 19–60) with a mean disease duration of 12.55 ± 10.25 years (SD; range 1–25) and healthy controls aged 39.42 ± 11.42 years (SD; range 19–58).The striatum was parcellated by targeting cortical regions that favored striosomes (pregenual anterior cingulate, posterior orbitofrontal, anterior insular cortex, and basolateral amygdala) and matrix-favoring areas (gyrus rectus, supplementary motor area, and primary sensory and motor cortex). The bilateral striatum was assessed for changes in volume using fractional anisotropy.

Results
Patients with dystonia showed a significant reduction of the left matrix-like voxels volume relative to controls ($p = 0.022$) with a moderate effect size (Cohen's $d = 0.640$) and a trend of decreased mean FA values in left Striosome-like voxels ($p = 0.063$) with a moderate effect size (cohen's $d = 0.510$). No difference was observed in the right matrix and striosome compartments. The disease duration showed a weak negative correlation with left Matrix-like voxels volume but was not significant (Pearson $r = -0.34$, $p = 0.076$).

Conclusions
By parcellating the striatum into striosome and matrix-like voxels, we showed that patients with idiopathic dystonia have a trend of diminished volume in striosome-like voxels, in agreement with anatomopathological findings of some genetic types of dystonia. Even in non-degenerative dystonias, volume differences may reflect an imbalance between striosome and matrix signaling, ultimately favoring the direct pathway. Studies with larger samples and different types of dystonia may improve our knowledge on this subject.

Funding source(s)
FAPESP grant #2021/14108-4 and CNPq grant # 402982/2021-5

Analysis Of Functional Connectivity Using Machine Learning And Deep Learning in EEG Data From Patients With Focal Dystonia

Caroline L. Alves[1,2], Artur José Marques Paulo[3], Danilo Donizete de Faria[4], João Ricardo Sato[5], Vanderci Borges[4], Sonia de Azevedo Silva[4,6], Henrique Ballalai Ferraz[4], Francisco Aparecido Rodrigues[1], Christiane Thielemann[2], Patrícia de Carvalho Aguiar[3,4]

[1]Universidade de Sao Paulo (USP), Sao Paulo, Brazil
[2] Aschaffenburg University of Applied Sciences, Aschaffenburg, Germany
[3] Hospital Israelita Albert Einstein, Sao Paulo, Brazil
[4] Universidade Federal de São Paulo, Sao Paulo, Brazil
[5]Universidade Federal do ABC, Sao Paulo, Brazil
[6]Hospital do Servidor Público Estadual de Sao Paulo, Sao Paulo, Brazil

Introduction

Recent evidence suggests a brain network disorder as the main explanation for the sensorimotor program dysregulation in dystonia. Current diagnostic approaches rely mainly on clinical features and some cases may pose diagnostic challenges with a lack of accuracy. The aim of this study is to apply novel machine learning (ML) and deep learning methods (convolutional neural networks-CNN) in order to identify a dystonia signature.

Methods

EEG from patients with idiopathic right upper limb focal dystonia (N= 20) and controls (N = 21) during resting-state, writing and finger-tapping were. As a data augmentation technique, time series were split into windows of 10 seconds, which were correlated to construct a connectivity matrix through eight distinct pairwise statistical metrics to feed our ML approach, determining the optimum metric for distinguishing brain connectivity patterns between groups. We used the Shapley (SHAP) value method to interpret the results [figure1].

Results

All the classifications resulted in excellent performances with AUC and an accuracy of 99%. The best metric was transfer of entropy. The primary connections related to dystonia were, in order of importance: C4-CP5, F3-FC5, C4-TP9. Regarding CNN, the best EEG task was resting, followed by finger-tapping.

Conclusions

Overall, our methodology was capable of capturing the brain alterations caused by dystonia with a high acuracy, despite a small sample size. Our technique outperformed existing research because we employed connectivity matrices, better representing EEG brain changes with less computation cost than raw time series. Using a low-cost technique such as the EEG, this method provides high accuracy in diagnosing focal right upper limb dystonia and can be further explored with other types of dystonia. It can also help to expand the knowledge regarding dystonia's pathophysiology with the potential to be applied to design brain-machine interfaces to treat this condition.

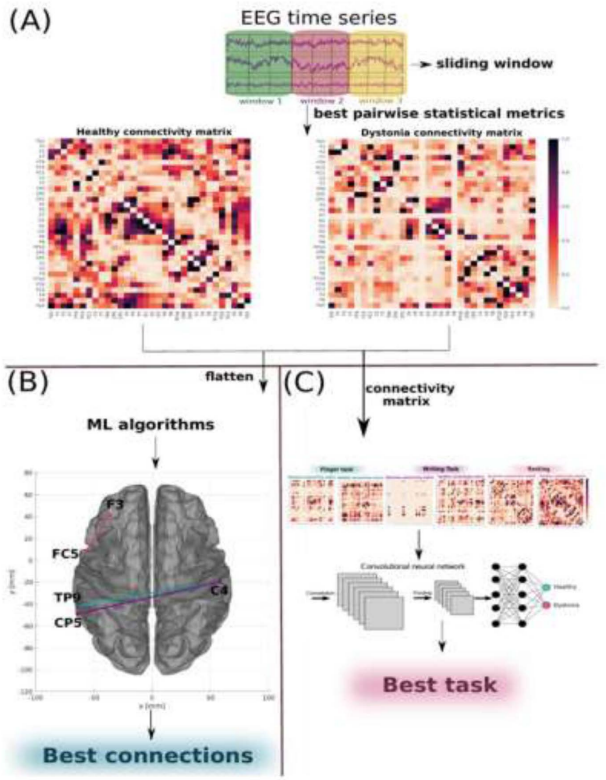


Figure 1 | **Methods for the diagnosis of dystonia using EEG data and Machine learning (ML)**
In (A), the EEG time series are split into windows (sliding window technique), then eight different pairwise statistical measures are employed to correlate EEG time series, which are examined by the ML algorithm to determine the best measure for capturing dystonia brain network changes. In (B), the best measure's connectivity matrices are fed into the ML to determine the best connections impacted by this disease. Finally, in (C), the connectivity matrices fed the Convolutional Neural Network method for evaluating the EEG task more critical for capturing brain alterations due to dystonia

Funding source(s)
FAPESP grant #2021/14108-4 and CNPq grant # 402982/2021-5

Deep Brain Stimulation Evoked Potentials (DBSEPs) In Children With Dystonia

Verity McClelland[1], Antonio Valentin[1], Eleonora Foddai[1], Tim Dennison[2], Jean-Pierre Lin[3]

[1]King's College London, London, United Kingdom
[2]University of Oxford, Oxford, United Kingdom
[3]Guys and St Thomas' NHS Foundation Trust, London, United Kingdom

Introduction
Pallidal Deep Brain Stimulation (DBS) is an established therapy for dystonia in adults and children. Stimulation parameters, including selection of active electrode contacts, are currently determined empirically. Recording the cortical response to the pallidal stimulus, using a technique called Deep Brain Stimulation Evoked Potentials (DBSEPs)[1,2] could provide a more objective measure of optimal contact selection. This study reports on the feasibility of conducting this investigation in children with genetic/idiopathic or acquired dystonia in a UK national referral centre for paediatric neuromodulation.

Materials and Methods
Fifteen young people (age 7-19years, mean 13.71) with dystonia participated, all with bilateral pallidal DBS implanted at least 6 months previously. Dystonia was genetic/idiopathic in eight and acquired in seven. Scalp EEG was recorded with an ASA (ANT-Neuro) system, using 10-20 international electrode placement and sampling at 5kHz. Impedances were reduced below 10kOhm. Contacts from the left and right stimulating electrode were tested separately, using 6Hz bipolar stimulation, with the other side turned off. Pairs of electrode contacts were tested sequentially using combinations of cathodal stimulation with an adjacent anode. Voltage was 2V, with pulse-width maintained at the patient's therapeutic level. Each combination was recorded for 3 minutes, giving >1000 stimuli per contact pair. Therapeutic settings were restored on study completion.

Offline, the DBS stimulus artefact was used to segment EEG data into epochs (10ms pre-stimulus, 150ms post-stimulus). Raw data were inspected manually and portions of data.

Results
Clear DBSEPs were obtained in 12/15 patients from one or both stimulation sides. Peak latency was 20ms. The largest amplitude response was recorded over the ipsilateral central/centro-parietal region in each patient. Both the peak latency and topography were in keeping with adult literature [1]. In 3/15 patients no convincing DBSEP was obtained from stimulation of either side.

Discussion/Conclusion
This study confirms the feasibility and tolerability of this technique in children with DBS for dystonia. The findings are consistent with adult studies [1], confirming reproducibility of the method. Future work will investigate the potential clinical application of DBSEPs to inform an objective and individualised approach to DBS contact and parameter selection.

References

[1] Tisch et al. 2008. Movement Disorders. 23:265-73.
[2] Bhanpuri et al 2014. Brain Stimulation 7:718-26.

Real-World Use Of Clinical Scales To Assess Botulinum Toxin Efficacy In Cervical Dystonia Treatment

Benjamin Wäschle[1,2], Denis Vézina[3], José Manuel Masso[4], Georg Comes[2], Holger Stark[1], Philipp Albrecht[5,6]

[1]Department of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Germany
[2]Merz Therapeutics GmbH, Frankfurt, Germany
[3]Merz Pharma Canada Ltd., Burlington, Ontario, Canada
[4]Merz Pharma España S.L., Madrid, Spain
[5]Department of Neurology, Heinrich Heine University Düsseldorf, Germany
[6]Department of Neurology, Maria Hilf Clinic, Mönchengladbach, Germany

Introduction
Botulinum toxin type A (BoNT/A) is the first-line treatment in symptomatic management of cervical dystonia (CD) and repeated injections are required for life-long symptom control. Treatment can be complex and documenting the effect is an important tool to optimize patient outcomes. A previously conducted survey showed the heterogeneity of efficacy scales used in routine clinical practice. This follow-up survey further characterizes real-world use of different scales.

Materials and Methods
38 centers in Canada, Poland, and Spain completed an online survey including questions about number of CD patients, frequency of injection sessions, type and frequency of efficacy scales, type of quality of life (QoL) scales, and safety.

Results and Discussion
The centers reported a total of 5968 CD patients currently treated with BoNT/A. Average number of injection sessions per year was 3.8 (3.6, 3.7, and 4.0 in Poland, Spain, and Canada, respectively). 29 of 38 centers documented patient-reported waning or duration of effect, and the type of guidance technique used; 71 % documented patient BoNT/A treatment in other indications (including Aesthetics) and 84 % documented suspected cause of treatment issues such as lack of effect. 27 centers used at least one type of efficacy scale while 11 centers did not routinely assess efficacy using a scale. Most frequently used was a 7-point Global Impression of Change Scale (GICS, patient or physician), reported by 21 centers who used the GICS in 81 % of their patients. The Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) was used in 14 centers and application rate varied between countries (15 % and 69 % in Spain and Poland, respectively). Only one center in Canada reported use of the TWSTRS. Various other scales were reported including the Tsui scale, Visual Analogue and Likert scales. Only 21 % used a questionnaire to assess QoL (SF-36, EQ-5D, CDC24).

This survey confirms the previously reported heterogeneity of efficacy scales and further gives evidence of the variability within centers how efficacy scales are applied. While the importance of quantifying quality of life is increasingly recognized, use of standardized patient reported outcomes remains the exception.

Funding Source
This survey was funded by Merz Therapeutics GmbH, Eckenheimer Landstrasse 100, 60318 Frankfurt.

Device-Led Social Cognition Measurement In Cervical Dystonia (Proof Of Concept)

Rory Miley[1], Shameer Rafee[1,2], Richard B. Reilly[1,2,3]

[1]The Trinity Centre for Biomedical Engineering, Trinity College, The University of Dublin, Ireland
[2]Dept of Neurology, St Vincent's University Hospital, Dublin, Ireland
[3]School of Engineering, Trinity College, The University of Dublin, Ireland

Introduction
Patients with cervical dystonia (CD) suffer from motor and non-motor symptoms. The non-motor symptoms include sensory, psychiatric and cognitive features. We have previously published studies on deficits in social cognition in patients with CD. One aspect of this is difficulty in recognising facial expression. This can affect interpersonal relationships; patients' understanding of the emotions of others, and provide insights into disease mechanism.

Several physiological signals (heart rate, galvanic skin response, blood pressure) show abnormal responses in patients with social cognition deficits. Their measurement may have potential in the diagnosis of social cognition. A wearable device to assess responses in certain conditions, such as during conversations with others, can track physiological changes in social cognitive abilities. Such a device, with an externally facing camera, can provide feedback for accurate recognition of facial expressions.

Objective
1) Design a device that integrates physiological signals and a camera to aid in diagnosing social cognition deficits and provide feedback to participants. 2) To test the system on volunteers with cervical dystonia and healthy volunteers.

Methods
Our device integrates a heart rate sensor, galvanic skin response sensor, a blood oxygen sensor and a camera on a portable single-board computer. Data is transferred via Wi-Fi to a workstation for real-time processing. We tested the device with healthy and cervical dystonia volunteers under a range of facial expression recognition tasks from a standardised database. A convoluted

neural network was used to automatically detect facial expressions and integrate the time-locked physiological signals.

Results
Our device reliably measures physiological signals. The camera facing the wearer detects facial expressions of the user and can face externally to analyse facial expressions of others. Emotional expressions are consistently captured and, using our machine learning algorithm, our device provides accurate feedback.

Conclusion
This proof-of-concept study provides evidence for a larger trial. We aim to recruit more patients with CD and healthy volunteers. No gold standard exists for social cognition measurements, and clinical interviews are time-consuming. Device-led measurements could act as diagnostic aids, reduce the burden of diagnosing social cognitive deficits and act as a therapeutic tool.

Funding source
The Trinity Centre for Biomedical Engineering, Trinity College, The University of Dublin.

The Role Of Physical Therapy In The Management Of Dystonia

Darcy Cooper

Duke Health System, Durham, NC, USA

When to Consider PT
PT may beneficial for individuals with focal dystonia with pain, impaired functional mobility of impaired gait. Consider PT when there are underlying musculoskeletal issues such as strength, range of motion, posture, flexibility and coordination deficits. PT can address poor biomechanics. PT can enhance the effects of botulinum toxin injections.

PT Evaluation
PTs will assess multiple systems including musculoskeletal (pain, ROM, strength, joint mobility, neural dynamics), sensory (laterality, sensory tricks, localization, proprioception), autonomic (vitals, breath assessment), motor control (biomechanics, coordination, speed), and psychosocial (stress, anxiety, coping strategies).

Goals
Pain reduction. Improve physical fitness and wellbeing. Address underlying musculoskeletal factors. Promote more optimal movement. Take an active role in managing the condition.

Common Treatment Strategies
Stop the abnormal movement (use of sensory tricks, change in position or environment or speed, break task into components). Quiet the nervous system. Promote positive health behaviors. Improve biomechanics. Improve sensory discrimination skills (including graded motor imagery). Retrain more optimal movement. Use of the OPTIMAL Theory of Motor Learning.

Research
PT + botulinum toxin injections may reduce future botulinum dose and increase duration of dose. This may be mediated through modulation of sensorimotor plasticity.[1]

No funding support.

Reference

[1] Hu W, Rundle-Gonzalez V, et al. A randomized study of botulinum toxin injections versus botulinum toxin plus physical therapy for treatment of cervical dystonia. *Parkinsonism and related disorders.* 2019;63:195-198.

Is Sinusoidal Head Tremor And Jerky Movements Characterized By Similar Basal Ganglia Neurophysiology In Dystonia?

Alexey Sedov[1], Indiko Dzhalagoniya[1], Svetlana Usova[1], Anna Gamaleya[2], Alexey Tomskiy[2], Aasef Shaikh[3]

[1]Semenov Research Center of Chemical Physics, Moscow, Russian Federation
[2]N. N. Burdenko National Medical Research Center of Neurosurgery, Moscow, Russian Federation
[3]Case Western Reserve University, Cleveland, USA

The relationship between two common movement disorders, dystonia and tremor, is controversial. Recent study showed biological differences in the basal ganglia network behavior in pure cervical dystonia or cervical dystonia combined with irregular head oscillations compared with cervical dystonia with sinusoidal oscillations. We tested the hypothesis that sinusoidal tremor has a different nature than jerky movements. We analized single unit activity of 1629 pallidal (GPi and GPe) cells, registered by microelectrode during DBS surgeries in 27 dystonic patients. We divided neurons into 3 groups – burst, pause, and tonic by means of an unsupervised clusterization. Then we calculated the firing rate, spike regularity, oscillation scores, burst and pause indexes, and other parameters of pallidal cells. We also performed spectral analysis of local field potentials (LFP) recorded by means of externalized LEADs on the second day after DBS surgeries in 5 dystonia patients. We compared parameters of pallidal activity between 3 groups: pure dystonia (Dys), dystonia with oscillatory tremor (OST) and dystonia with jerky movements (Jerky). We found robust differences in both firing rate and firing pattern of pallidal cells between OST and Jerky groups as well as between OST and Dys group and no differences between Dys and Jerky group. We found significant increase of pause cells percentage in both GPi and GPe nuclei. Burst and pause cells in OST group were also characterized by lower firing rate, higher irregularity and more bursty pattern compared to the other two groups. The differences were observed in GPi in cervical dystonia and in GPe in generalized dystonia. Spectral analysis of LFP showed significantly higher theta and alpha oscillations in the OST group and higher low beta activity in the Dys group. Our results prove hypothesis that pure dystonia and dystonia with jerky movement have similar neurophysiological mechanisms while oscillatory tremor could be an additional symptom, having a different origin. On the other hand, our data showed that theta-alpha activity, which has been considered as a biomarker for dystonia, appears to be a biomarker for dystonic tremor. The study was funded by the Russian Science Foundation (23-15-00487).

Funding source(s)
Russian Science Foundation (Grant 23-15-00487).

Lost Of Pallidal Multifractal Complexity Is Regained During DBS In Patients With Dystonia

Ulia Semenova[1], Indiko Dzhalagoniya[1] (Presenting), Anna Gamaleya[2], Alexey Tomskiy[2], Alexey Sedov[1,3]

[1]N.N.Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow, Russian Federation
[2]N. N. Burdenko National Scientific and Practical Center for Neurosurgery, Moscow, Russian Federation; Moscow Institute of Physics and Technology, Dolgoprudny, Moscow Region, Russian Federation

The internal segment of globus pallidus (GPi) is the most common target for surgical treatment of dystonia. Currently, low-frequency 3-12 Hz oscillations in the globus pallidus are considered the only potential biomarker of pathological activity in dystonia (Neumann et al., 2017). However, it remains unclear what rearrangements of the pattern temporal organization are behind the emergence of these rhythms. It is assumed that the emergence

of simpler dynamics can be seen as a degradation of multifractal complexity.

Previously, we have shown that dystonic BFMDRS symptom severity significantly correlated with the width and the shape of the multifractal spectrum (Semenova et al., 2021). An increase in severity scores was accompanied by a decrease of multifractal spectrum width and rise of its asymmetry.

In this study, we examined how these characteristics change during DBS. The data were recorded during planned neurosurgical operations at the National Medical Research Center for Neurosurgery N.N. Burdenko for implantation of stimulating electrodes (DBS). Four patients underwent externalization of LEAD electrodes for postoperative 16-channel recording of local potentials (LFP) of the globus pallidus. We calculated the width and asymmetry of LFPs multifractal spectra estimated based on wavelet leaders at rest and during stimulation.

In all patients we observed substantial increase of multifractal spectrum width and restoration of its symmetry during DBS. We localized pallidal areas associated with the largest change in the width of the multifractal spectrum in response to DBS. Using LEAD-DBS software we modeled VTAs corresponding to the most efficacious DBS and looked at how these areas overlap. For all patients, the thesholded peak intensities of increase in the multifractal spectrum width was within the optimal VTA or significantly overlapped with it. We also found that the wider the spectrum became during the DBS, the better the clinical effect was observed in follow-up study.

In sum, our data indicate the promise of using multifractal characteristics of pallidal neuronal activity as biomarkers of pathological activity in dystonia, as well as for evaluating and predicting the clinical effect of DBS.

Funding source(s), including NIH support, if any
This study was supported by Russian Science Foundation (23-25-00406).

References

Neumann W, Horn A, Ewert S, et al. A localized pallidal physiomarker in cervical dystonia. Ann Neurol. 2017;82(6):912-924. doi:10.1002/ana.25095

Semenova U, Popov V, Medvednik R, Tomskiy A, Sedov A. Multifractal spectrum width of pallidal activity correlates with dystonic severity [abstract]. Mov Disord. 2021; 36 (suppl 1)

Music, Stress, And Childhood Trauma – Differences In Stress-Reactivity Between Musician's Dystonia Patients And Healthy Musicians And Their Association With Childhood Adversities

Stine Alpheis[1,3,4], Christopher Sinke[2], Julian Burek[2], Tillmann Krüger[2], Eckart Altenmüller[1], Daniel S. Scholz [3,4]

[1] Institute of Music Physiology and Musicians' Medicine, University of Music, Drama and Media, Hannover, Germany
[2] Hannover Medical School, Hannover, Germany
[3] Department of Musicians' Health, University of Music Lübeck, Lübeck, Germany
[4] Department of Neurology, University of Lübeck, Lübeck, Germany

Musician's dystonia (MD) is a task-specific focal dystonia characterized by muscle cramps and impaired voluntary motor-control whilst playing a musical instrument. Even though several risk factors have been established, the exact pathophysiology remains unknown. Recently, the network hypothesis predominated, stating that MD is caused by dysfunctional neural networks in sensory-motor, basal ganglia, cerebellar and limbic loops. Additionally, adverse childhood experiences (ACE) and altered stress reactivity have been discussed as possible influencing factors.

We hypothesize that MD patients have experienced more ACE and therefore show increased stress reactivity. We expect altered activation in stress-related networks (amygdala, hippocampus), and in areas related to cognitive control and self-evaluation (prefrontal cortex, insula, precuneus).

Forty MD patients and 39 matched healthy musicians were compared using functional magnetic resonance imaging (fMRI) and psychological questionnaires. To

induce stress, the Montreal Imaging Stress Task was administered. Whole-brain analysis and Regions of Interest (ROI) analysis were performed. Parameter estimates from the ROI analysis were compared between groups and correlated with the Childhood Trauma Questionnaire (CTQ).

MD patients reported significantly higher CTQ-scores than healthy controls (W=581, p = .025). Across the 66 participants (MD=33) whose data was useable for fMRI analysis, increased activity of visual association and temporal areas was observed under stress, but no increased activation of the limbic system. The ROI analysis revealed reduced activity of the left cerebellar cortex (t(64) = 2.85, p = .006) and the precuneus (t(64) = 2.07, p = .042) in dystonia patients, and a negative correlation between ACE and right precuneus activity across all participants (r(64) = -.25, p = .043).

We could not confirm that stress processing in the limbic network is altered in MD patients. However, we observed reduced activity in the left Crus I of the cerebellum, an area involved in working memory and attentional processing, and in the precuneus, associated with social cognition and emotional processing. This could indicate that MD patients show blunted neurological reactions in general, which might affect sensorimotor integration or inhibition during movement generation.

Furthermore, higher rates of ACE among MD patients suggest their importance during the taking of medical history of MD patients.

Funding sources
No external funding was used for this study.

Altered Inhibitory And Excitatory Signaling Within The Sensorimotor Network Is Associated With Motor And Neuropsychiatric Symptoms In Blepharospasm

Caileigh Dintino[1], Matthew Barrett[1], Dean Krusienski[2], Christopher Groth[3], Brian D Berman[1]

[1]Virginia Commonwealth University Department of Neurology, Richmond, USA
[2]Virginia Commonwealth University Department of Biomedical Engineering, Richmond, USA
[3]University of Iowa Department of Neurology, Iowa City, USA

Objective
Determine if concentrations of inhibitory and excitatory molecules within the sensorimotor cortex, basal ganglia and cerebellum underlie motor and non-motor symptoms in blepharospasm (BSP).

Background
A variety of investigative lines suggest an imbalance between inhibition and excitation in neuronal circuits contributes to the pathophysiology of movement disorders including dystonia. Magnetic resonance spectroscopy (MRS) with modern spectral editing methods provides estimates of levels of gamma-aminobutyric acid (GABA) and glutamate- the most abundant inhibitory and excitatory neurotransmitters in the brain, respectively. Previous GABA MRS studies had mixed results but suggest GABA levels may be abnormal within sensorimotor networks in focal hand and cervical dystonia. Neuropsychiatric symptoms of depression and anxiety have been associated with decreases in GABA, but such relationships have not previously been reported in those with BSP.

Design/Methods
Nine BSP patients (7F; 66.45 ± 9.5 yrs) underwent MRS on a 3T MRI using the MEGA-PRESS pulse sequence and voxels placed in the left sensorimotor cortex, left basal ganglia (lentiform nucleus), and right cerebellum. Concentrations of GABA (GABA+/Water) and glutamate+-glutamine (Glx/Water) levels were derived using Gannet software (v3.3.0). BSP severity was assessed using the Blepharospasm Severity Rating Scale, Burke-Fahn-Marsden Rating Scale (BFM-eyes), and Blepharospasm Disability Index (BDI). Neuropsychiatric symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS) and Beck Depression Inventory II (BDI). Pearson's coefficient was used to test for correlations between neurometabolite levels and clinical scale scores. Significance was defined as a $p<0.05$.

Results
BSP severity (BFM-eyes), ($r=-0.738$, $p=0.050$) negatively correlated with GABA levels in the cerebellum while BSP disability (BDI) positively correlated with Glx levels in the sensorimotor cortex ($r=0.791$, $p=0.016$) and basal ganglia ($r=0.809$, $p=0.012$). Glx levels in the basal ganglia positively correlated with severity of anxiety (HADS-A; $r=0.821$, $p=0.010$) and depression (BDI-II; $r=0.843$, $p=0.006$).

Conclusions
Loss of inhibition in the cerebellum was associated with increased motor severity in BSP, while increased excitation within the basal ganglia was associated with increased severity of anxiety and depressive symptoms as well as self-rated degree of disability from BSP. These findings suggest that altered inhibitory and excitatory neuronal activity within the sensorimotor network contributes to the pathophysiology of dystonia and may underlie motor and non-motor symptoms in BSP.

Funding
This research was made possible in part by a grant from VCU School of Medicine's Value Efficiency Teaching And Research (VETAR) program.

Pathophysiology Of Dyt1 Dystonia Is Mediated By Spinal Cord Dysfunction

Amanda M. Pocratsky[1*], [†]Filipe Nascimento[1], [†]M. Görkem Özyurt[1], Ian J. White[3], Roisin Sullivan[4], Benjamin J. O'Callaghan[4], Calvin C. Smith[1], Sunaina Surana[1,5], Marco Beato[2], Robert M. Brownstone[1*]

[1]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, University College London; London, WC1N 3BG, UK
[2]Department of Neuroscience, Physiology, and Pharmacology, University College London; London, WC1E 6BT, UK
[3]Laboratory for Molecular Cell Biology, University College London; London, WC1E 6BT, UK
[4]Department of Molecular Neuroscience, UCL Queen Square Institute of Neurology, University College London; London, WC1N 3BG, UK.
[5]UK Dementia Research Institute, University College London; London, WC1E 6BT, UK
[†]These authors contributed equally to this work

Dystonia is a circuit disorder wherein dysfunction arising within and between supraspinal centres leads to downstream abnormal muscle contractions and disorganised movements. Given that spinal circuits directly organise and produce movements, we sought to determine whether spinal circuit dysfunction contributes to dystonia pathophysiology, focusing on a prevalent form of primary dystonia: DYT1-*TOR1A*.

We developed a new model wherein exons 3-5 are flanked by flippase-sensitive recognition sites (Tor1a-frt). Through multigenerational breeding of Tor1a-frt with the caudalising Cdx2::FlpO mouse, we produced a biallelic "double" conditional knockout of *Tor1a* in the caudal neuraxis whilst sparing the brain ("spinal Tor1a d-cko"). After confirming the site-specificity of the d-cko, we used a suite of techniques to investigate the dystonic-like phenotype and its underlying pathophysiology, including: video recordings, electromyography (EMG) recordings, extracellular recordings from isolated spinal cords, and intracellular patch-clamp recordings from motoneurons.

Video recordings revealed that spinal *Tor1a* d-cko mice develop a striking early-onset dystonic-like phenotype that mimics DYT1-*TOR1A*. Motor issues emerged

early in the hindlimbs and then - over postnatal maturation - generalised caudo-rostrally to affect the trunk and forelimbs. EMG recordings revealed that spinal *Tor1a* d-cko mice bear the pathophysiological biosignatures of dystonia: involuntary muscle contractions at rest, disorganised activity during volitional movements, and co-contractions. These pathophysiological biosignatures are produced by dysfunctional spinal circuits as revealed by extracellular ventral root recordings from hindlimb motor pools in isolated spinal cords. To unravel circuit-specific dysfunction, we investigated the monosynaptic reflex. Extracellular ventral root recordings from hindlimb motor pools following dorsal root stimulation revealed that the caudal-most reflexes were affected first and then - throughout postnatal maturation - the more rostral reflexes became impaired. Focusing individual motoneurons, intracellular patch-clamp recordings revealed that motoneurons are smaller. Following dorsal root stimulation, motoneurons showed altered excitatory post-synaptic currents that bore the same pathophysiological signatures detected in the motoneuron pools: reduced amplitude, increased latency, and multiple asynchronous peaks. That is, spinal *Tor1a* d-cko mice show altered sensory-motor integration, another key biosignature of dystonia.

The spinal knockout of *Tor1a* reproduces the pathophysiology of DYT1-*TOR1A*, uncovering a key role for spinal circuit dysfunction in early-onset generalised dystonia.

Funding source(s), including NIH support, if any
Dystonia Medical Research Foundation DMRFPRF-2022-3
European Molecular Biology Organization: ALTF 495-2018
Wellcome: 110193/Z/15/Z
Wellcome: 221610/Z/20/Z
Wellcome: 218278/Z/19/Z
Medical Research Council: MR/V003607/1
Medical Research Council: MR/R011494
Medical Research Council: MC/U12266B
Brain Research UK: none
Biotechnology and Biological Sciences Research Council: BB/S005943/1
Royal Society: NIF\R1\192316
Human Frontier Science Program: LT000220/2017-L

Dopamine-Acetylcholine Interplay At The Pallidal-Amygdala Circuit In A DYT1 Mouse Model Of Dystonia

Giuseppe Sciamanna[1,2], Maria Meringolo[1,2], Annalisa Tassone[2], Giulia Ponterio[2], Ilham El Atiallah[2,3], Martina Montanari[2,3], Giuseppina Martella[2], Paola Bonsi[2], Antonio Pisani[4,5]

[1]Unicamillus International University of Health Sciences, Rome, Italy
[2]Santa Lucia Foundation IRCCS, Rome, Italy
[3]University of Rome TorVergata, Rome, Italy
[4]University of Pavia, Pavia, Italy. 5IRCCS Fondazione C. Mondino, Pavia, Italy

DYT1 dystonia is an early-onset, hyperkinetic movement disorder caused by a deletion in the gene TOR1A, which encodes the protein torsinA. Abnormal firing activity of external globus pallidus (GPe,) has been reported both in dystonic patients and animal models, supporting an altered corticostriatal-GPe transmission. Because of its mutual projections to all Basal Ganglia nuclei, GPe is centrally placed in the motor selection process. Phasic changes in the firing activity of GPe neurons are associated with initiation of active movements, as well as with amplitude and direction of movement. Thus, alteration of neuronal activity of GPe neurons may unbalance whole BG activity and motor control. Interestingly, GPe has been reported to have a newly identified connection with structures involved in fear-and anxiety-related behaviors such as amygdala and in particular the CeA region. Non-motor symptoms, such as depression and anxiety, are commonly observed in some form of dystonia in addition to the characteristic motor symptoms. Experimental research in both patients and rodents suggested a massive link between torsinA and dopaminergic signaling. Dopamine has a strong impact on the GPe by also modulating a class of pallidal cholinergic interneurons (ChAT+). Deficits in dopaminergic signaling have been documented in GPe of both DYT1 dystonia rodent models and patients as well, pointing at this subcortical area as a crucial region that, when altered, could brings

about motor and cognitive dysfunctions by also affecting the amygdala.

Results
By electrophysiological patch-clamp recording and Biochemical investigation by western blotting analysis we found an unbalanced autonomous firing rate of ChAT+ neurons, with a reduced efficacy of dopamine DRD2-mediated response in Tor1a+/ΔGAG. Levels of dopamine transporter (DAT) in GPe tissue from Tor1a+/ΔGAG result significantly reduced. Finally, neurons of CeA from Tor1a+/ΔGAG show a clear increase in excitatory spontaneous synaptic currents (EPSCs).

Discussion
Our data show that dopaminergic signaling affect the GPe excitability through also cholinergic functional interplay. We found for the first time alterations of excitability of amygdala neurons, an anxiety-related area to date not considered in dystonia pathophysiology. By the pallidal-amygdala connection, GPe could have pivotal role underlying both motor and cognitive dysfunctions of dystonia.

Funding source(s), including NIH support, if any
Italian Ministry of Health, Grant number GR-2018-12365733

Investigating Abnormal Neurodevelopment During A Critical Window Of Vulnerability In An Invertebrate Model Of Dystonia

Simon A. Lowe[1], Abigail D. Wilson[1], James E.C. Jepson[1]

[1]Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom

An emerging body of evidence suggests that altered neurodevelopment during critical windows of vulnerability is key to the pathogenesis of inherited forms of dystonia [1]. For example, a recent paper demonstrated the existence of a critical window in a mouse model of *DYT1*, caused by *Tor1A* loss of function (LOF) [2]. Suppressing torsinA expression specifically during embryogenesis caused dystonia-like phenotypes in adult mice, while *restoring* torsinA expression in juvenile *Tor1A*-LOF mice rescued the phenotypes [2]. However, it is unclear to what extent similar pathogenic processes are involved in genetically distinct forms of dystonia, and using a similar rodent-based approach to investigate each of the large number of mutations associated with dystonia would be extremely resource-intensive and time consuming. Here invertebrate models of dystonia are a powerful tool. The rapid lifespan, relatively low cost of experimentation, and extremely powerful genetic toolkit available in the fruit-fly *Drosophila* melanogaster make it an ideal system for exploratory research, particularly for rare and recently discovered forms of dystonia.

Dystonia in the context of Paroxysmal non-Kinesigenic Dyskinesia (PNKD3) is caused by gain-of-function point mutations in *KCNMA1* [3]. The majority of research into PNKD3 has focussed on *acute* alterations in neuronal excitability as a mechanism for motor dysfunction [3]. We recently generated a *Drosophila* PNKD3 model with an equivalent mutation in a highly conserved *KCNMA1* homologue *slo* (*slo*^{E366G}), which displays profound motor defects [4]animal models harboring corresponding mutations are lacking. Here we utilize the fruit fly, Drosophila, to study a PxD linked to a gain-of-function (GOF. Here, I demonstrate that expression of SLO^{E366G} during a short critical window during neurodevelopment is necessary and sufficient to cause permanent motor defects in the adult fly, while acute adult expression has no deleterious effect.

I then describe alterations to neurodevelopmental processes occurring during this critical window, showing that 1) highly stereotyped spontaneously-generated neuronal activity occurring at this time [5] is suppressed, and 2) brain-wide expression of a key pre-synaptic protein is permanently reduced. Finally, I present preliminary data suggesting that *raising* neuronal activity during the critical window may partially rescue the motor phenotypes. I therefore hypothesise that the pathogenic mutations acts by decreasing spontaneous neuronal activity during a critical neurodevelopmental window, causing permanent changes in synaptic structure and function.

Taken together these data present compelling evidence from an invertebrate model that PNKD3-dystonia is essentially a neurodevelopmental disorder. This insight has clear therapeutic implications, and makes an important contribution to the emerging understanding of the importance of critical windows to dystonia pathogenesis.

Funding

This work was funded by the Dystonia Medical Research Foundation in the form of the 2020 Postdoctoral Research Fellowship Award. The authors declare no commercial funding or conflicts of interest.

References

[1] J. Li, S. Kim, S. S. Pappas, and W. T. Dauer, 'CNS critical periods: implications for dystonia and other neurodevelopmental disorders', *JCI Insight*, vol. 6, no. 4, Feb. 2021, doi: 10.1172/jci.insight.142483.

[2] J. Li, D. S. Levin, A. J. Kim, S. S. Pappas, and W. T. Dauer, 'TorsinA restoration in a mouse model identifies a critical therapeutic window for DYT1 dystonia', *J Clin Invest*, vol. 131, no. 6, pp. e139606, 139606, Mar. 2021, doi: 10.1172/JCI139606.

[3] W. Du *et al.*, 'Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder', *Nat Genet*, vol. 37, no. 7, pp. 733–738, Jul. 2005, doi: 10.1038/ng1585.

[4] P. Kratschmer *et al.*, 'Impaired Pre-Motor Circuit Activity and Movement in a Drosophila Model of KCNMA1-Linked Dyskinesia', *Movement Disorders*, vol. 36, no. 5, pp. 1158–1169, 2021, doi: 10.1002/mds.28479.

[5] O. Akin, B. T. Bajar, M. F. Keles, M. A. Frye, and S. L. Zipursky, 'Cell-type-Specific Patterned Stimulus-Independent Neuronal Activity in the Drosophila Visual System during Synapse Formation', *Neuron*, vol. 101, no. 5, pp. 894-904.e5, Mar. 2019, doi: 10.1016/j.neuron.2019.01.008.

Single-nuclear RNA-seq Reveals The Involvement Of Glutamatergic Interneurons In DYT6 Dystonia

Wenxu Zheng[1], Yuchao Chen[1], Thomas Ott[1], Olaf Riess[1,2], Fubo Cheng[1, 2]

[1]Institute of Medical Genetics and Applied Genomics, University of Tuebingen, 72076 Tuebingen, Germany
[2]NGS competence center Tuebingen, University of Tuebingen, 72076 Tuebingen, Germany

Mutations in *THAP1* gene are responsible for DY6 dystonia. Until now, the pathogenesis of DYT6 dystonia is not well characterized. In our previous study, we generated a THAP1 heterozygous knock-out rat model for DYT6 dystonia and found involvement of chemical synaptic transmission and nervous system development pathways in DYT6 rat model using bulk RNA-seq. However, the gene expression changes in DYT6 rats at single-neuron level are not clear so far.

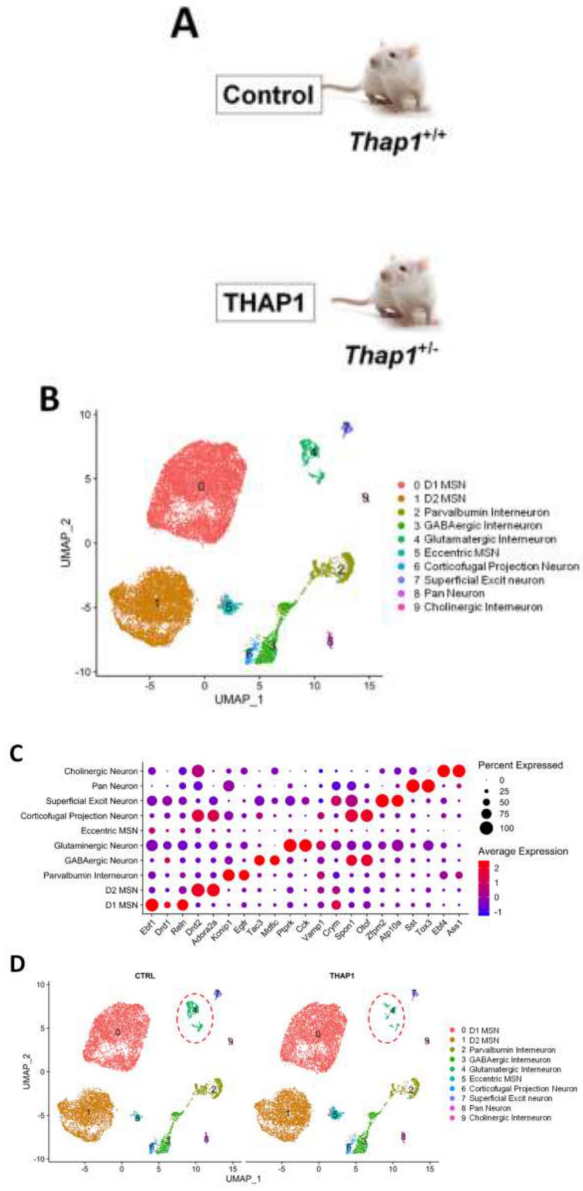


Figure 1 | **snRNA-seq analyses show changes of rat striatal neurons in *Thap1*$^{+/-}$ rats**. (A) Wild-type and *Thap1*$^{+/-}$ rats were used for this study. (B) A total of 10 different cell clusters were identified by Seurat (V4) clusters analysis. (C) Heatmap shows the expression of cell makers from each cluster. (D) Comparative snRNA-seq analysis reveals loss of glutamatergic interneurons in the striatum of *Thap1*$^{+/-}$ rats (red circle).

In this study, we performed single-nuclear RNA-seq (snRNA-seq) to characterize gene expression change at single-neuron level. The rat striatum tissues from 9-month-old wild-type and *Thap1*$^{+/-}$ rats were used for snRNA-seq analysis (Fig. 1A). Striatal single-neurons were sorted by using fluorescence activated cell sorting (FACS) and anti-NeuN antibody.

In rat striatum, we annotated 10 different neuron populations (Fig. 1B and 1C). The two largest groups of neurons are D1 medium spiny neuron (D1 MSN) and D2 MSN. Cell number of glutamatergic interneurons, which specifically express Ptprk and Cck, is significantly reduced in *Thap1*$^{+/-}$ rat striatum compared to the control rat striatum (Fig. 1C and 1D).

Our observations indicate the reduction of glutamatergic interneurons in striatum of *Thap1*$^{+/-}$ rat, which might be associated to the pathogenesis of DYT6 dystonia.

Funding source
This work was supported by Deutsche Forschungsgemeinschaft (DFG) (FC: CH2339/5-1 AOGJ690451)

Disrupting eIF2α Signaling Evokes Dystonia-Like Movements

Sara A Lewis, Jacob Forstrom, Jennifer Tavani, Sergio Padilla-Lopez, Michael C Kruer

Phoenix Children's Hospital, Phoenix, USA. University of Arizona College of Medicine, Phoenix, USA

The molecular and cellular mechanisms linking genetic risk and circuit changes in dystonia has remained enigmatic. One potential link is the cellular stress-response protein, eIF2α. However, whether eIF2α can drive changes leading to dystonia is unknown. With cytotoxic stressors, eIF2α is phosphorylated (eIF2α-P) to decrease global protein translation and express stress response genes. Regulation of phosphorylation of eIF2α-P regulates synaptic strength in circuits, with eIF2α-P promoting long-term depression/decreased strength and de-phosphorylation favors long-term potentiation/strengthening. We find altering eIF2α signaling produces abnormal posturing and disordered movements in a drosophila model, recapitulating the dystonia-like dyskinetic movements previously observed in the DYT1 fly model. We analyzed cell type specificity, altered neuronal excitability, and synaptic connectivity in flies with increased/decreased eIF2α-P.

We used loss-of-function alleles of eIF2α kinase (PEK) to increase and phosphatase (PPP1R15A) to decrease eIF2α-P. We used Gal4 drivers for cell-type specific overexpression of the eIF2α-P kinase, phosphatase, and htorΔE (DYT1). Alterations to eIF2α-P were confirmed via western blot. Motor function assays performed in 14-day old adult flies. Axon terminal size was assayed using anti-HRP to visualize neuron membrane and phalloidin for muscle.

Genetically increasing or decreasing eIF2α-P impairs locomotion measured by distance traveled. Overexpression of the stress response gene, ATF4, also impaired locomotion. We found suppressing eIF2α-P specifically in glutamatergic, dopaminergic, and D1-type neurons decreased distance traveled. Suppressing eIF2α-P or overexpressing human DYT1 allele htorΔE in cholinergic neurons elicited dyskinetic dystonia-like movements with mechanosensory stimulation. Decreased or increased of eIF2α-P in D2-receptor neurons disrupted motor control, elicited dyskinetic movements, and altered distance traveled. Finally, elevated eIF2α-P correlated with increased axon terminal size.

Our findings support a crucial role of eIF2α-P regulation in motor control, with key roles for dopaminergic signaling, particularly D2 receptors, and cholinergic neurons. Dyskinetic movements occur in mutant animals with either increased or decreased eIF2α-P, providing proof of principle data that perturbations in the eIF2α axis directly impact motor function. Enhanced synaptic connectivity is a potential structural basis linking disrupted eIF2α-P and increased ATF4 with changes in motor control. Overall, molecular alterations in eIF2α-P can create neuron/circuit changes and result in dystonia-like movements.

Funding
SL: CPARF PRG 06621, MCK: NIH NINDS R01NS106298

Central Pattern Generator Dysfunction Is A Common Phenomenon Across Diverse *Drosophila* Models Of Inherited Dystonia

Abigail D. Wilson, James E.C. Jepson

Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom

Dystonia is a highly debilitating movement disorder with limited therapeutic options. Mutations in a broad range of genes with distinct functions have been linked to inherited dystonia. However, whether there are common pathophysiological processes shared across diverse forms of dystonia remains unclear, as are the key neural circuits responsible for dystonic movements. The genetically tractable model *Drosophila* provides a unique platform to address these questions.

Abnormal co-contraction of antagonistic muscles is a central feature of dystonia, and reduced reciprocal inhibition within spinal central pattern generator (CPG) circuits has also been reported in dystonia patients[1,2]. Therefore, we assessed whether CPG activity was disrupted in a range of *Drosophila* dystonia models. GCaMP-based optical imaging was used to record CPG activity in the ex vivo ventral nerve cord (analogous to the spinal cord) of larval *Drosophila* harbouring mutations in an array of dystonia-related genes: TOR1A/*Torsin*; KCNMA1/*slo*; TBC1D24/*skywalker* and LAMB1/*LanB1*.

All dystonia-linked genes that were assessed exhibited dysfunctional CPG activity compared to control. The KCNMA1/*Slo* gain-of-function mutant[3] exhibited extremely disrupted CPG activity with no forward waves and almost no fictive turns. The TBC1D24/*skywalker* *Drosophila* model of exercise-induced dystonia[4] and the LAMB1/*LanB1* overexpression model associated with dystonia-linked movement disorders in mouse models[5] exhibited a decrease in forward wave propagation with a decrease in the number of fictive turns. Finally, a *Torsin* knockout *Drosophila* model of TOR1A (DYT1) dystonia[6] also exhibited a decrease in the forward wave propagation time. CPG-driven forward wave propagation disruption appears to be a commonality across *Drosophila* models of dystonia (Figure 1).

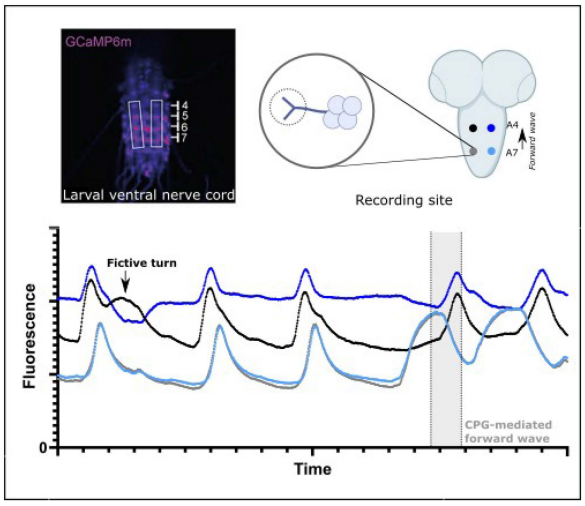


Figure 1 | CPG-driven peristaltic behaviour recorded from *Drosophila* ventral nerve cord motor neurons

Drosophila models of dystonia provide an effective platform to assess CPG dysfunction. Our work suggests that CPG dysfunction is a common process disrupted by distinct dystonia-linked mutations. Through ongoing experiments, we aim to define the cellular basis of this phenomenon.

Funding sources

MRC Senior Non-Clinical Fellowship (MR/V03118X/1) to Prof. James Jepson.

References

1. Balint, B. *et al. Nat. Rev. Dis. Primer* **4**, 25 (2018).
2. Mir, P. *et al. Brain J. Neurol.* **128**, 291–299 (2005).
3. Kratschmer, P. *et al. Mov. Disord.* **36**, 1158–1169 (2021).
4. Lüthy, K. *et al. Brain* **142**, 2319–2335 (2019).
5. Liu, Y. B. *et al. eLife* **4**, e11102 (2015).
6. Wakabayashi-Ito, N. *et al. PloS One* **6**, e26183 (2011).

Cerebellar Nuclei Neuron Activity Drives Dystonic Attacks In Mice

Alejandro G. Rey Hipolito[2,5], Roy V. Sillitoe[1-5]

[1]Department of Pathology & Immunology, BCM
[2]Department of Neuroscience, BCM
[3]Department of Pediatrics, BCM
[4]DDMT Graduate Program, BCM
[5]Jan and Dan Duncan Neurological Research Institute, TCH, Houston, Texas, USA

Dystonia is a neurological disorder characterized by painful, involuntary muscle contractions as exemplified by its hallmark symptom: dystonic twisting postures. At best, episodes of dystonic posturing transiently disable an isolated body part following a specific cue like alcohol or stress; at worst, severe attacks seize the entire body randomly or chronically, destroying a livelihood. While the triggers for a dystonic attack are critical for diagnosis and treatment, it is unclear where in the brain the physiological mechanism for attacks originates and how it arises. Among the motor regions of the brain that are involved in dystonia, a growing number of studies implicate cerebellar nuclei neuron (CNN) dysfunction in dystonia, but these studies were limited by methods that target many neural populations or indirectly alter CNN function. Thus, it is unclear which cerebellar neurons and circuits are critical for inducing attacks, complicating their therapeutic utility. As CNNs serve as the primary output from the cerebellum to many areas implicated in dystonia, we hypothesized that the CNN pathway may play a role in inducing dystonic attacks. To test this, we devised an optogenetic approach to selectively manipulate CNNs in mice with intermittent and spontaneous dystonia (*Ptf1a*^{Cre};*Vglut2*$^{fx/fx}$;*Rosa*$^{lsl\text{-}ChR2}$) while quantifying dystonia-like behaviors with electromyography, tremor monitors, and a dystonia rating scale. In these mice, photoactivation of CNNs initiated dystonic attacks on-demand. These data suggest that CNN pathway hyperactivity mediates attack onset in dystonia, a mechanistic insight with therapeutic implications for patients suffering from dystonia.

Funding source(s), including NIH support, if any
This work was supported by Baylor College of Medicine (BCM), Texas Children's Hospital, The Hamill Foundation, and the National Institutes of Neurological Disorders and Stroke (NINDS) R01NS100874, R01NS119301, and R01NS127435 to Dr. Roy V. Sillitoe. Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number P50HD103555 for use of the Cell and Tissue Pathogenesis Core and In Situ Hybridization Core (the BCM IDDRC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Support was also provided by a Dystonia Medical Research Foundation (DMRF) grant to RVS.

Adult Loss Of Gnal In The Striatum Or Cerebellum Causes Dystonia Phenotypes In Mice

Nicole Chambers, Dominic Hall, Douglas Nabert, Morgan Kaplan, Sarah Garan, Tiffany Curry, Lauren Sanchez, Mark S Moehle

Department of Pharmacology and Therapeutics and Center for Translational Research in Neurodegeneration, University of Florida College of Medicine, Gainesville, FL, US

Loss of function mutations in *GNAL*, which encodes for a unique alpha subunit of heterotrimeric G protein, Gαolf, are causative for an adult onset form of dystonia. Gαolf has a unique expression profile where it replaces Gαs in the striatum and is expressed in Purkinje Cells of the cerebellum. Both of these nuclei are consistently linked to dystonia pathophysiology, and suggests that Gαolf may critically regulate these cells and the circuits they are involved in to mechanistically cause dystonia

symptoms. However, current models of *GNAL* linked dystonia do not have overt dystonic phenotypes, which makes tying cellular and circuit dysfunction to distinct dystonia phenotypes challenging. Using a new model of *GNAL* where we floxed exons 3 and 4 of *Gnal*, we used viral delivery of cre or well established cre lines to selectively remove *Gnal* embryonically or in adulthood in specific cell populations. Interestingly, we found that viral mediated adult knockout, but not embryonic, knockout, of *Gnal* causes dystonic like motor phenotypes in mice when removed from the cerebellum or striatum of *Gnal* flx/flx mice. These behavioral phenotypes correlate to altered intrinsic properties of striatal spiny projection neurons as determined through patch-clamp electrophysiology. These findings suggest that loss of Gαolf expression causes dysregulated striatal or cerebellar function which leads to subsequent basal ganglia circuit dysfunction to cause dystonia. Adult mediated knockout of Gnal may serve as a unique model to mechanistically determine the underlying circuit dysfunction that causes dystonia, and as a novel testing platform for new therapeutic strategies for adult onset dystonia.

Striatal Synaptic Endophenotype In The Tor1a$^{+/\Delta gag}$ Mouse Model Of DYT1 Dystonia

Giulia Ponterio[1], Gaia Faustini[2], Ilham El Atiallah[1], Giuseppe Sciamanna[3], Maria Meringolo[3], Annalisa Tassone[1], Paola Imbriani[1], Silvia Cerri[4], Giuseppina Martella[1], Paola Bonsi[1], Arianna Bellucci[2], Antonio Pisani[4,5]

[1]FONDAZIONE SANTA LUCIA IRCCS, ROME, Italy
[2]Department of Molecular and Translational Medicine, University of Brescia, BRESCIA, Italy
[3]UniCamillus Saint Camillus International University of Health Sciences, ROME, Italy
[4]IRCCS Fondazione Mondino PAVIA, Italy
[5]Department of Brain and Behavioral Sciences, University of Pavia, PAVIA, Italy

Introduction

Impairment of synaptic activity is a hallmark of movement disorders such as dystonia. In particular, DYT1 dystonia is characterized by reduced penetrance and several endophenotypes converging on synaptic dysfunction have been shown in different experimental models. Intriguingly, torsinA (TA), the protein causative of DYT1 dystonia, has been found to interact with alpha-synuclein (α-Syn). Both proteins act as molecular chaperones and control synaptic machinery. Despite such evidence, the role of α-Syn in dystonia has never been investigated. We explored whether α-Syn and N-ethylmaleimide sensitive fusion attachment protein receptor proteins (SNAREs), that are known to be modulated by α-Syn, may be involved in DYT1 dystonia synaptic dysfunction.

Materials and Methods

We used electrophysiological and biochemical techniques to study synaptic alterations in the dorsal striatum of the Tor1a$^{+/\Delta gag}$ mouse model of DYT1 dystonia.

Results

In the Tor1a$^{+/\Delta gag}$ DYT1 mutant mice, we found a significant reduction of α-Syn levels in whole striata, mainly involving glutamatergic corticostriatal terminals. Strikingly, the striatal levels of the vesicular SNARE VAMP-2, a direct α-Syn interactor, and of the transmembrane SNARE synaptosome-associated protein 23 (SNAP-23), that promotes glutamate synaptic vesicles release, were markedly decreased in mutant mice. Moreover, we detected an impairment of miniature glutamatergic postsynaptic currents (mEPSCs) recorded from striatal spiny neurons, in parallel with a robust alteration in release probability. Finally, we also observed a significant reduction of TA striatal expression in α-Syn null mice.

Discussion

Our data demonstrate an unprecedented relationship between TA and α-Syn, and reveal that α-Syn and SNAREs alterations characterize the synaptic dysfunction underlying DYT1 dystonia (Ponterio et al., *Movement Disorders* 2022).

Funding

This work was partially supported by Cure Dystonia Now, and the United States Department of Defense grant W 81 XWH 20 1 0446 The Italian Ministry of Health supported this project, under the framework of the European Joint Programme on Rare Diseases (EJPRD) EJP RD 1352019 In addition, this project received funding from the European Union's Horizon 2020 research and innovation program under EJP RD COFUND EJP No 825575 EurDyscover

I declare that all authors consent to publication

Spike-Triggered Adaptive Closed-Loop Cerebellar Deep Brain Stimulation (DBS) For Dystonia

Linda H. Kim[1,5], Amanda M. Brown[1,5], Tao Lin[1,5], Roy V. Sillitoe[1-5]

[1]Department of Pathology & Immunology, BCM
[2]Department of Neuroscience, BCM
[3]Department of Pediatrics, BCM
[4]DDMT Graduate Program, BCM
[5]Jan and Dan Duncan Neurological Research Institute, TCH, Houston, Texas, USA

Despite diverse manifestations and causes, current hypotheses suggest that dystonia arises from a faulty network involving the cerebral cortex, basal ganglia, and cerebellum. Cerebellar nuclei (CN) neurons, the main output of the cerebellum, may be particularly susceptible to dystonia-related insults. *Here, I leverage this CN sensitivity to develop a "signature signal" therapeutic approach for restoring movement in dystonia*. We previously generated *Ptf1a$^{Cre/+}$;Vglut2$^{fl/fl}$* mutant mice with selective loss of glutamatergic neurotransmission at climbing fiber to Purkinje cell synapses. In this model, abnormal Purkinje cell "burst" activity drives similar erratic firing downstream in the connected CN neurons, resulting in severe early-onset dystonia. Irregular firing of the CN is a common abnormality among several models of dystonia, regardless of the initial insult and mechanism of action. We also showed that pharmacological inhibition and DBS of the CN in *Ptf1a$^{Cre/+}$;Vglut2$^{fl/fl}$* mice reduced many, but not all, dystonia symptoms. *A major problem is that the current chronic stimulation strategies fail to account for the progression of aberrant neuronal dynamics in the cerebellum and the greater motor network in real-time*. **To address this, I am testing the hypothesis that the unique pathophysiological CN signals can serve as robust biomarkers for triggering an adaptable closed-loop DBS response to restore movement in the *Ptf1a$^{Cre/+}$;Vglut2$^{fl/fl}$* mouse model of dystonia.** A closed-loop approach with robust triggers and feedback signals will aid in identifying specific neurophysiological parameters that underlie different features of dystonia, such as twisting postures, co-/over-contractions, and initiation and progression of tremor; the goal is to provide accurate dialing of DBS for specific disease symptoms. This work ultimately models unique pathophysiological CN signals that could drive different aspects of dystonia and addresses a universal problem with chronic DBS, which is the failure to adapt in real-time to changing neuronal dynamics. Therefore, a major advance that our approach offers is an online and progressive "dosing" of DBS that monitors the current state of disease initiation, progression, and severity with millisecond precision. *This work will address a major gap in treatment options for dystonia by defining disease-specific neural targets for designing a customizable therapy that is self-controlled with great precision.*

Funding source(s), including NIH support, if any
This work was supported by Baylor College of Medicine (BCM), Texas Children's Hospital, The Hamill Foundation, and the National Institutes of Neurological Disorders and Stroke (NINDS) R01NS100874, R01NS119301, and R01NS127435 to RVS. Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number P50HD103555 for use of the Cell and Tissue Pathogenesis Core and In Situ Hybridization Core (the BCM IDDRC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. AMB was supported by F31NS101891. Support was also provided by a Dystonia Medical Research Foundation (DMRF) grant to RVS and a DMRF fellowship (Mahlon DeLong Young Investigator Award) to LHK.

Dyt1 Dystonia: Neurophysiological Aspects

Dzhalagoniya Indiko Z.[1], Usova Svetlana V.[1], Gamaleya Anna A.[2], Tomskiy Alexey A.[2], Sedov Alexey S.[1]

[1] N.N. Semenov Federal Research Center for Chemical Physics Russian Academy of Sciences, Moscow, Russian Federation, icp@chph.ras.ru
[2] N.N. Burdenko National Medical Research Center for Neurosurgery, Moscow, Russian Federation

The aim of this study was a comparative analysis of the neuronal activity of globus pallidus in patients with DYT1 dystonia and idiopathic dystonia.

The DYT1 mutation leads to dysfunction of the torsin A protein, resulting in increased activation of striatum neurons that suppress the globus pallidus. We wonder how DYT1 mutation can affect the electrophysiological properties of neuronal activity in both segments of globus pallidus (GPe and GPi).

The data were obtained during stereotactic implantation of electrodes for deep brain stimulation (DBS). During the operations we performed microelectrode recording of the single unit activity in the GPe and the GPi in 5 patients with DYT1 and in 6 patients without DYT1. We performed offline analysis of neuronal activity parameters and visualized neurons localization in Lead-DBS. All neurons were divided into 3 neuron activity types (burst, pause and tonic) depending on neuron activity pattern by using unsupervised clusterization method.

In total, the activity of 662 neurons were analyzed. We found a significantly reduced firing rate, burst index and burst rate in both segments of the globus pallidus in DYT1 patients. Also, we found significantly increased interburst interval, preburst interval and pause index. Clusterization showed that in the GPe the differences between DYT1 and non-DYT1 neuron activity parameters were significant only in burst neurons, while in the GPi the same differences were observed in both burst and pause neurons. The visualization showed diversity of pause neurons' localization in the GPe.

Obtained results indicate that DYT1 neurons tend to be more 'pausy' and less 'bursty'. Also, the similarity of changes in neural activity in GPe and GPi points on a common pathological focus for these structures, which may be located in the striatum. Difference in the location of pause neurons in the GPe may be due to strong striatal influence which alters the activity pattern of the neurons.

The study was funded by the Russian Science Foundation project 23-25-00406.

I hereby confirm my consent to publish this abstract in the Dystonia journal.

Funding source
Russian Science Foundation project 23-25-00406.

Luteolin Disrupts The Interaction Between PKR And PACT To Prevent Pathological And Maladaptive ISR In DYT-PRKRA.

Kenneth Frederick, Rekha C. Patel

Department of Biological Sciences, University of South Carolina, Columbia, SC, USA

DYT-PRKRA is caused by mutations in the PRKRA gene [1], which encodes for PACT, the protein activator of interferon-induced, double-stranded RNA (dsRNA)-activated protein kinase PKR [2]. PACT causes PKR's catalytic activation by a direct binding in response to stress signals and activated PKR phosphorylates the translation initiation factor eIF2α. Phosphorylation of eIF2α is the central regulatory event that is part of the integrated stress response (ISR), an evolutionarily conserved signaling network essential for adapting to environmental stresses [3]. A dysregulation of either the level or the duration of eIF2α phosphorylation causes the normally pro-survival ISR to become pro-apoptotic. Our research previously established that eight reported DYT-PRKRA mutations lead to enhanced PACT-PKR interactions causing hyperactivation of PKR, dysregulation of ISR and an increased sensitivity to apoptosis [4, 5]. In the present study, we wanted to determine if disrupting the heightened PACT-PKR interaction in DYT-PRKRA patient cells can bring homeostasis and protect from increased apoptosis. We previously identified luteolin, a plant flavonoid, as an inhibitor of the PACT-PKR interaction using high-throughput screening of chemical libraries [6]. We determined the ability of luteolin to protect cells using biochemical and molecular techniques to study the ISR and apoptosis in DYT-PRKRA. Our results indicate that luteolin is markedly effective in disrupting the pathological PACT-PKR interactions to protect DYT-PRKRA cells against apoptosis, thus suggesting a therapeutic option for using luteolin for DYT-PRKRA and possibly other diseases resulting from enhanced PACT-PKR interactions.

Funding source(s), including NIH support, if any

This work was supported by a Department of Defense through the grants W81XWH-18-1-0088, W81XWH-22-1-0526 to R. P. and a pilot grant to R. P. from the NIH COBRE Center for Dietary Supplements & Inflammation Targeted Therapeutics at University of South Carolina supported by grant P01AT00396 and a SPARC Grant from University of South Carolina Vice President's Office to K. F. Opinions, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

References

[1] Camargos, S., et al., DYT16, a novel young-onset dystonia-parkinsonism disorder: identification of a segregating mutation in the stress-response protein PRKRA. Lancet Neurol, 2008. 7(3): p. 207-215.

[2] Patel, R.C. and G.C. Sen, PACT, a protein activator of the interferon-induced protein kinase, PKR. EMBO J, 1998. 17(15): p. 4379-4390.

[3] Pakos-Zebrucka, K., et al., The integrated stress response. EMBO Rep, 2016. 17(10): p. 1374-1395.

[4] Vaughn, L.S., et al., Altered Activation of Protein Kinase PKR and Enhanced Apoptosis in Dystonia Cells Carrying a Mutation in PKR Activator Protein PACT. J Biol Chem, 2015. 290(37): p. 22543-22557.

[5] Burnett, S.B., et al., Dystonia 16 (DYT16) mutations in PACT cause dysregulated PKR activation and eIF2α signaling leading to a compromised stress response. Neurobiol Dis, 2020. 146: p. 105135.

[6] Dabo, S., et al., Inhibition of the inflammatory response to stress by targeting interaction between PKR and its cellular activator PACT. Sci Rep, 2017. 7(1): p. 16129.

Application Of Exome Sequencing To Solve The Genetic Etiology In A Large Dystonia Sample

Mirja Thomsen[1], Fabian Ott[2], Sebastian Loens[3], Gamze Kilic-Berkmen[4], Ai Huey Tan[5], Shen-Yang Lim[5], H. A Jinnah[4], Tobias Bäumer[3], Hauke Busch[2], Christine Klein[1], Katja Lohmann[1], on behalf of the DysTract consortium and the Dystonia Coalition

[1]Institute of Neurogenetics, University of Luebeck, Luebeck, Germany
[2]Medical Systems Biology Division, Institute of Experimental Dermatology, University of Luebeck, Luebeck, Germany
[3]Institute of Systems Motor Science, CBBM, University of Luebeck, Luebeck, Germany
[4]Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA
[5]Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Introduction

Dystonia is a rare movement disorder with relevant clinical and genetic heterogeneity. Despite its high heritability (>25%), the genetic etiology in most patients remains elusive. Using a large patient sample, this study aims to further elucidate the genetic causes underlying dystonia.

Materials and Methods

About 2,000 dystonia patients were mainly selected from two large dystonia registries (DysTract [https://www.isms.uni-luebeck.de/en/research/dystract/] and the Dystonia Coalition [https://dystonia-foundation.org/research/dystonia-coalition/]) based on inconclusive prior hot spot screening for about 300 known pathogenic variants in dystonia genes or GenePanel analysis. Whole-exome sequencing was performed at a median coverage of 102 reads. As a first step, we searched for rare variants (MAF<0.001) in genes previously implicated in dystonia (n=412). Variants were Sanger confirmed and tested for segregation when possible.

Results

We identified 206 patients (10.4%) with (likely) pathogenic variants (according to the VarSome ACMG classifier; version 11.6.4) in 54 genes (**Figure 1**). The mean age at onset in this group was 22.3 ± 17.7 years; 38.8% presented with generalized, 14.6% with segmental, and 33.5% with focal dystonia. Additionally, we found 311 patients (15.8%) with variants of uncertain significance in 60 dystonia-related genes.

Genes with >10 mutation carriers included *GCH1, SGCE, THAP1,* and *VPS16,* while the GAG deletion in *TOR1A* was excluded by prescreening. At least four variants occurred de novo (in *GNB1*, *IRF2BPL*, *KCNN2*, and *KMT2B*), supporting pathogenicity.

Discussion

This study demonstrates the value of exome sequencing in establishing diagnoses in dystonia, a disease for which variants in more than 400 genes have been linked

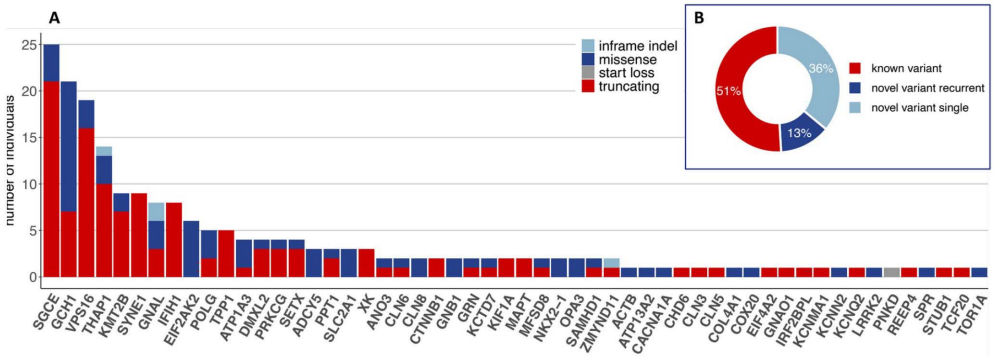


Figure 1 | **Landscape of genetic etiologies in the dystonia sample.**
(A) By using exome sequencing, a total of 206 possible diagnoses were found, representing 54 distinct genetic forms. Most variants were heterozygous and found in genes linked to dominantly inherited diseases. Biallelic variants were detected in COX20, GCH1, OPA3, SETX, and SPR. Only variants classified as (likely) pathogenic according to ACMG criteria were considered disease-causing. Truncating variants include frameshift, stop gained and splice site variants. (B) Percentage of patients with variants that were previously published in the literature (known variant) or not previously published (novel) occurring in a single or >1 independent patients (recurrent)

to diverse clinical expressions. Despite prescreening and selecting presumably mutation-negative patients, we found (likely) pathogenic variants in 10.4% of patients in well-established and newly identified dystonia candidate genes. Additional analyses of our exome datasets will likely further expand the growing list of dystonia genes.

Funding sources
Grant References: DFG (LO1555/10-1)

Striatal Cholinergic Transmission In A Mouse Model With Inducible Dystonia

Mariangela Scarduzio[1,2], David G Standaert[1,2]

[1]Center for Neurodegeneration and Experimental Therapeutics, [2]Department of Neurology, UAB, Birmingham, AL, USA

Introduction

Our previous research identified paradoxical excitatory responses to dopamine D2 receptor (D2R) activation in striatal cholinergic interneurons (ChIs) as a shared endophenotype across genetic mouse models of dystonia. However, these models lacked overt dystonic motor symptoms, leaving a gap in our understanding of the role of ChI activity in dystonic movements. To address this, we investigated a transgenic mouse model of paroxysmal non-kinesiogenic dystonia (PNKD), displaying motor symptoms akin to human hyperkinetic conditions upon exposure to caffeine.

Methods

To study the relationship between cholinergic transmission and dystonia, we employed ex vivo slice physiology and in vivo monitoring of striatal acetylcholine (ACh) via fiber photometry during behavioral assessments.

Results

Both PNKD and wild-type (WT) mice exhibited increased locomotion in response to caffeine, with PNKD mice uniquely displaying complex dystonia. Fiber photometry revealed sustained elevated ACh rhythms in the delta frequency band during caffeine-induced hyperlocomotion in both genotypes. However, this rhythm was disrupted in PNKD mice upon the onset of abnormal movements. A similar disruption occurred in both genotypes following quinpirole injections, known for its inhibitory effects on movement.

Ex vivo studies on striatal slices revealed caffeine's inhibitory effect on ChI firing in both PNKD and WT animals. In contrast, quinpirole induced paradoxical excitation of ChI firing exclusively in PNKD mice, mirroring findings in other dystonia models. Interestingly, in the presence of caffeine, quinpirole's effects on ChI activity reversed: it became excitatory in WT-ChIs and inhibitory in PNKD-ChIs, resembling caffeine-induced alterations in ACh dynamics seen *in vivo*.

Discussion

In the PNKD model, caffeine triggered dystonic symptoms while disrupting the striatal delta ACh rhythm and reversing D2R-mediated ChI excitation. Conversely, caffeine stimulated locomotion in WT animals while elevating ACh rhythm and switching D2R control of ChI activity from inhibitory to excitatory. These findings highlight the dynamical role of D2Rs in modulating ChI activity and suggest that the D2R "paradoxical excitation" observed in non-phenotypic dystonia models may serve as a compensatory or protective mechanism, preventing the manifestation of movement abnormalities and becoming evident when lost, as in the PNKD model. This insight enhances our comprehension of dystonia pathophysiology, emphasizing the interplay of cholinergic and dopaminergic signaling in this context.

Funding Sources

DMRF Basic and Clinical Aspects of Dystonia grant 2020

In vivo Evidence Of An Imbalance Between The Direct And Indirect Basal Ganglia Pathways Of Freely Moving DYT-TOR1A Dystonic Mice

Filipa França de Barros[1,3], Marcelo D. Mendonça[1,2,3], Diogo Soares Melo[1,2], Susanne Knorr[4], Lisa Rauschenberger[4], Chi Wang Ip[4], Rui Costa[5], Albino J. Oliveira-Maia[12,3], Joaquim Alves da Silva[1,3]

[1]Champalimaud Foundation, Champalimaud Research, Lisbon, Portugal
[2]Champalimaud Clinical Centre, Champalimaud Foundation, Lisbon, Portugal
[3]NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisbon, Portugal
[4]University Hospital Würzburg, Department of Neurology, Würzburg, Germany
[5]Zuckerman Mind Brain Behavior Institute, Departments of Neuroscience and Neurology, New York, United States of America

Introduction

Dystonia is a movement disorder characterized by involuntary muscle contractions. Theoretical models of dystonia suggest that these abnormal movements could emerge due to an imbalance in pathways of the basal ganglia, a group of nuclei within the brain that regulate motor control. The striatum is at the core of these pathways since it receives cortical and subcortical inputs that will ultimately guide motor behavior. These models propose that a higher activity in the direct pathway (responsible for action selection, involving D1 medium spiny neurons (MSNs)) and a lower activity in the indirect pathway (responsible for inhibiting competing actions, involving D2 MSNs) is at the core of dystonia. In patients with dystonia, this abnormal activity pattern would give rise to an impaired action selection and result in the over-activation of antagonistic pairs of muscles. Yet, there is a lack of *in vivo* studies dissecting the activity of D1 or D2 MSNs in dystonia.

Material and Methods

DYT-TOR1AΔGAG knock-in and wild-type (WT) mice expressing Cre recombinase under the control of the dopamine D1 or A2A receptors (for D2 populations) were included. Using *in vivo* calcium imaging, MSNs activity was recorded in freely moving animals, during an open field test, while behavior was assessed by high-resolution video and head-mounted accelerometers. Assessments were performed weekly, before and up to nine weeks after a standardized sciatic nerve crush lesion (SNCL) – a procedure known to induce dystonic-like movement in genetically-predisposed animals. Dystonia-like movements were also assessed using a tail suspension test (TST).

Results

No significant differences were found between DYT1-TOR1A and WT mice in the overall time spent locomoting or the speed ranges attained in each group. In the TST, although not all DYT1-TOR1A mice developed persistent dystonia-like movements of the hindlimb submitted to the SNCL, the group had on average dystonia scores higher than controls. Analyses of neuronal activity did not reveal generalized changes in the activity of D1-or D2 -MSNs during self-paced movement. However, when we compared the activity of neurons that were specifically modulated by movement initiation after the SNCL, the activity of D1 MSNs significantly increased, while the activity of D2 MSNs showed a decreasing trend, as dystonic-like movements developed in DYT-TOR1A but not in WT mice.

Discussion
Our observations shed light into the pathophysiology of DYT-TOR1A dystonia by revealing an imbalance between the D1 and D2 pathways at movement onset, compatible with the focused selection and inhibition model of the basal ganglia.

Funding source(s)
This work is financed by national funds through the FCT - Foundation for Science and Tecnology, I.P., under the project 2022.02008.PTDC and grant EurDyscover-EJPRD19-135, funded by FCT(EJPRD/0001/2019) through the European Joint Program for Rare Diseases. FFdB received funding from 2022-2023 Graduate Women in Science National Fellowship and a Postdoctoral Research Fellowship from the Dystonia Medical Research Foundation (DMRF-PRF-2023-1).

Omics Investigation On The Brain Of A DYT-TOR1A Mouse Model Exposed To A 2nd Hit

Colette Reinhold[1], Susanne Knorr[1], Lisa Rauschenberger[1], Rhonda McFleder[1], Jens Volkmann[1], Chi Wang Ip[1]

[1]University hospital- Neurology, Wuerzburg, Germany

Background
Dystonia is a rare movement disorder characterized by involuntary muscle contractions, such as twisting or cramps. DYT-TOR1A is the most common inherited form of dystonia caused by a GAG deletion in the TOR1A gene and has a disease penetrance of only 30%. Although the gene responsible for the disease is known, the pathophysiology remains unclear. The low penetrance indicates a gene-environment interaction, trigged by a genetic and/or environmental factor. Previous studies have shown that a peripheral nerve crush, acting as the environmental trigger, induces a dystonia like phenotype in genetically predisposed rodents. In this study, we performed a sciatic nerve crush in a DYT-TOR1A KI mouse model, carrying the human TORA1 mutation, to study the role of the DYT-TOR1A mutation and the gene-environment interaction by multi-omics in three different brain regions.

Methods
Dystonia like movements (DLM), after sciatic nerve crush injury of the right hindlimb were assessed by a deep learning network during the tail suspension test (TST). Pathophysiological pathways were investigated using Omics technologies in the ipsilateral cerebellum, and contralateral striatum and cortex.

Results
The nerve-injured DYT-TOR1A KI animals had more DLM per minute than DYT-TOR1A KI naive mice during the 12 weeks experiments. The highest score was reached three weeks after nerve crush injury and started to decrease four to five weeks after nerve crush injury. Omics analysis revealed translational changes in the ipsilateral cerebellum, but not in the contralateral cortex and striatum of nerve-injured wildtype animals. Interestingly, the nerve crush injury induces changes in DYT-TOR1A KI mice, with translational regulation in the contralateral striatum and cortex, but not in the ipsilateral cerebellum.

Conclusion
The nerve-injured DYT-TOR1A KI mice showed a more severe clinical phenotype, compared to the DYT-TOR1A KI naive mice. The Omics investigation suggests that translation regulation in the cerebellum, acts as a rescue mechanism in nerve-injured wildtype mice. However, cortical, and striatal translational regulation might be the underlying cause for the dystonic phenotype in DYT-TOR1A KI nerve injured mice.

Funding source(s)
This project was funded by the European Union's Horizon 2020 research and innovation programme under the EJP RD COFUND-EJP N° 825575 (EurDyscover) and by the VERUM foundation. Moreover, C.W.I. receives funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) Project-ID 424778381-TRR 295 (A01, A06) and the Interdisciplinary Center for Clinical Research (IZKF) at the University of Würzburg (S-506, N-362).

Effects Of A Peripheral Nerve Injury On The Dystonic Phenotype And Striatal Synaptic Function Of A DYT1 Mouse Model

Maria Meringolo[1,2], Giuseppina Martella[1], Martina Montanari[1], Ilham El Atiallah[1], Giulia Ponterio[1], Annalisa Tassone[1], Giuseppe Sciamanna[1,2], Susanne Knorr[3], Lisa Rauschenberger[3], Chi Wang Ip[3], Antonio Pisani[4], Paola Bonsi[1]

[1]Fondazione Santa Lucia IRCCS, Rome, Italy
[2]Unicamillus University, Rome, Italy
[3]University Hospital of Würzburg, Würzburg, Germany
[4]University of Pavia and IRCCS Mondino Foundation, Pavia, Italy

Gene-environment interactions may be relevant in the pathogenesis of hereditary forms of dystonia with reduced penetrance, such as DYT1 (DYT-TOR1A), DYT6 (DYT-THAP1), DYT25 (DYT-GNAL). Based on the hypothesis of a "second hit", proposing that the manifestation of dystonia results from the interplay of an intrinsic predisposition and an environmental trigger, a dystonia-like phenotype has been disclosed in genetic rodent models by exposing them to a sensorimotor stressor, the compression of the sciatic nerve. We utilized electrophysiological recordings from striatal slices of the Tor1a$^{+/\Delta gag}$ mouse model of DYT1 dystonia, at different time-intervals after a 15 second-long compression of the sciatic nerve, to determine the synaptic correlates of the dystonia-like phenotype. We performed surgery on Tor1a$^{+/\Delta gag}$ mice aged 30 postnatal days. The tail suspension test (TST) was performed 24 hours before and after surgery, and then weekly for additional 8 weeks. The video recordings of the TST sessions were analyzed with an automated system. Mice subjected to sciatic nerve compression showed abnormal postures of the injured hind limb, similar to human peripheral pseudodystonia. Tor1a$^{+/\Delta gag}$ mice showed an increased frequency and duration of dystonic movements, compared to their wildtype littermates. This difference was statistically significant from 4 weeks post-injury. Both sham and injured mutant Tor1a$^{+/\Delta gag}$ mice showed similar striatal electrophysiological alterations: loss of long-term depression of corticostriatal synaptic transmission (LTD) and reduced pause-response evoked in cholinergic interneurons (ChIs) by a thalamic stimulation mimicking salient sensory stimuli. Notably, 2 weeks after injury, at the peak of dystonia-like movements, also wildtype littermates showed similar striatal dysfunctions, LTD impairment and reduced ChIs pause-response, which, however, were fully rescued 4 weeks after surgery, in parallel with dystonia-like postures. These results support a relationship between an altered thalamo-cortical synaptic integration in the striatum and the manifestation of a dystonic phenotype.

Funding source
The Italian Ministry of Health supported this project, under the framework of the European Joint Programme on Rare Diseases (EJP RD) (EJP RD-1352019). In addition, this project received funding from the European Union's Horizon 2020 research and innovation program under EJP RD COFUND-EJP No. 825575 (EurDyscover).

Investigating The Dysregulation Of ISR Pathway By Antipsychotics As A Possible Cause Of Drug-Induced Dystonia (DID)

Tricia A. Simon, Rekha C. Patel

Department of Biological Sciences, University of South Carolina, Columbia, SC, USA

A maladaptive integrated stress response (ISR) involving dysregulation of the eukaryotic translation initiation factor α (eIF2α) mediated signaling is observed in DYT-*PRKRA* patient cells (1,2). Additionally, recent research has indicated that dysregulated eIF2α signaling is one of the convergent mechanisms in etiologically diverse dystonias (3). Dystonia is observed as a side effect during

antipsychotic therapy and people often discontinue their medications due to side effects (4). In this study, we investigated if ISR pathway is also dysregulated in response to antipsychotic drugs and could contribute to drug-induced dystonia (DID). Using human lymphoblasts, we investigated the ability of antipsychotic drugs to modulate ISR. This was done by using western blot analysis to study induction of the transcription factor ATF4 after endoplasmic reticulum (ER) stress with or without prior treatment with the antipsychotic drugs. Our results indicate that the antipsychotic drugs alter the ISR by either changing the intensity or the duration of the response. Based on our previous research with DYT-*PRKRA*, we also tested the ability of a natural plant flavonoid, luteolin, to restore normal ISR in the presence of antipsychotics. Our results indicate that luteolin can alleviate the ISR dysregulation caused by antipsychotic drugs, thereby suggesting its possible application in avoiding DID. Precise regulation of eIF2α signaling in neurons is critical and skewing of ISR pathway in either direction can have significant negative consequences (5). We plan on testing the effect of antipsychotic drugs on neurite outgrowth and maintenance using cultured neurons.

Funding source(s), including NIH support, if any
This work was supported by a Department of Defense through the grant W81XWH-22-1-0526 to R. P. and a pilot grant to R. P. from the NIH COBRE Center for Dietary Supplements & Inflammation Targeted Therapeutics at University of South Carolina supported by grant. Opinions, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

References

1. Burnett, S. B., Vaughn, L. S., Sharma, N., Kulkarni, R., and Patel, R. C. (2020) Dystonia 16 (DYT16) mutations in PACT cause dysregulated PKR activation and eIF2α signaling leading to a compromised stress response. *Neurobiology of disease* **146**, 105135.

2. Vaughn, L. S., Bragg, D. C., Sharma, N., Camargos, S., Cardoso, F., and Patel, R. C. (2015) Altered Activation of Protein Kinase PKR and Enhanced Apoptosis in Dystonia Cells Carrying a Mutation in PKR Activator Protein PACT. *The Journal of biological chemistry* **290**, 22543-22557.

3. Gonzalez-Latapi, P., Marotta, N., and Mencacci, N. E. (2021) Emerging and converging molecular mechanisms in dystonia. *Journal of neural transmission (Vienna, Austria : 1996)* **128**, 483-498.

4. Mehta, S. H., Morgan, J. C., and Sethi, K. D. (2015) Drug-induced movement disorders. *Neurologic clinics* **33**, 153-174.

5. Bellato, H. M., and Hajj, G. N. (2016) Translational control by eIF2α in neurons: Beyond the stress response. *Cytoskeleton (Hoboken)* **73**, 551-565.

Investigating The Molecular And Cellular Basis Of Epsilon-Sarcoglycan-Related Myoclonus-Dystonia In An iPSC-derived Neuronal Model

Karen Grütz[1], Philip Seibler[1], Enrico Glaab[2], Anne Weissbach[1,3], Sokhna-Aida Diaw[1], Francesca Carlisle[4], Derek Blake[4], Christine Klein[1], Katja Lohmann[1], Anne Grünewald[1,2]

[1]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany
[2]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux, Luxembourg
[3]Institute of Systems Motor Science, University of Lübeck, Lübeck, Germany
[4]Division of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff, United Kingdom

SGCE-related myoclonus-dystonia (M-D) underlies the epigenetic process of imprinting, leading to reduced penetrance upon maternal transmission of a pathogenic variant. We previously demonstrated that induced pluripotent stem cell (iPSC)-derived cortical neurons with pathogenic *SGCE* variants can serve as an adequate disease model for *SGCE*-related M-D, enabling the investigation of functional properties, such as the cellular localization of epsilon-sarcoglycan (encoded by *SGCE*). Interestingly, epsilon-sarcoglycan has been linked to the dystrophin-associated glycoprotein complex (DGC), which is located at the plasma membrane and varies in composition.

iPSC lines of two M-D patients with pathogenic variants in *SGCE* (c.298T>G, p.Trp100Gly and c.304C>T, p.Arg102Ter) and two control iPSC lines were differentiated into mature cortical neurons. Localization of brain-specific epsilon-sarcoglycan was investigated by cell-surface biotinylation and Western blotting in controls and the missense variant line with and without proteasomal inhibition (MG132). The nonsense variant

was excluded since no protein was detectable in previous analyses. RNA samples of all four iPSC-derived cortical neuron lines were subjected to transcriptome analysis. Candidate transcripts were validated by quantitative real-time PCR (qPCR).

Upon biotin treatment, brain-specific epsilon-sarcoglycan was detected in the membrane fraction of controls. The missense-variant protein was detected in whole-cell lysates but not at the cell surface. Incubation with MG132 increased levels of epsilon-sarcoglycan, but the location at the plasma membrane could not be restored. Transcriptome analysis revealed that of the DGC components *SGCA*, *SGCB*, *SGCG*, *SGCD*, and *SGCZ*, only *SGCD* and *SGCZ* were upregulated in neurons with *SGCE* variants with foldchanges and p-values of 20.96; 7.64*10-6 and 14.67; 5.05*10-4, respectively. Validation by qPCR indicated only small expression changes of these two genes.

The endogenous M-D model studied here indicates that the brain-specific isoform of epsilon-sarcoglycan is indeed localized at the cell surface in control neurons but not in patient-derived cells. Proteasomal inhibition increases the amount of epsilon-sarcoglycan but does not affect the cellular localization of the missense variant. mRNA expression analyses revealed that *SGCD* and *SGCZ* are upregulated in lines with pathogenic *SGCE* variants, indicating a possible compensation in the composition of the DGC. Further analyses are warranted to comprehensively expand these preliminary findings.

Funding source(s), including NIH support, if any
The study was supported by intramural funds from the University of Lübeck and the Dystonia Medical Research Foundation.

Individual-Specific Brain Functional Connectivity Mapping Of Therapeutic Response In Task-Specific Focal Dystonia

Gordon EM[1], Matthews K[1], Meyer AC[1], Norris SA[1,2]

[1]Washington University School of Medicine, Departments of Radiology[1] and Neurology

Introduction
Isolated task-specific dystonia occurs during specific activities, but not at rest. This allows for neuroimaging study with reduced potential state-related confounds, particularly when considering studies of treatment response. Current therapeutic gold standards for task-specific dystonia rely on recurring botulinum toxin injections. Therapeutic advancement requires improved understanding of neural mechanisms related to adaptive treatment response. Resting-state fMRI measures brain network functional connectivity (FC), where botulinum toxin therapy may alter FC in patients with non-task-specific dystonia, but intra-scan movement potentially confounds interpretation. Furthermore, group averaging in prior studies combine regions with and without dysfunction across patients and may fail to detect effects localized to individual-specific brain regions. The objective of this study is to determine therapeutic effects of botulinum toxin on functional connectivity in individuals with task-specific dystonia.

Materials and Methods
We conducted a treatment response application of precision functional mapping (PFM) in patients with task-specific dystonia (writer's cramp & laryngeal dystonia). PFM involves repeated collection of fMRI data in the same patient to characterize individual-specific brain network organization without averaging across patients. We collected 125 minutes rs-fMRI/40 minutes task fMRI in each patient, both before and after successful therapeutic botulinum toxin. Tasks included impairment-specific motor tasks (vocalization; hand movement). Within each individual, we compared task activation and rest-

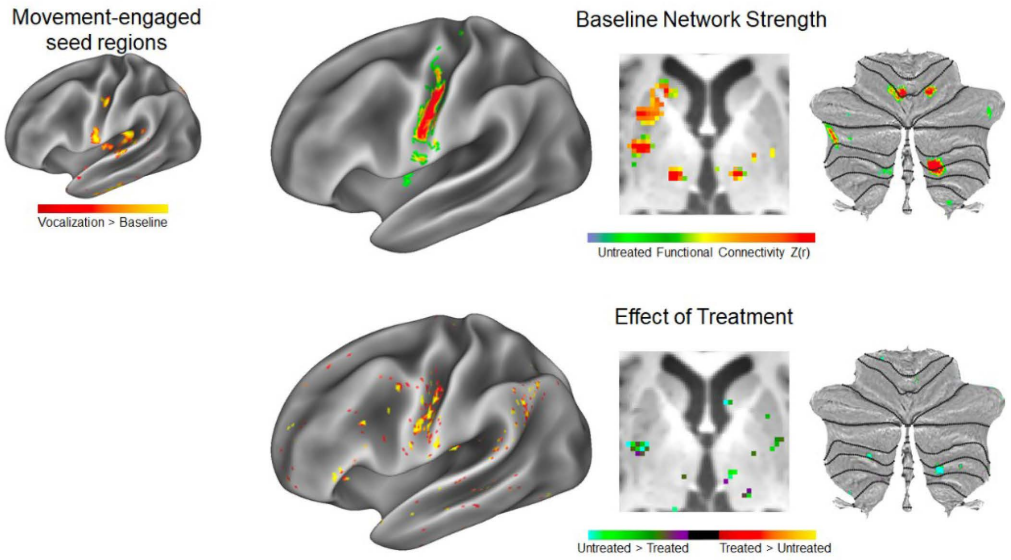


Figure 1 | Examining effects of botulinum toxin treatment on the vocalization-related brain network in laryngeal dystonia. Left: In a single individual patient with laryngeal dystonia, precision, high-data fMRI scanning during a vocalization task revealed vocalization-related cortical regions in central sulcus as a dual representation (dorsal/ventral). Right, top: Resting-state functional connectivity of those vocalization-related motor regions in the same individual revealed an integrated network in motor cortex and in putamen, thalamus, and cerebellum. Right, bottom: Compared to the untreated state, botulinum toxin treatment of this individual increased this functional connectivity in motor cortex but decreased connectivity in striatum, thalamus, and cerebellum

ing-state FC before and after successful therapeutic botulinum toxin.

Results

PFM allowed us to 1) determine the task activated somatomotor functional network in individuals, and 2) detect highly localized changes in individual's task engagement and brain network connectivity that related to successful therapeutic intervention. In pre-treatment conditions, the impairment-specific motor task engaged localized regions of M1 that were more strongly engaged after treatment. These task-related regions exhibited strong FC to somatomotor, striatal, and cerebellar regions. After successful botulinum toxin treatment, FC increased within cortex but decreased in subcortical structures (Figure 1).

Discussion

PFM in task-specific focal dystonia offers advantages to understand individualized network alterations in response to successful botulinum toxin treatment, and thus sheds light on pathophysiologic mechanisms. Altered treatment related FC in somatotopically specific regions might represent potential target engagement sites or future therapeutic trials.

Funding source(s), including NIH support

Dysphonia International

NIH, R01NS124789

Authors Index

N

O

P

Q

R

S

T

U

V

W

Y

Z

DYSTONIA

Dystonia is the official journal of
the Dystonia Medical Research Foundation

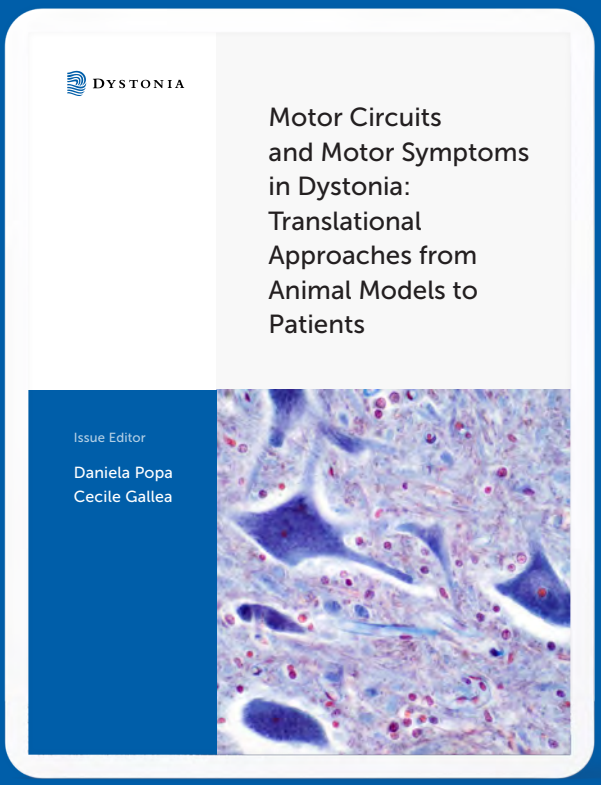
The mission of the DMRF is to advance research for more treatments and ultimately a cure, to promote awareness and education, and to support the needs and well being of affected individuals and families.

Discover more about the journal

See more →

fro.ntiers.in/dyst
publishingpartnerships.frontiersin.org

Contact
dystonia.office@frontierspartnerships.org



frontiers