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Electromyographic evidence in support of a knock-in mouse model of DYT1 dystonia

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Abstract

Background—DYT1 dystonia is an autosomal dominant movement disorder characterized by abnormal, often repetitive, movements, and postures. Its hallmark feature is sustained or intermittent contractions of muscles involving co-contractions of antagonist muscle pairs. The symptoms are relieved with the anticholinergic drug trihexyphenidyl. The primary mutation is a trinucleotide deletion (GAG) in *DYT1/TOR1A*, which codes for torsinA. Previous studies showed that (1) heterozygous *Dyt1* GAG knock-in mice, which have an analogous mutation in the endogenous gene, exhibit motor deficits and altered corticostriatal synaptic plasticity in the brain, and (2) these deficits can be rescued by trihexyphenidyl. However, brain imaging studies suggest that the *Dyt1* knock-in mouse models non-manifesting mutation carriers of DYT1 dystonia.

Objectives—To examine the hallmark features of DYT1 dystonia in the *Dyt1* knock-in mice by analyzing muscular activities.

Methods—Wireless telemetry devices with biopotential channels were implanted to the bicep and the rectus femori muscles in the *Dyt1* knock-in mice, and the muscular activities were recorded before and after trihexyphenidyl administration.

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Results—(1) Consistent with DYT1 dystonia patients, the *Dyt1* knock-in mice showed sustained contractions and co-contractions of the antagonistic bicep femoris and rectus femoris. (2) The abnormal muscle contractions were normalized by trihexyphenidyl.

Conclusions—The results suggest that the motor deficits in the *Dyt1* knock-in mice are likely produced by the abnormal muscle contractions and the *Dyt1* knock-in mice can potentially be used as a manifesting disease model to study the pathophysiology and to develop novel therapeutics.

Keywords

Anticholinergic; dystonia; DYT1; electromyography; muscle contraction

Introduction

Dystonia is the third most common movement disorder after Parkinson's disease and essential tremor. The disorder is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements and postures¹. DYT1 dystonia (Oppenheim's dystonia) is the most common type of autosomal dominant, isolated dystonia. Patients typically begin to present with lower limb dystonia between 5 and 28 years of age². The symptoms progressively move upwards and generalize to the rest of the body. DYT1 dystonia is primarily caused by a trinucleotide GAG deletion in the *DYT1/TOR1A* gene with reduced penetrance. TorsinA belongs to the AAA+ (ATPases associated with diverse cellular activities) superfamily³, and has a molecular chaperon-like activity^{4,5}. TorsinA is involved in recycling and releasing of pre-synaptic neurotransmitter vesicles, and mutations may influence cytoplasmic Ca²⁺ dynamics and neuronal plasticity⁶⁻⁹. However, the mechanism by which mutant torsinA causes DYT1 dystonia is unclear.

While several animal models of DYT1 dystonia exist¹⁰, two independently developed knock-in models faithfully recapitulate the trinucleotide deletion seen commonly in patients^{11,12}. Both lines of *Dyt1* GAG knock-in (KI) mice do not display overt dystonic symptoms. However, they exhibit altered locomotor behaviors, including abnormal gait, increased locomotor activity, and motor deficits in the beam-walking test^{11,13}. Furthermore, the motor deficits in *Dyt1* KI mice can be rescued by trihexyphenidyl (THP)¹⁴, an anticholinergic commonly used to alleviate symptoms in DYT1 dystonia patients¹⁵. *Dyt1* KI mice showed an impaired corticostriatal long-term depression (LTD), which is a form of synaptic plasticity that can be restored to WT levels by THP¹⁴. However, as the *Dyt1* KI mice do not have overt dystonic symptoms, it is unclear whether the mice model manifesting DYT1 dystonia patients or non-manifesting mutation carriers.

A patient study revealed that manifesting DYT1 dystonia patients and non-manifesting DYT1 dystonia mutation carriers have reduced integrity in cerebellothalamic white matter tracts¹⁶. The non-manifesting mutation carriers have a unique disruption in thalamocortical white matter tracts, which suggest it blocks the appearance of an overt dystonia phenotype in them. A follow-up study was conducted on *Dyt1* KI mice at 18 weeks of age, and demonstrated altered cerebellothalamocortical tract changes along with alterations in the brainstem that connect the basal ganglia to the cerebellum¹⁷. The authors, consequentially, suggested that the *Dyt1* KI mice are a model of non-manifesting mutation carriers of DYT1

dystonia. However, it is worth noting that a previous study determined that motor symptoms do not present in the other line of *Dyt1* KI mice until approximately 6 months of age¹⁸. Therefore, it is unknown whether at an older age the mice will present with an imaging phenotype similar to manifesting DYT1 dystonia patients.

We sought to address the issue of whether the *Dyt1* KI mice are a model of manifesting DYT1 dystonia or non-manifesting mutation carriers using a different approach. EMG recordings detect the electrical potential generated by muscle groups¹⁹. In mice, EMG is generally recorded by implanting an electrode directly into a muscle group. As the hallmark clinical feature of dystonia is sustained muscle contractions or intermittent co-contractions of antagonistic muscles, we used this technique to investigate whether the *Dyt1* KI mice exhibited sustained or intermittent muscle contractions characteristic of clinical dystonia. The study also tested whether these abnormal muscle contractions can be normalized by the anticholinergic THP.

Methods

Mice

Dyt1 KI mice and littermate WT controls were generated and genotyped as previously described^{11, 20}. Mice were housed in a vivarium with 12 hours of light, 12 hours of dark with *ad libitum* access to food and water. All experiments were conducted in compliance with the USPHS Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Use and Care Committee at the University of Alabama at Birmingham and the University of Florida.

Electromyography

EMG recordings were performed as previously described²¹. In brief, each mouse was implanted with a wireless telemetry device (F20-EET, Data Sciences International), which weighed approximately 3.9 g and 1.9 cm³ in volume, with two biopotential channels. Each biopotential channel had two leads with an outside diameter of approximately 0.3 mm. Ten *Dyt1* KI mice and 6 wild-type (WT) littermate controls of approximately 6 months of age were used. The mice were deeply anesthetized by a mixture of ketamine and xylazine and maintained using isoflurane during the surgical implantation. One side of the skin near the hind limb was shaved and a small vertical cut (approximately 1 cm) was made. The bicep femoris, which is involved in knee flexion, and the rectus femoris, which is involved in knee extension, were visually localized. The biopotential leads were then inserted into the muscle, fastened with sutures, and glued in place. The wireless transmitter body was then placed under the skin of the back of the mouse alongside any excess wire. The incision was then closed with suture, antibiotics were applied to the site, and the mouse was placed in a housing cage on top of a heating pad, which was monitored for temperature, to aid in recuperation from the surgery. After mice awoke from anesthesia and were freely walking around with ease, they were returned to a housing room to recover from surgery for an additional 24 to 48 hrs. After this recovery period the wireless transmitters were turned on using a magnet, and EMG data was acquired at 1,000 Hz by a connected computer using Dataquest A.R.T. software (Data Sciences International). After one day of recording in their

home cage, a random subset of 8 *Dyt1* KI mice and 4 WT littermate controls were injected intraperitoneally daily with a 0.8 mg/kg dose of THP in saline and recorded for two additional days. Data during the 12 hr nocturnal period, which relates to the wake period for mice, were subsequently analyzed.

Spectral analysis

The 12-hr blocks of wake-period data were divided into 1 s intervals. Due to technical issues with the transmitter, intervals from partial recordings were analyzed for two mice. Intervals contaminated by artifacts were excluded from further analysis. Thus, an average of 72% of trials was retained. Power spectral density (PSD) using Fast Fourier transforms was calculated to examine the overall temporal structures within each EMG recording. To quantitatively compare the PSDs across conditions, mean power frequency was calculated as the sum of product of the EMG power spectrum and frequency divided by the total sum of the power spectrum, and the median frequency was calculated as the frequency at which the EMG power spectrum is divided into two regions with equal amplitude^{22, 23}.

Analysis of sustained contractions

A protocol was designed in the NeuroScore software package (Data Sciences International) for the detection of sustained contractions (band-pass filtered at 10-100 Hz). The protocol assessed sustained contractions as being 3 times baseline activity for at least 10 s with a 2 s joint interval. An investigator blind to the genotype of the animal determined the value of the baseline activity. One WT animal was excluded from data analysis for the first day of recording after THP because of a technical issue with the transmitter. Due to the non-normality of the data, a Mann-Whitney rank-sum test was used to determine significance between groups ($p < 0.05$). For simplicity of graphical representation, the data was normalized to the WT level and presented using a box-whisker plot.

Analysis of co-contractions

The cross-correlation between the agonist and antagonist EMGs was computed for each interval for lags up to ± 100 ms to assess co-contractions. To examine the frequency of occurrence of co-contractions for each animal, the histogram of the zero-lag ($\tau=0$) cross-correlation was calculated, shown as a percentage of the total number of intervals for each mouse, and color-coded by treatment condition for comparison. The zero-lag cross-correlation distribution was normalized using Fisher z-transformation, and the peak value, or absolute value of the median correlation coefficient, was tested using paired t-test for statistical significance ($p < 0.05$).

Results

General electromyographic characteristics

Dyt1 KI and WT littermate mice were implanted with a wireless telemetry system and EMG activities were subsequently recorded (Fig. 1A). In addition to normal muscle contractions (Fig. 1B), there were clear observations of sustained contractions of muscles and co-contractions of antagonistic muscles in *Dyt1* KI mice (Fig. 1C, D). There was no statistical difference in the power spectral density (Fig. 1E, H), mean frequency (Fig. 1F, I), or median

frequency (Fig. 1G, J) between *Dyt1* KI and WT mice before or after injection of THP. Therefore, this suggests that there are no gross differences in EMG architecture between the genotypes.

Sustained contractions and amelioration by trihexyphenidyl in *Dyt1* KI mice

A significant increase in the number of sustained contractions was observed in the *Dyt1* KI mice compared to WT mice ($p < 0.05$, Fig. 2A). To determine the effects of a classic DYT1 dystonia treatment on muscle activity, *Dyt1* KI and WT mice were injected with THP, an anticholinergic, and recorded the effects on electromyographic activity. No significant differences in sustained contractions were observed between *Dyt1* KI and WT mice ($p > 0.05$, Fig. 2B).

Co-contractions and amelioration by trihexyphenidyl in *Dyt1* KI mice

We examined *Dyt1* KI and WT mice for the presence of intermittent co-contractions, a characteristic clinical feature of dystonia in humans. The EMG normalized cross-correlation at zero lag ($\tau = 0$), which is an indicator of the occurrence of co-contractions²⁴, was higher in *Dyt1* KI than in WT mice ($p < 0.05$, Fig. 3A). The percent distribution of zero-lag cross-correlation coefficients across all trials in each animal further demonstrated that co-contractions, represented by correlation coefficients greater than 0, were more likely to occur in *Dyt1* KI mice than in WT mice (Fig. 3B). This is statistically shown to be true in Figure 3E, where the absolute values of the median correlation coefficient (i.e. peak values in Fig. 3B) were shown to be statistically different between *Dyt1* KI and WT mice (red and blue markers, respectively, $p < 0.05$, paired t-test). Furthermore, THP treatment shifted the distribution of zero-lag cross-correlation in KI mice significantly (Fig. 3C and 3D) towards a peak correlation coefficient of zero (i.e. no co-contractions) to the point they were statistically indistinguishable from WT mice, suggesting an amelioration of co-contractions in *Dyt1* KI mice by THP ($p > 0.05$, paired t-test, Fig. 3E).

Discussion

DYT1 dystonia is a genetic movement disorder characterized by sustained muscle contractions and co-contractions. Anticholinergic THP treatment provides significant relief in humans. *Dyt1* KI mice, which exhibit motor deficits and impairment of corticostriatal synaptic plasticity and THP relief of these deficits, provide a promising preclinical model of dystonia. However, there are disagreements over whether the motor deficits in the *Dyt1* KI mice are equivalent to dystonic symptoms in DYT1 dystonia patients. We examined the *Dyt1* KI mice for sustained muscle contractions and co-contractions of antagonist muscle pairs with wireless EMG recording. *Dyt1* KI mice exhibited both sustained muscle contractions and co-contractions of an antagonistic muscle pair, which were subsequently ameliorated by THP. The results can be seen as providing further evidence supporting the face validity and utility of the *Dyt1* KI mice as a disease model of manifesting DYT1 dystonia.

The level of torsinA is decreased in both fibroblasts derived from a DYT1 dystonia patient and the brain of *Dyt1* KI mice^{12, 18, 25}, suggesting that a partial loss of torsinA function

derived from the GAG mutation may contribute to the clinical symptoms of DYT1 dystonia. Consistently, a novel candidate drug increased striatal torsinA levels and rescued motor deficits in *Dyt1* KI mice ⁵. In addition, both *Dyt1* knockdown ²⁶ and *Dyt1* heterozygous knockout mice show motor deficits ⁷. To further test this loss-of-function hypothesis, it will be of value in future studies to examine *Dyt1* knockdown and heterozygous knockout mice for co-contractions and sustained contractions. Furthermore, alterations of motor performance were reported in distinct brain region-specific *Dyt1* conditional knockout mice ^{25, 27-32}. EMG analysis of these models will elucidate how the loss of torsinA function in individual brain regions contributes to dystonia.

Additionally, our result of increased co-contractions in *Dyt1* KI mice is similar to phenotypes observed in EMG analysis of models that have an overt dystonic phenotype. For instance in the tottering mice, a classic model of episodic ataxia type 2 that has dystonic features, there is an increase in co-contractions of muscles compared to controls ³³. Likewise, a model of inherited deficiency of bilirubin metabolism has an increase in co-contractions and dystonic postures after sulfadimethoxine treatment ³⁴. However, there was a study conducted in a model of severe ataxia with dystonia, in which there was no correlation between muscle activity, neuronal firing, and abnormal gait ³⁵. It is worth noting that in this particular study EMG analysis was conducted using a neck muscle, rather than a leg muscle, and that abnormal gait is not specific to dystonia. More closely relatable to our studies is a pharmacological model of DYT12 dystonia. DYT12 dystonia is caused by a mutation in a particular neuronal Na⁺/K⁺-ATPase. Fremont and colleagues injected ouabain, an inhibitor of Na⁺/K⁺-ATPases, into the cerebellum of mice and observed dystonic postures and co-contractions of antagonistic muscles ³⁶. Future studies of other genotypic mouse models of dystonia, including DYT6 ³⁷, 11 ³⁸⁻⁴¹, and 12 ⁴²⁻⁴⁵ dystonias, which were previously characterized, could benefit from EMG analysis.

The mechanism by which THP can normalize sustained muscle contractions and co-contractions in *Dyt1* KI mice is not known. While THP is known to cause sedation in certain conditions in patients ⁴⁶, in rodents THP treatment increases motor activity instead ^{47, 48}. Therefore, the effect of THP treatment shown here likely was not resulted from a sedative effect of THP. We speculate, based on previous findings that reduced D2R levels on cholinergic interneurons result in increased release of acetylcholine, over-activation of muscarinic acetylcholine receptors, and inhibition of Ca_v1.3 channels. This therefore results in the reduction of calcium influx, causing corticostriatal LTD deficits, and abnormal muscle contractions and motor deficits. However, further studies are needed to test and refine this circuit hypothesis ^{2, 14, 49}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord.* 2013; 28(7):863–873. [PubMed: 23649720]
2. Breakefield XO, Blood AJ, Li Y, Hallett M, Hanson PI, Standaert DG. The pathophysiological basis of dystonias. *Nat Rev Neurosci.* 2008; 9(3):222–234. [PubMed: 18285800]
3. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet.* 1997; 17(1):40–48. [PubMed: 9288096]
4. Caldwell GA, Cao S, Sexton EG, Gelwix CC, Bevel JP, Caldwell KA. Suppression of polyglutamine-induced protein aggregation in *Caenorhabditis elegans* by torsin proteins. *Hum Mol Genet.* 2003; 12(3):307–319. [PubMed: 12554684]
5. Cao S, Hewett JW, Yokoi F, et al. Chemical enhancement of torsinA function in cell and animal models of torsion dystonia. *Dis Model Mech.* 2010; 3(5-6):386–396. [PubMed: 20223934]
6. Yokoi F, Cheetham CC, Campbell SL, Sweatt JD, Li Y. Pre-synaptic release deficits in a DYT1 dystonia mouse model. *PLoS One.* 2013; 8(8):e72491. [PubMed: 23967309]
7. Yokoi F, Chen HX, Dang MT, et al. Behavioral and electrophysiological characterization of Dyt1 heterozygous knockout mice. *PLoS One.* 2015; 10(3):e0120916. [PubMed: 25799505]
8. Kakazu Y, Koh JY, Ho KW, Gonzalez-Alegre P, Harata NC. Synaptic vesicle recycling is enhanced by torsinA that harbors the DYT1 dystonia mutation. *Synapse.* 2012; 66(5):453–464. [PubMed: 22213465]
9. Iwabuchi S, Kakazu Y, Koh JY, Harata NC. Abnormal cytoplasmic calcium dynamics in central neurons of a dystonia mouse model. *Neurosci Lett.* 2013; 548:61–66. [PubMed: 23748075]
10. Oleas J, Yokoi F, DeAndrade MP, Pisani A, Li Y. Engineering animal models of dystonia. *Mov Disord.* 2013; 28(7):990–1000. [PubMed: 23893455]
11. Dang MT, Yokoi F, McNaught KS, et al. Generation and characterization of Dyt1 DeltaGAG knock-in mouse as a model for early-onset dystonia. *Exp Neurol.* 2005; 196(2):452–463. [PubMed: 16242683]
12. Goodchild RE, Kim CE, Dauer WT. Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. *Neuron.* 2005; 48(6):923–932. [PubMed: 16364897]
13. Song CH, Fan X, Exeter CJ, Hess EJ, Jinnah HA. Functional analysis of dopaminergic systems in a DYT1 knock-in mouse model of dystonia. *Neurobiol Dis.* 2012; 48(1):66–78. [PubMed: 22659308]
14. Dang MT, Yokoi F, Cheetham CC, et al. An anticholinergic reverses motor control and corticostriatal LTD deficits in Dyt1 DeltaGAG knock-in mice. *Behav Brain Res.* 2012; 226(2):465–472. [PubMed: 21995941]
15. Jankovic J. Treatment of dystonia. *Lancet Neurol.* 2006; 5(10):864–872. [PubMed: 16987733]
16. Argyelan M, Carbon M, Niethammer M, et al. Cerebellothalamocortical connectivity regulates penetrance in dystonia. *J Neurosci.* 2009; 29(31):9740–9747. [PubMed: 19657027]
17. Ulug AM, Vo A, Argyelan M, et al. Cerebellothalamocortical pathway abnormalities in torsinA DYT1 knock-in mice. *Proc Natl Acad Sci U S A.* 2011; 108(16):6638–6643. [PubMed: 21464304]
18. Yokoi F, Yang G, Li J, DeAndrade MP, Zhou T, Li Y. Earlier onset of motor deficits in mice with double mutations in Dyt1 and Sgce. *J Biochem.* 2010; 148(4):459–466. [PubMed: 20627944]
19. Whelan PJ. Electromyogram recordings from freely moving animals. *Methods.* 2003; 30(2):127–141. [PubMed: 12725779]
20. Yokoi F, Dang MT, Miller CA, et al. Increased c-fos expression in the central nucleus of the amygdala and enhancement of cued fear memory in Dyt1 DeltaGAG knock-in mice. *Neurosci Res.* 2009; 65(3):228–235. [PubMed: 19619587]

21. DeAndrade MP, Johnson RL, Unger EL, et al. Motor restlessness, sleep disturbances, thermal sensory alterations and elevated serum iron levels in Btbd9 mutant mice. *Hum Mol Genet.* 2012; 21(18):3984–3992. [PubMed: 22678064]
22. Oskoei MA, Hu H. Support vector machine-based classification scheme for myoelectric control applied to upper limb. *IEEE Trans Biomed Eng.* 2008; 55(8):1956–1965. [PubMed: 18632358]
23. Phinyomark A, Phukpattaranont P, Limsakul C. Feature reduction and selection for EMG signal classification. *Expert Systems with Applications.* 2012; 39:7420–7431.
24. Calderon DP, Fremont R, Kraenzlin F, Khodakhah K. The neural substrates of rapid-onset Dystonia-Parkinsonism. *Nat Neurosci.* 2011; 14(3):357–365. [PubMed: 21297628]
25. Yokoi F, Dang MT, Li Y. Improved motor performance in Dyt1 DeltaGAG heterozygous knock-in mice by cerebellar Purkinje-cell specific Dyt1 conditional knocking-out. *Behav Brain Res.* 2012; 230(2):389–398. [PubMed: 22391119]
26. Dang MT, Yokoi F, Pence MA, Li Y. Motor deficits and hyperactivity in Dyt1 knockdown mice. *Neurosci Res.* 2006; 56(4):470–474. [PubMed: 17046090]
27. Yokoi F, Dang MT, Mitsui S, Li J, Li Y. Motor deficits and hyperactivity in cerebral cortex-specific Dyt1 conditional knockout mice. *J Biochem.* 2008; 143(1):39–47. [PubMed: 17956903]
28. Yokoi F, Dang MT, Li J, Standaert DG, Li Y. Motor deficits and decreased striatal dopamine receptor 2 binding activity in the striatum-specific Dyt1 conditional knockout mice. *PLoS One.* 2011; 6(9):e24539. [PubMed: 21931745]
29. Liang CC, Tanabe LM, Jou S, Chi F, Dauer WT. TorsinA hypofunction causes abnormal twisting movements and sensorimotor circuit neurodegeneration. *J Clin Invest.* 2014; 124(7):3080–3092. [PubMed: 24937429]
30. Pappas SS, Darr K, Holley SM, et al. Forebrain deletion of the dystonia protein torsinA causes dystonic-like movements and loss of striatal cholinergic neurons. *Elife.* 2015; 4:e08352. [PubMed: 26052670]
31. Zhang L, Yokoi F, Jin YH, et al. Altered Dendritic Morphology of Purkinje cells in Dyt1 DeltaGAG Knock-In and Purkinje Cell-Specific Dyt1 Conditional Knockout Mice. *PLoS One.* 2011; 6(3):e18357. [PubMed: 21479250]
32. Sciamanna G, Hollis R, Ball C, et al. Cholinergic dysregulation produced by selective inactivation of the dystonia-associated protein torsinA. *Neurobiol Dis.* 2012; 47(3):416–427. [PubMed: 22579992]
33. Scholle HC, Jinnah HA, Arnold D, et al. Kinematic and electromyographic tools for characterizing movement disorders in mice. *Mov Disord.* 2010; 25(3):265–274. [PubMed: 20077474]
34. Chaniary K, Baron M, Rice A, Wetzel P, Shapiro S. Electromyographic characterization in an animal model of dystonia. *Mov Disord.* 2008; 23(8):1122–1129. [PubMed: 18442136]
35. Hisatsune C, Miyamoto H, Hirono M, et al. IP3R1 deficiency in the cerebellum/brainstem causes basal ganglia-independent dystonia by triggering tonic Purkinje cell firings in mice. *Front Neural Circuits.* 2013; 7:156. [PubMed: 24109434]
36. Fremont R, Calderon DP, Maleki S, Khodakhah K. Abnormal high-frequency burst firing of cerebellar neurons in rapid-onset dystonia-parkinsonism. *J Neurosci.* 2014; 34(35):11723–11732. [PubMed: 25164667]
37. Ruiz M, Perez-Garcia G, Ortiz-Virumbrales M, et al. Abnormalities of motor function, transcription and cerebellar structure in mouse models of THAP1 dystonia. *Hum Mol Genet.* 2015; 24(25):7159–7170. [PubMed: 26376866]
38. Yokoi F, Dang MT, Mitsui S, Li Y. Exclusive paternal expression and novel alternatively spliced variants of epsilon-sarcoglycan mRNA in mouse brain. *FEBS Lett.* 2005; 579(21):4822–4828. [PubMed: 16099459]
39. Yokoi F, Dang MT, Li J, Li Y. Myoclonus, motor deficits, alterations in emotional responses and monoamine metabolism in epsilon-sarcoglycan deficient mice. *J Biochem.* 2006; 140(1):141–146. [PubMed: 16815860]
40. Yokoi F, Dang MT, Yang G, et al. Abnormal nuclear envelope in the cerebellar Purkinje cells and impaired motor learning in DYT11 myoclonus-dystonia mouse models. *Behav Brain Res.* 2012; 227(1):12–20. [PubMed: 22040906]

41. Yokoi F, Dang MT, Zhou T, Li Y. Abnormal nuclear envelopes in the striatum and motor deficits in DYT11 myoclonus-dystonia mouse models. *Hum Mol Genet.* 2012; 21(4):916–925. [PubMed: 22080833]
42. Moseley AE, Williams MT, Schaefer TL, et al. Deficiency in Na,K-ATPase alpha isoform genes alters spatial learning, motor activity, and anxiety in mice. *J Neurosci.* 2007; 27(3):616–626. [PubMed: 17234593]
43. DeAndrade MP, Yokoi F, van Groen T, Lingrel JB, Li Y. Characterization of Atp1a3 mutant mice as a model of rapid-onset dystonia with parkinsonism. *Behav Brain Res.* 2011; 216(2):659–665. [PubMed: 20850480]
44. Ikeda K, Satake S, Onaka T, et al. Enhanced inhibitory neurotransmission in the cerebellar cortex of the Atp1a3-deficient heterozygous mice. *J Physiol.* 2013
45. Sugimoto H, Ikeda K, Kawakami K. Heterozygous mice deficient in Atp1a3 exhibit motor deficits by chronic restraint stress. *Behav Brain Res.* 2014; 272:100–110. [PubMed: 24983657]
46. Mink, JW. Dystonia (DRD, Primary and Secondary). In: Singer, Harvey S.; K, EH.; Hartman, Adam L.; Crawford, Thomas O., editors. *Treatment of Pediatric Neurologic Disorders*. Boca Raton, FL: CRC Press, Taylor & Francis Group, LLC; 2005. p. 139-144.
47. Sipos ML, Burchnell V, Galbicka G. Dose-response curves and time-course effects of selected anticholinergics on locomotor activity in rats. *Psychopharmacology (Berl).* 1999; 147(3):250–256. [PubMed: 10639682]
48. Fish EW, Krouse MC, Stringfield SJ, Diberto JF, Robinson JE, Malanga CJ. Changes in sensitivity of reward and motor behavior to dopaminergic, glutamatergic, and cholinergic drugs in a mouse model of fragile X syndrome. *PLoS One.* 2013; 8(10):e77896. [PubMed: 24205018]
49. Oleas, J.; Yokoi, F.; DeAndrade, MP.; Li, Y. Rodent Models of Autosomal Dominant Primary Dystonia. In: LeDoux, MS., editor. *Movement Disorders: Genetics and Models*. Second. New York: Academic Press Elsevier; 2015. p. 483-505.

Fig 1.

Representative EMG traces of dystonic muscle phenotypes examined. **A.** Schematic representation of the experimental design. **B.** An EMG trace of a normal muscle contraction from a WT mouse (rectus femoris). **C.** An EMG trace of a sustained muscle contraction from a *Dyt1* KI mouse (rectus femoris). **D.** An EMG trace of a co-contraction of the agonistic muscle pair from a *Dyt1* KI mouse. The left traces were obtained from the rectus femoris and the biceps femoris in the same scale as **B** and **C**. The yellow-shaded traces on the right were enlarged to show co-contractions at the faster time scale on the right. The arrows show the agonist and antagonist muscle activities coincide. Averaged power spectra before (**E**) and after THP treatment (**H**) for WT and KI mice ± 1 STD (shaded regions) from both agonist and antagonist muscles. Mean power frequency for KI and WT mice before (**F**) and after THP treatment (**I**) shows no significant difference between KI and WT mice. Median power frequency for KI and WT mice before (**G**) and after THP treatment (**J**) shows no significant difference between KI and WT mice.

Fig 2.

Sustained contractions in *Dyt1* KI and WT mice and the effect of THP treatment.

Examination of sustained contractions revealed a significantly increased number in *Dyt1* KI mice compared to WT mice (**A**). After treatment of *Dyt1* KI and WT mice with THP, there was no statistical difference between groups on the first or second day of treatment (**B**). Data is presented using a box and whisker plot, where the box represents the 25th to 75th percentile, the horizontal line within the box represents the median, and the whiskers extend to include the minimum and maximum value. *: $p < 0.05$.

Fig. 3.

Co-contractions in *Dyt1* KI and WT mice and the effect of THP treatment. **A.** Averaged EMG normalized cross-correlation before THP treatment revealed a significant difference at lag $\tau = 0$ between WT and KI mice, which was indicative of co-contractions in KI mice. **B.** Individual Fisher transformed histograms of cross-correlation coefficients at $\tau = 0$ for KI mice ($n = 7$) and WT mice ($n = 4$). **C, D.** After THP treatment there was a general normalization of lag at $\tau = 0$ between WT and KI mice. **E.** Comparison of the absolute values of the median (peak) correlation coefficients at $\tau = 0$ from panels (**B**) and (**D**). Circle markers represent individual peak values, black dashes show the means of the values plotted. Gray lines pair each mouse before and after treatment. The KI mice before THP treatment showed significantly high absolute value of the median correlation coefficient in comparison to other three groups ($p < 0.05$). On the other hand, WT mice showed no significant alteration before and after THP treatment ($p = 0.41$). *: $p < 0.05$.