HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2022 April 09.

Published in final edited form as:

Behav Brain Res. 2021 April 09; 403: 113137. doi:10.1016/j.bbr.2021.113137.

Investigating the role of striatal dopamine receptor 2 in motor coordination and balance: insights into the pathogenesis of DYT1 dystonia

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Abstract

DYT1 or DYT-TOR1A dystonia is early-onset, generalized dystonia. Most DYT1 dystonia patients have a heterozygous trinucleotide *GAG* deletion in *DYT1* or *TOR1A* gene, with a loss of a glutamic acid residue of the protein torsinA. DYT1 dystonia patients show reduced striatal dopamine D2 receptor (D2R) binding activity. We previously reported reduced striatal D2R proteins and impaired corticostriatal plasticity in *Dyt1* Δ*GAG* heterozygous knock-in (*Dyt1* KI) mice. It remains unclear how the D2R reduction contributes to the pathogenesis of DYT1 dystonia. Recent knockout studies indicate that D2R on cholinergic interneurons (Chls) has a significant role in corticostriatal plasticity, while D2R on medium spiny neurons (MSNs) plays a minor role. To determine how reduced D2Rs on ChIs and MSNs affect motor performance, we generated ChI- or MSN-specific D2R conditional knockout mice (*Drd2* ChKO or *Drd2* sKO). The striatal ChIs in the *Drd2* ChKO mice showed an increased firing frequency and impaired quinpirole-induced inhibition, suggesting a reduced D2R function on the ChIs. *Drd2* ChKO mice had an age-dependent deficient performance on the beam-walking test similar to the *Dyt1* KI mice. The *Drd2* sKO mice, conversely, had a deficit on the rotarod but not the beam-walking test. Our findings suggest that D2Rs on Chls and MSNs have critical roles in motor control and balance. The similarity of the beam-walking deficit between the *Drd2* ChKO and *Dyt1* KI mice supports our earlier notion that D2R reduction on striatal ChIs contributes to the pathophysiology and the motor symptoms of DYT1 dystonia.

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Author contributions: (1) Research Project: A. Conception, Y. Liu, DEV, Y. Li; B. Organization, Y. Liu, DEV, Y. Li; C. Execution, Y. Liu, HX, FY, Y. Li; (2) Statistical Analysis: A. Design, Y. Liu, Y. Li; B. Execution, Y. Liu, Y. Li; C. Review and Critique, Y. Liu, HX, FY, DEV, Y. Li; (3) Manuscript Preparation: A. Writing of the First Draft, Y. Liu; B. Review and Critique, Y. Liu, HX, FY, DEV, Y. Li.

Declarations of interest: None.

Financial Disclosures of all authors: Nothing to report.

Keywords

DYT1/DYT-TOR1A dystonia; dopamine D2 receptor; cholinergic interneurons; medium spiny neurons; corticostriatal plasticity

1. Introduction

Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements, postures, or both (Albanese et al., 2013). It can be caused by genetic mutations, brain injury, or the side effects of drugs. DYT1 or DYT-TOR1A dystonia (OMIM: #128100) is the most common type of early-onset generalized dystonia (Bressman et al., 2000) with symptoms onset from 5 to 28 years old. The majority of the individuals affected by DYT1 dystonia share a trinucleotide deletion (Δ*GAG*) in the exon 5 of the *DYT1* or *TOR1A* gene, leading to a loss of a glutamate amino acid residue for torsinA (torsinA$^{\Delta E}$) (Klein and Ozelius, 2002). Other mutations in the gene were also reported in rare cases (Leung et al., 2001; Zirn *et al.*, 2008; Ritz et al., 2009; Calakos et al., 2010; Cheng et al., 2014; Harata, 2014; Vulinovic et al., 2014; Dobricic et al., 2015). Moreover, recent reports suggest that homozygous mutation in *DYT1* causes developmental delay and motor deficits (Kariminejad et al., 2017; Reichert et al., 2017; Isik et al., 2019). As a member of the AAA+ (ATPases associated with diverse cellular activities) superfamily (Ozelius et al., 1997), torsinA is expressed at a higher level during early development of the brain (Xiao et al., 2004; Siegert et al., 2005; Vasudevan et al., 2006), especially for the neurons located in the basal ganglia (Augood et al., 1999; Shashidharan et al., 2000). In DYT1 dystonia patients, mutant torsinA might exert a dominant-negative effect and decrease wildtype torsinA activity due to a homo-oligomeric feature of AAA+ proteins (Konakova and Pulst, 2005; Pham et al., 2006; Zhao et al., 2013; Demircioglu et al., 2016; Chase et al., 2017).

It is generally believed that dystonia is not a muscle disease but is caused by brain deficits. Deep brain stimulation (DBS) targeting the globus pallidus internus (GPi) or subthalamic nucleus (STN) ameliorates the dystonia symptom (Sun et al., 2007; Panov et al., 2013; Guzzi et al., 2016; Cury et al., 2018; Wu et al., 2019), suggesting a functional alteration of the basal ganglia circuits. The striatum functions in controlling movement based on the balanced modulation of striatal circuits between cholinergic interneurons (ChIs) and the dopaminergic terminals from the substantia nigra (Stoof et al., 1992; Pisani et al., 2007; Aosaki et al., 2010). Growing evidence suggests that an alteration of the cholinergic systems plays a critical role in the pathophysiology of DYT1 dystonia (Eskow Jaunarajs et al., 2015). Anticholinergic drugs such as trihexyphenidyl (THP) are clinically effective in treating DYT1 dystonia, indicating an abnormal cholinergic tone in DYT1 dystonia. Decreased dopamine D2 receptor (D2R) binding activity and expression level in the striatum have been identified in DYT1 dystonia patients (Augood et al., 2002; Asanuma et al., 2005) and in multiple lines of *Dyt1* rodent models (Napolitano et al., 2010; Yokoi et al., 2011; Dang et al., 2012; Bonsi et al., 2019; Yokoi et al., 2020b), whereas high levels of D2Rs are present on the ChI in wild type mice (Le Moine et al., 1990). Activation of D2R slows the spontaneous firing of ChIs and acetylcholine release (Maurice et al., 2004; Chuhma et al.,

2014). Previous studies showed a paradoxical excitation in response to quinpirole, a D2R agonist, in multiple DYT1 dystonia genetic models (Sciamanna et al., 2011; Grundmann et al., 2012; Sciamanna et al., 2012; Martella et al., 2014; Eskow Jaunarajs et al., 2015; Scarduzio et al., 2017), suggesting D2R alterations contribute to the DYT1 pathophysiology. However, whether the reduction of striatal D2R is the result or cause of DYT1 dystonia is not known.

Animal models are essential for investigating the pathophysiology of human disease and contribute to the development of effective treatments. DYT1 animal models do not have overt dystonia, but they do show impairments in motor coordination and balance with the beam-walking and rotarod tests (Dang et al., 2005; Sharma et al., 2005; Dang et al., 2006a; Grundmann et al., 2007; Yokoi *et al.*, 2008; Zhao et al., 2008; Page et al., 2010; Sciamanna et al., 2012; Song et al., 2012; Yokoi et al., 2015; Sciamanna et al., 2020; Yokoi et al., 2020b). *Dyt1/Tor1a* Δ*GAG* heterozygous knock-in *(Dyt1* KI*)* mice have the corresponding in-frame trinucleotide *GAG* deletion in the endogenous *Dyt1* and model DYT1 patients (Dang et al., 2005; Goodchild et al., 2005). *Dyt1* KI mice exhibit the reduced level of striatal D2R protein (Dang et al., 2012; Bonsi et al., 2019), elevated extracellular acetylcholine (Scarduzio et al., 2017; Downs et al., 2019), impaired long-term depression (LTD) in the corticostriatal pathway (Dang et al., 2012; Maltese et al., 2014; Martella et al., 2014), abnormal sustained co-contractions of agonist and antagonist muscles (DeAndrade et al., 2016), and motor deficits in the beam-walking test (Dang et al., 2005; Song et al., 2012). The deficits in the LTD, beam-walking, and muscle co-contractions can be ameliorated by THP (Dang et al., 2012; Maltese et al., 2014; DeAndrade et al., 2016). Since D2R on ChIs have been identified as a key regulator of the striatal bidirectional synaptic plasticity (Wang et al., 2006; Augustin et al., 2018) and the dopamine/acetylcholine balance (Kharkwal et al., 2016), we proposed a model that links reduced D2R signaling on ChIs, the elevation of acetylcholine level, impairment of LTD, and motor abnormality in DYT1 dystonia (Dang et al., 2012). To further test the role of D2R on the ChIs in the pathogenesis of DYT1 dystonia directly, we used ChI-specific *Drd2* conditional knockout mice (*Drd2* ChKO). We characterized their biochemical, electrophysiological, and behavioral phenotypes. We also analyzed MSN-specific *Drd2* conditional knockout mice (*Drd2* sKO) to explore the role of D2R on MSNs in motor performance. Finally, we characterized an individual line of *Dyt1* KI mice for their motor performance to compare with the *Drd2* ChKO and sKO mice.

2. Materials and methods

All experiments were carried out by investigators blind to the genotypes and in compliance with the ARRIVE guidelines and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All animal experiments were approved by the Institution Animal Care and Use Committee at the University of Florida. The mice were housed with *ad libitum* access to food and water with 12 hours-light and 12 hours-dark.

2.1 Animals

Chat-cre mice were obtained by crossing ChAT-IRES-Cre::*frt-neo-frt* mice (Jackson Lab. Stock No: 006410) (Rossi et al., 2011) with flippase (FLP) deleter strain (Jackson Lab. Stock No: 003946; ROSA26::FLPe knock-in) (Farley et al., 2000). The recombinase FLP recognizes flippase recognition target (*frt*) sites and removed *neomycin* cassette in ChAT-IRES-Cre::*frt-neo-frt* mice by FLP-mediated recombination (Sauer and Henderson, 1988). *Chat-cre* mice can be used for deleting the floxed gene in ChIs, without disrupting endogenous *Chat* expression (Rossi et al., 2011). *Drd2 loxP*−/− mice (Bello et al., 2011), which have floxed exon 2 of the *Drd2* gene, were imported (Jackson Lab. Stock No: 020631) and bred with *Chat-cre*+/− mice. Recombination between these two *loxP* sites leads to the loss of D2R activity (Bello et al., 2011). *Chat-cre*+/−*Drd2 loxP*+/− (double heterozygous, ChDHet) mice were generated by crossing the *Chat-cre*+/− mice and *Drd2 loxP*−/− mice. *Drd2* ChKO mice, ChDHet mice, and their control (CT) littermates (*Drd2 loxP*−/− or *Drd2 loxP*+/−) were generated by crossing the ChDHet mice with *Drd2 loxP*−/− mice. The MSN-specific *Drd2* KO mice were generated by breeding *Drd2 loxP* mice with *Rgs9-cre* mice (Dang et al., 2006b), to produce *Rgs9-cre*+/−*Drd2 loxP*+/− (sDHet) which were used for breeding with homozygous *Drd2* loxP mice to generate the sKO, sDHet, and CT littermates. *Dyt1* KI mice (*Tor1a*^{tm2Wtd}/J; Jackson Lab. stock no. 025637) were bred as previously described (Goodchild et al., 2005).

Genotyping for *Drd2* ChKO, *Drd2* sKO, and *Dyt1* KI mice was performed by PCR using a set of Drd2loxpF (5_- TCTCCCTCATCTCTGGACTCA −3_) and Drd2loxpR (5_-TGGGAAAGGGCTACAGCA −3_) primers for *Drd2 loxP*, a pair of creA (5_-ATCTCCGGTATTGAAACTCCAGCGC-3_) and cre6 (5_-CACTCATGGAAAATAGCGATC- 3_) primers for *cre* (Campos et al., 2004), and a set of 22117 (5_-GTGCATCAGAGTGGAGATGC- 3_) and 22118 (5_-CAGGAGCTTCAGGTCCATGT- 3_) primers for *Dyt1* KI (Goodchild et al., 2005). Tail DNA was used as the template.

A minimum number of animals was used following the principles of the 3Rs (Replacement, Reduction, and Refinement). We further used systematic heterogenization of study samples and conditions recommended by others (Voelkl et al., 2020). The mice were first used for behavioral tests, followed by Western blot analysis and electrophysiology, which were delayed due to the pandemic.

2.2 Behavioral analysis

Motor behaviors were assessed by semi-quantitative assessments, accelerated rotarod tests, and beam-walking, in this order. Mice were allowed to rest for at least 4 days in between the behavioral tests. A group of 8 *Drd2* ChKO, 6 ChDHet, and 11 CT littermates of either sex was tested two times at 3 and 7 months of age (Fig. 4A). Another male cohort of 12 *Drd2* sKO, 6 sDHet, and 11 CT littermates was tested for motor performance at the average age of 6 months (P121-P270). The last group of 21 male *Dyt1* KI and 19 WT littermates was also tested for motor performance at the average age of 4.5 months (P108-P150). All motor behaviors were tested within the last 8 h of the light period after acclimation to a sound-attenuated testing room for 1 h. Body form assessment of motor disorders was performed as

described earlier (Fernagut et al., 2002; Dang et al., 2005). Mice were placed individually on the table. Truncal distortion and balance adjustments to a postural challenge were examined. Truncal distortion was assessed as the flexed posture. The postural challenge was observed by flipping the mouse onto its back, and its capability of righting was noted.

The accelerated rotarod test assesses mice's ability to maintain balance and coordination on an accelerating rotating rod (Ugo Basile) as previously described (Sciamanna et al., 2012). The apparatus started at an initial speed of 4 rpm, and then each mouse was put on the same slot one by one. The rod speed was gradually increased at a rate of 0.2 rpm/s. The latency to fall was measured with a cutoff time of 3 min at a final rate of 40 rpm. Mice were tested for three trials at about 1 h intervals on each day for 2 days.

The beam-walking test was performed as described earlier (Dang et al., 2005; Dang et al., 2012). The mice were trained to transverse a medium square beam (14 mm wide) in three consecutive trials each day for 2 days, and they were tested twice each on the medium square beam and a medium round beam (17 mm diameter) on the third day. The mice were then tested twice, each on a small round beam (10 mm diameter) and a small square beam (7 mm wide) on the fourth day. The number of hind paw slips on each side was counted by investigators blind to the genotypes. All 4 beams were 100 cm long, and the slips traversing the middle 80 cm were counted.

2.3 Western blot analysis

Two groups of mice were used for Western blot analysis. 5 *Drd2* ChKO mice, 3 ChDHet mice, and 5 controls of either sex at an average of 10 months of age were used in the ChKO group. Moreover, 4 *Drd2* sKO male mice with its 4 sDHet, and 5 control littermates at an average of 10 months of age were used in the sKO group. The striata were dissected and were homogenized in 200 μl of ice-cold lysis buffer, and the proteins were extracted in 1% Triton X-100 buffer and quantified as previously described (Yokoi et al., 2020a; Yokoi et al., 2020b). Protein concentration was measured by Bradford assay (Bradford, 1976). Protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Millipore Immobilon-FL transfer polyvinylidene difluoride (PVDF) membranes. The membranes were processed as previously described (Yokoi et al., 2015). The PVDF membranes were blocked with LI-COR Odyssey blocking buffer and incubated at 4°C overnight with goat anti-choline acetyltransferase (ChAT) antibody (EMD Millipore, AB144P; 1:1,000 dilution), rabbit anti-acetylcholinesterase (AChE) antibody (Santa Cruz, sc-11409; 1:1,000 dilution), mouse Anti-D2R antibody (Santa Cruz, sc-5303; 1:500 dilution), or rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruz, sc-25778; 1:1,000 dilution). LI-COR IRDye 800CW donkey anti-goat IgG (H+L) or LI-COR IRDye 680RD donkey anti-rabbit IgG (H+L) were used as appropriate at a dilution of 1:15,556. The signals were captured with an LI-COR Odyssey imaging system and analyzed with Image Studio Lite Ver. 5.2. The density of the corresponding band signals was normalized to those of GAPDH. GAPDH is a reasonable western blot loading control in neuronal tissues (Bauer et al., 2009; Bangaru et al., 2012). GAPDH was chosen in the current study because of the proper molecular weight and stability between ages (Yu et al., 2011). Western blot analysis was performed in duplicate.

2.4 Electrophysiology

Electrophysiological recordings of striatal ChIs were obtained from 3 *Drd2* ChKO, 4 ChDHet, and 2 CT littermates of either sex at an average of 12 months of age, as described previously (Lyu et al., 2019). The animals were anesthetized by the inhalation of isoflurane, decapitated, and the brains were rapidly removed and briefly chilled in an ice-cold cutting solution containing (in mM) 190 Sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1 $CaCl_2$, 10 $MgCl_2$, and 10 D-glucose and was oxygenated with 95% O_2–5% CO_2 (pH 7.35~7.45). Coronal brain slices 300 μm-thick were cut with a Vibratome (LEICA VT 1000S, Leica Microsystems, Wetzlar, Germany) in the same ice-cold cutting solution. Slices were first incubated on a brain slice keeper (AutoMate Scientific, Inc. Berkeley, CA) and covered by a thin layer of artificial cerebrospinal fluid (ACSF) containing (in mM) 127 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 10 D-glucose and were constantly oxygenated with 95% O_2 and 5% CO_2 (pH 7.35~7.45) at 35°C for 60 min, followed by incubation at room temperature. After incubation of at least 60 min, a slice was then transferred to a submerged recording chamber with the continuous flow (2 ml/min) of ACSF. All experiments were carried out at 32°C under visual guidance using an inverted microscope equipped with infrared differential interference contrast (IR-DIC) videomicroscopy (Axioskop-FS; Carl Zeiss, Jena, Germany) and a 40× water-immersion lens.

Cholinergic interneurons were identified based on morphology and size, as they are irregularly polygonal with large cell soma (>20 μm), and were further confirmed by characteristic electrophysiological properties observed in whole-cell current-clamp recordings (Oswald et al., 2009). The patch electrodes had a resistance of 5-10 MΩ when filled with a K-gluconate-based solution containing the following intracellular solution (in mM): 112.5 K-gluconate, 4 NaCl, 17.5 KCl, 0.5 $CaCl_2$, 5 MgATP, 1 NaGTP, 5 EGTA, 10 HEPES, 0.1% Biocytin; with pH 7.2 (osmolality 270-280 mOsm/l). While approaching the cell, positive pressure was applied to the patch electrode. The seal (> 5 GΩ) between the recording pipette and the cell membrane was obtained by applying suction to the electrode. The spontaneous action potentials were recorded in the voltage-clamp mode that maintained an average of 0 pA holding current with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). After recording the baseline with cell-attached mode, subsets of the neurons were investigated further with the bath application of D2R agonist quinpirole (10 μM, 90 seconds) followed by a washout for 5 min with ACSF. ChIs were recovered with an average recovery time of 2 min.

The recording data was acquired using pClamp 10 software and further analyzed by the Mini Analysis Program (Synaptosoft). The electrophysiological recordings and analysis were performed by investigators who were blind to the genotypes. The spontaneous firing rates of 30 seconds before quinpirole treatment and the last 30 seconds during the application of quinpirole were compared to determine the quinpirole effect. Signals were filtered at 5 kHz and digitized at 10 kHz with a DigiData 1440 (Molecular Devices).

2.5 Statistics

All data were tested for normality using the JMP Pro software. Electrophysiological recording data were analyzed using the student's t-test. The Western blot signals and the latency to fall in the accelerated rotarod test were analyzed by either the mixed model ANOVA (SAS) for normally distributed data or GENMOD procedure (SAS) with a log link for gamma distribution for not normally distributed data (Yokoi et al., 2020b). Slip numbers of the medium round, small round, and small square beams in the beam-walking test were analyzed by using the GENMOD procedure (SAS) with a negative binomial distribution (Dang et al., 2005). Sex, age, body-color, and body weight were used as covariates in all behavioral analyses. Two-way interactions between genotypes and other covariates were explored before final analysis. Significance was assigned at $p \leq 0.05$.

3. Results

3.1 Generation of Drd2 ChKO mice

To investigate whether decreased D2R on ChIs causes motor deficits similar to *Dyt1* KI mice (Dang et al., 2005; Dang et al., 2012), *Drd2* ChKO was generated as described (Figure 1). The *Drd2* ChKO mice were obtained with Mendel's ratio ($p = 0.999$, Chi-Square test), suggesting that *Drd2* ChKO mice are neither embryonic nor neonatal lethal. *Drd2* ChKO mice grew up to adulthood without noticeable developmental delay.

3.2 Impaired quinpirole mediated inhibition in Drd2 ChKO mice

To assess the knockout of D2R, we measured the spontaneous firing properties of the ChIs in *Drd2* ChKO and its modulation by D2R agonist quinpirole. The firing frequency in both *Drd2* ChKO and ChDHet mice was significantly increased compared with control mice (Fig. 2B, Mean ± S.E.M.: CT: 2.31 ± 0.45 Hz, n = 11 cells/2 mice; ChKO: 4.84 ± 0.69 Hz, n = 12 cells/3 mice; ChDHet: 4.11 ± 0.70 Hz, n = 9 cells/4 mice; CT and ChKO: $p = 0.006$; CT and ChDHet: $p = 0.0497$; ChKO with ChDHet: $p = 0.47$, student's t-test). After quinpirole treatment, the firing frequency was significantly decreased in all genotypes (Fig. 2C, After quinpirole application: Mean ± S.E.M.: CT: 1.01 ± 0.36 Hz, $p = 0.005$; ChKO: 3.49 ± 0.60 Hz, $p = 0.001$; ChDHet: 3.03 ± 0.58 Hz, $p = 0.013$, student's t-test). However, the ratio of the firing frequency (after/prior treatment) showed a significant increase in *Drd2* ChKO and ChDHet mice compared with CT mice (Fig. 2D, Mean ± S.E.M.: CT: 0.38 ± 0.11; ChKO: 0.68 ± 0.06; ChDHet: 0.74 ± 0.08; CT and ChKO: $p = 0.03$; CT and ChDHet: $p = 0.02$; ChKO with ChDHet: $p = 0.55$, student's t-test). There was no change of resting membrane potential of ChIs in *Drd2* ChKO and ChDHet mice compared to the control mice (Supplemental Figure 1). These results indicate the successful knockout of D2Rs on ChIs.

3.3 Normal striatal cholinergic enzyme levels in *Drd2* ChKO mice

To examine whether selective knockout of D2R on ChIs leads to an altered striatal cholinergic system, we determined the protein level of the striatal ChAT and AChE, which are two key metabolic enzymes of acetylcholine (Fig. 3A–B). There were no significant difference in either ChAT (Fig. 3A, Mean ± S.E.M.: CT: 0.16 ± 0.012; ChKO: 0.14 ± 0.012; ChDHet: 0.12 ± 0.017; CT and ChKO: $p = 0.34$; CT and ChDHet: $p = 0.11$; ChKO with

ChDHet: $p = 0.40$, $F_{[2,11]} = 1.57$, $p = 0.25$, ANOVA) or AChE (Fig. 3B, Mean ± S.E.M.: CT: 1.95 ± 0.096; ChKO: 2.14 ± 0.096; ChDHet: 1.81 ± 0.16; CT and ChKO: $p = 0.21$; CT and ChDHet: $p = 0.47$; ChKO with ChDHet: $p = 0.11$, $F_{[2,11]} = 1.80$, $p = 0.21$, ANOVA) between *Drd2* ChKO mice (n = 5), ChDHet (n = 3), and *Drd2 loxP* mice (n = 5). The results indicate that there was no ChI neurodegeneration in *Drd2* ChKO striatum, which is consistent with most of the DYT1 dystonia mouse models.

3.4 Reduced striatal D2R levels in *Drd2* sKO mice

To assess the knockout of D2R in MSNs, we measeured the protein level of strial D2R in *Drd2* sKO mice (n = 4), which showed significantly reduced D2R expression level compared with their sDHet (n = 4) and control (n = 5) littermates (Fig. 3C, Mean ± S.E.M.: CT: 0.20 ± 0.01; sKO: 0.14 ± 0.0096; sDHet: 0.24 ± 0.04; CT and sKO: $p < 0.001$; CT and sDHet: $p = 0.41$; sKO with sDHet: $p = 0.003$, GENMOD with a gamma distribution). The results indicate the successful knockout of D2Rs on MSNs.

3.5 Impaired motor coordination and balance in Drd2 ChKO mice

We next determined the motor performance of the *Drd2* ChKO mice. *Drd2* ChKO mice and ChDHet mice exhibited strong righting reflexes when tipped on their side and did not show any overt dystonic behavior in the body form assessment. We further performed rotarod and beam-walking tests at 3 months of age. There were no differences between *Drd2* ChKO mice (n = 8), control mice (n = 11), and the ChDHet mice (n = 6) on the rotarod (Fig. 4B–C) and beam-walking tests (Fig. 4D). However, at 7 months of age, *Drd2* ChKO and ChDHet mice displayed excessive slips on the beam-walking test (Fig. 4G, Mean ± S.E.M.: CT: 1.02 ± 0.26 slips; ChKO: 2.27 ± 0.51 slips; ChDHet: 2.27 ± 0.44 slips; CT and ChKO: $p = 0.007$; CT and ChDHet: $p = 0.001$; ChKO with ChDHet: $p = 0.988$, GENMOD with a negative binomial distribution), suggesting an age-dependent impairment in motor performance. There was no significant difference between *Drd2* ChKO mice, ChDHet mice, and control mice on the accelerated rotarod test across 6 trials (Fig. 4E–F, Mean ± S.E.M.: CT: 145.25 ± 16.82 sec; ChKO: 129.82 ± 11.83 sec; ChDHet: 125.39 ± 23.25 sec; CT and ChKO: $p = 0.30$; CT and ChDHet: $p = 0.46$; ChKO with ChDHet: $p = 0.87$, GENMOD with a gamma distribution).

3.6 Impaired motor coordination and balance in the Drd2 sKO mice

MSNs can regulate the activity of ChIs (Lim et al., 2014). D2Rs on MSNs in the indirect pathway also contribute to synaptic plasticity in the striatum (Wang et al., 2006; Shen et al., 2008; Augustin et al., 2018). To further study the role of striatal D2R in the DYT1 dystonia, we generated MSN-specific *Drd2* conditional knockout mice to test motor coordination and balance. *Drd2* sKO mice were bred with the same breeding strategy with *Drd2* ChKO (Fig. 1) by using *Rgs9-cre* mice that induce recombination restricted to MSNs (Dang et al., 2006b; Lyu et al., 2019). *Drd2* sKO mice displayed significantly reduced latency to fall on the rotarod test across 6 trials (Fig. 5A–B, Mean ± S.E.M.: CT: 111.44 ± 7.94 sec; sKO: 82.00 ± 8.13 sec; sDHet: 112.18 ± 9.32 sec; CT and sKO: $p = 0.018$; CT and sDHet: $p = 0.95$; sKO and sDHet: $p = 0.021$; $F_{[2,29]} = 3.89$, $p = 0.032$, ANOVA). Conversely, there was no difference between *Drd2* sKO mice, the sDHet mice, and the control littermates on the beam-walking test (Fig. 5C, Mean ± S.E.M.: CT: 6.44 ± 2.20 slips; sKO: 7.09 ± 1.81 slips;

sDHet: 3.88 ± 0.85 slips; CT and sKO: $p = 0.83$; CT and sDHet: $p = 0.30$; sKO with sDHet: $p = 0.09$, GENMOD with a negative binomial distribution).

3.7 Impaired motor coordination and balance in the Dyt1 KI mice

To further examine the altered motor coordination and balance we observed in *Drd2* ChKO, *Drd2* sKO, and our *Dyt1* KI mice (Dang et al., 2005), we performed rotarod and beam walking tests in another *Dyt1* knock-in model with the trinucleotide deletion commonly seen in DYT1 patients (Goodchild et al., 2005). In the accelerated rotarod test, the *Dyt1* KI mice showed no significant difference compared to WT littermates across all 6 trials (Fig. 6B, Mean ± S.E.M.: WT: 115.08 ± 6.29 sec; *Dyt1* KI: 100.81 ± 7.06 sec; $p = 0.16$, GENMOD with a gamma distribution), but they had a significantly shorter time to fall in the 3 trials on the first day (Fig. 6A, $p = 0.048$). With the beam-walking test, the *Dyt1* KI mice displayed a 119% increase of slips over their WT littermates (Fig. 6C, Mean ± S.E.M.: WT: 0.23 ± 0.07 slips; *Dyt1* KI: 0.51 ± 0.12 slips; $p = 0.016$, GENMOD with a negative binomial distribution). Taken together, the *Dyt1* KI mice displayed significant motor coordination and balance deficits.

4. Discussion

Both animal models and DYT1 dystonia patients show decreased striatal D2R binding or receptor protein levels. The D2R reduction appears to occur also in DYT6 and DYT11 dystonia (Beukers et al., 2009; Carbon et al., 2009; Zhang et al., 2012; Frederick et al., 2019). However, whether D2R reduction causes dystonia or is a consequence of dystonia is not clear. This is complicated further by the multiple locations of D2R in the striatum. D2Rs are present on at least five different types of neurons or axon terminals in the striatum: indirect-pathway MSNs, ChIs, a subset of GABA interneurons, and afferents to the striatum from midbrain dopaminergic neurons and prefrontal cortical neurons (Dobbs et al., 2017). Compared with the pharmacological methods, which may easily affect all these locations, genetic mouse models can precisely knockout D2Rs only on one subset of neurons. Here, we used genetic approaches to investigate the role of D2R reduction on ChIs and MSNs in the pathogenesis of DYT1 dystonia. We found that selective deletion or reduction of D2Rs from ChIs resulted in increased ChI firing rate, impaired quinpirole-mediated inhibition, and age-dependent motor deficits in beam-walking, which is a characteristic motor deficit found across multiple lines of DYT1 and other dystonia mouse models. Interestingly, knocking out D2Rs, but not reduction, from MSNs, produced a rotarod deficit, a motor deficit found in several DYT1 dystonia mouse models. Finally, rotarod and beam walking tests in another line of *Dyt1* KI mice showed similar motor deficits as the *Drd2* ChKO mice. Overall, these results indicate that knocking out or reduction of D2Rs in the ChI alone could lead to impaired coordination and balance, similar to *Dyt1* KI mice. Our results highlight the importance of D2R activity on ChIs to the pathogenesis of DYT1 dystonia.

Selective knockout or reduction of D2Rs on ChI led to age-dependent beam-walking deficits similar to *Dyt1* KI mice. Excessive slips were apparent only in 6-month-old *Dyt1* KI mice, not in 3-month-old mice (Dang et al., 2005). The age-dependent motor deficits have been shown in various DYT1 animal models (reviewed in Richter and Richter, 2014). Even

though it is challenging to compare mice's life phases with humans, a recent study suggested that 3-month-old mice are equivalent to 21-year-old humans while 7-month-old mice are equivalent to 30-year-old humans (Wang et al., 2020). Therefore the age-dependent beam-walking deficits suggest that motor deficits start to develop between 3 to 6 months of age. This is consistent with the human study that most DYT1 dystonia symptoms begin before the age of 26 years old (Opal et al., 2002). *Drd2* ChKO and ChDHet mice did not show a deficit on the rotarod, which is also consistent with the normal rotarod performance in the *Dyt1* KI mice (Dang et al., 2005) and the *Dyt1* KI line (Goodchild et al., 2005) used in this study. ChDHet mice displayed a beam-walking deficit suggesting that reduction of D2R expression on ChIs is sufficient to induce typical coordination and balance deficits in *Dyt1* KI mice. Overall, the similarity of the motor performance between *Drd2* ChKO, ChDHet, and both two *Dyt1* KI mouse lines supports what we proposed in an earlier study that reduced D2R on ChIs leads to impaired motor performance in DYT1 dystonia (Dang et al., 2012). There are limitations to this study. *Chat-cre* line can also affect cholinergic neurons outside of the striatum like pedunculopontine, laterodorsal tegmental nuclei, basal forebrain, and the spinal cord (Wang and Morales, 2009; Ballinger et al., 2016). However, D2R expression on those cholinergic neurons is low, and the contribution to the motor phenotypes remains to be investigated.

Decreased D2R binding activity and expression level in the striatum has been found in DYT1 dystonia patients and animal models (Augood et al., 2002; Asanuma et al., 2005; Napolitano et al., 2010; Yokoi et al., 2011; Dang et al., 2012; Bonsi et al., 2019; Yokoi et al., 2020b). Besides, reduced D2R has been found in other dystonia patients and animal models, such as DYT6 (Carbon et al., 2009), DYT11 (Beukers et al., 2009; Zhang et al., 2012), cranial or hand dystonia (Perlmutter et al., 1997), and other related movement disorders like Parkinson's disease (Albin et al., 1989). Furthermore, the *DRD2* variants are related to myoclonus dystonia (Klein et al., 1999) and cervical dystonia (van der Weijden et al., 2020), highlighting that D2R is critical in motor control.

ChIs only account for 1 to 2% of striatal neurons, and most neurons in the striatum are MSNs. We further explored motor performance when D2Rs were selectively manipulated in the MSNs. *Drd2* sKO and sDHet mice did not display overt dystonia, consistent with other animals with striatal D2R reduction (Lemos et al., 2016; Shana et al., 2020). Furthermore, we found that *Drd2* sKO and sDHet mice did not have a beam-walking deficit. Conversely, *Drd2* sKO, but not sDHet mice, showed a rotarod deficit. Interestingly, other groups used *Adora2A-cre* transgenic mice instead of *Rgs9-cre* to direct *cre*-mediated recombination in indirect-pathway MSNs (Lemos et al., 2016; Shana et al., 2020), and the knockout mice showed impaired performance on the rotarod test. These results suggest that indirect pathway MSN-specific D2R knockout mice have a rotarod deficit, similar to a subset of *Dyt1* dystonia models (Sharma et al., 2005; Grundmann et al., 2007; Sciamanna et al., 2012; Sciamanna et al., 2020; Yokoi et al., 2020b). We conclude that D2R on MSNs also contributes to the coordination and balance deficits in *Dyt1* KI mice. It is interesting that MSNs also modulate nociception (Rooney and Sewell, 1989; Zarrindast and Moghaddampour, 1989; Verma and Kulkarni, 1993; Wall et al., 2013), and *Dyt1* KI mice show sensory deficit (Richter et al., 2017). Alteration of sensory perception will likely contribute to the motor performance in the rotarod and beam walking tests.

Rotarod and beam-walking tests are useful for determining motor coordination and balance deficits (Buccafusco, 2009). However, the challenge to animals is slightly different. Rotarod readout includes all 4 limbs, while the beam walking test is only focused on the hindlimbs. Furthermore, a previous study showed that only a 30% GABA-A receptor occupancy by benzodiazepine agonists was needed in order to observe motor deficits on the beam compared to over 70%-90% receptor occupancy for deficits on the rotarod (Stanley et al., 2005). Therefore, as motor coordination and balance measurements, the beam walking test is more sensitive than the rotarod test and is ideal for detecting subtle motor deficits in dystonia animal models. In the current study, knocking out D2R on ChI altered the firing of ChI, which in turn affected the modulatory effect of acetylcholine on both direct and indirect pathway MSNs. Knocking out D2R on MSNs, on the other hand, only affected the modulatory effect of DA on the indirect pathway MSNs. These differences may account for the different motor performance we observed in *Drd2* ChDHet and sDHet mice.

In our previous studies, we found impaired corticostriatal LTD and sustained co-contraction of hindlimb muscles in *Dyt1* KI mice. THP can restore LTD and beam-walking deficits and decrease muscle co-contraction (Dang et al., 2012; DeAndrade et al., 2016). These results suggest that impaired corticostriatal LTD could be correlated with the motor deficits in *Dyt1* KI mice. Interestingly, a recent study using *Drd2* conditional knockout mice replicated the LTD deficit found in *Dyt1* KI mice and showed that D2Rs on ChIs plays a major role in LTD, while the D2Rs on the indirect pathway MSNs only have a weaker modulatory effect on LTD induction (Augustin et al., 2018). Using similar *Drd2* conditional knockout mice, we recapitulated the motor coordination and balance deficits found in *Dyt1* KI mice. Together, these results suggest that knocking out of striatal D2Rs alone in wild type mice could mimic the motor phenotypes of *Dyt1* KI mice, highlighting the importance of D2R in DYT1 pathophysiology. These results also further support that corticostriatal LTD deficits contribute to motor coordination and balance deficits in *Dyt1* KI mice (Dang et al., 2012). Correcting the corticostriatal LTD deficits might be able to treat the DYT1 dystonia patients (Maltese et al., 2014; Martella et al., 2014; Maltese et al., 2018). Interestingly, enhanced eIF2α signaling can restore the impaired corticostriatal LTD in *Dyt1* KI mice and improve neonatal survival of *Dyt1* homozygous KI mice (Rittiner et al., 2016). The eIF2α pathway integrates stress response. It is required for long-term synaptic plasticity (Trinh and Klann, 2013) by regulating the ATF4 and the CREB-dependent transcription and downstream synaptic plasticity-related genes. Therefore, it will be interesting to investigate whether correcting LTD deficit via either the eIF2α signaling pathway or D2Rs on ChIs can rescue the motor deficits in *Dyt1* KI mice and provide symptom relief in DYT1 patients.

5. Conclusion

Reduced striatal D2R is found in both DYT1 patients and animal models. Selective knocking out or reduction of D2R on ChI alone led to the same age-dependent beam walking deficit observed in *Dyt1* KI mice. In contrast, the removal of D2R, but not the reduction of D2R, on MSNs led to the rotarod deficit found in a small subset of DYT1 animal models. The results suggest that D2R on ChIs is critical in the pathophysiology of DYT1 dystonia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the University of Florida Animal Care Services staff for animal care, Alexis Ernst, Kyle Singh, Gracie Korkmaz, Maisha Anika, and Caroline Comeau, for their technical assistance.

Funding

This study was supported by Tyler's Hope for a Dystonia Cure, Inc., National Institutes of Health [grants NS54246, NS72872, NS74423, NS75012, NS82244, NS111498, and NS118397], and Bachmann-Strauss Dystonia & Parkinson Foundation Inc.

Abbreviations

AAA+	ATPases associated with diverse cellular activities
AChE	acetylcholinesterase
ACSF	artificial cerebrospinal fluid
ChAT	choline acetyltransferase
ChDHet	*Chat-cre Drd2 loxP* double heterozygote mice
ChI	cholinergic interneuron
CT	control
D2R	dopamine D2 receptor
DBS	deep brain stimulation
***Drd2* ChKO**	*Drd2* ChI-specific KO mice
***Drd2* sKO**	*Drd2* MSN-specific KO mice
***Dyt1* KI mice**	*Dyt1* ΔGAG heterozygous knock-in mice
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPi	globus pallidus internus
KO	knockout
LTD	long-term depression
MSN	medium spiny neuron
PVDF	polyvinylidene difluoride
sDHet	*Rgs9-cre Drd2 loxP* double heterozygote mice
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

STN	subthalamic nucleus
THP	trihexyphenidyl
WT	wild type

References

Albanese A, Bhatia K, Bressman SB, DeLong MR, Fahn S, Fung VS, et al. Phenomenology and classification of dystonia: a consensus update. Mov Disord 2013; 28(7): 863–73. [PubMed: 23649720]

Albin R, Young A, Penney J. The functional anatomy of basal ganglia disorders. Trends in neurosciences 1989; 12(10).

Aosaki T, Miura M, Suzuki T, Nishimura K, Masuda M. Acetylcholine-dopamine balance hypothesis in the striatum: an update. Geriatr Gerontol Int 2010; 10 Suppl 1: S148–57. [PubMed: 20590830]

Asanuma K, Ma Y, Okulski J, Dhawan V, Chaly T, Carbon M, et al. Decreased striatal D2 receptor binding in non-manifesting carriers of the DYT1 dystonia mutation. Neurology 2005; 64(2): 347–9. [PubMed: 15668438]

Augood SJ, Hollingsworth Z, Albers DS, Yang L, Leung JC, Muller B, et al. Dopamine transmission in DYT1 dystonia: a biochemical and autoradiographical study. Neurology 2002; 59(3): 445–8. [PubMed: 12177384]

Augood SJ, Martin DM, Ozelius LJ, Breakefield XO, Penney JB Jr., Standaert DG, Distribution of the mRNAs encoding torsinA and torsinB in the normal adult human brain. Ann Neurol 1999; 46(5): 761–9. [PubMed: 10553994]

Augustin SM, Chancey JH, Lovinger DM. Dual Dopaminergic Regulation of Corticostriatal Plasticity by Cholinergic Interneurons and Indirect Pathway Medium Spiny Neurons. Cell Rep 2018; 24(11): 2883–93. [PubMed: 30208314]

Ballinger E, Ananth M, Talmage D, Role L. Basal Forebrain Cholinergic Circuits and Signaling in Cognition and Cognitive Decline. Neuron 2016; 91(6).

Bangaru M, Park F, Hudmon A, McCallum J, Hogan Q. Quantification of gene expression after painful nerve injury: validation of optimal reference genes. Journal of molecular neuroscience : MN 2012; 46(3).

Bauer D, Haroutunian V, McCullumsmith R, Meador-Woodruff J. Expression of four housekeeping proteins in elderly patients with schizophrenia. Journal of neural transmission (Vienna, Austria : 1996) 2009; 116(4).

Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ, et al. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. Nat Neurosci 2011; 14(8): 1033–8. [PubMed: 21743470]

Beukers R, Booij J, Weisscher N, Zijlstra F, van AT, Tijssen M. Reduced striatal D2 receptor binding in myoclonus-dystonia. European journal of nuclear medicine and molecular imaging 2009; 36(2).

Bonsi P, Ponterio G, Vanni V, Tassone A, Sciamanna G, Migliarini S, et al. RGS9-2 rescues dopamine D2 receptor levels and signaling in DYT1 dystonia mouse models. EMBO Mol Med 2019; 11(1).

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248–54. [PubMed: 942051]

Bressman SB, Sabatti C, Raymond D, de Leon D, Klein C, Kramer PL, et al. The DYT1 phenotype and guidelines for diagnostic testing. Neurology 2000; 54(9): 1746–52. [PubMed: 10802779]

Buccafusco J Methods of Behavior Analysis in Neuroscience. 2009.

Calakos N, Patel VD, Gottron M, Wang G, Tran-Viet KN, Brewington D, et al. Functional evidence implicating a novel TOR1A mutation in idiopathic, late-onset focal dystonia. J Med Genet 2010; 47(9): 646–50. [PubMed: 19955557]

Campos V, Du M, Li Y. Increased seizure susceptibility and cortical malformation in beta-catenin mutant mice. Biochemical and biophysical research communications 2004; 320(2).

Carbon M, Niethammer M, Peng S, Raymond D, Dhawan V, Chaly T, et al. Abnormal striatal and thalamic dopamine neurotransmission: Genotype-related features of dystonia. Neurology 2009; 72(24).

Chase AR, Laudermilch E, Schlieker C. Torsin ATPases: Harnessing Dynamic Instability for Function. Front Mol Biosci 2017; 4: 29. [PubMed: 28553638]

Cheng FB, Feng JC, Ma LY, Miao J, Ott T, Wan XH, et al. Combined occurrence of a novel TOR1A and a THAP1 mutation in primary dystonia. Mov Disord 2014; 29(8): 1079–83. [PubMed: 24862462]

Chuhma N, Mingote S, Moore H, Rayport S. Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling. Neuron 2014; 81(4): 901–12. [PubMed: 24559678]

Cury R, Kalia S, Shah B, Jimenez-Shahed J, Prashanth L, Moro E. Surgical treatment of dystonia. Expert review of neurotherapeutics 2018; 18(6).

Dang MT, Yokoi F, Cheetham CC, Lu J, Vo V, Lovinger DM, et al. An anticholinergic reverses motor control and corticostriatal LTD deficits in Dyt1 DeltaGAG knock-in mice. Behav Brain Res 2012; 226(2): 465–72. [PubMed: 21995941]

Dang MT, Yokoi F, McNaught KS, Jengelley TA, Jackson T, Li J, et al. Generation and characterization of Dyt1 DeltaGAG knock-in mouse as a model for early-onset dystonia. Exp Neurol 2005; 196(2): 452–63. [PubMed: 16242683]

Dang MT, Yokoi F, Pence MA, Li Y. Motor deficits and hyperactivity in Dyt1 knockdown mice. Neurosci Res 2006a; 56(4): 470–4. [PubMed: 17046090]

Dang MT, Yokoi F, Yin HH, Lovinger DM, Wang Y, Li Y. Disrupted motor learning and long-term synaptic plasticity in mice lacking NMDAR1 in the striatum. Proc Natl Acad Sci U S A 2006b; 103(41): 15254–9. [PubMed: 17015831]

DeAndrade MP, Trongnetrpunya A, Yokoi F, Cheetham CC, Peng N, Wyss JM, et al. Electromyographic evidence in support of a knock-in mouse model of DYT1 Dystonia. Mov Disord 2016; 31(11): 1633–9. [PubMed: 27241685]

Demircioglu FE, Sosa BA, Ingram J, Ploegh HL, Schwartz TU. Structures of TorsinA and its disease-mutant complexed with an activator reveal the molecular basis for primary dystonia. Elife 2016; 5.

Dobbs L, Lemos J, Alvarez V. Restructuring of basal ganglia circuitry and associated behaviors triggered by low striatal D2 receptor expression: implications for substance use disorders. Genes, brain, and behavior 2017; 16(1).

Dobricic V, Kresojevic N, Zarkovic M, Tomic A, Marjanovic A, Westenberger A, et al. Phenotype of non-c.907_909delGAG mutations in TOR1A: DYT1 dystonia revisited. Parkinsonism Relat Disord 2015; 21(10): 1256–9. [PubMed: 26297380]

Downs AM, Fan X, Donsante C, Jinnah HA, Hess EJ. Trihexyphenidyl rescues the deficit in dopamine neurotransmission in a mouse model of DYT1 dystonia. Neurobiol Dis 2019; 125: 115–22. [PubMed: 30707939]

Eskow Jaunarajs KL, Bonsi P, Chesselet MF, Standaert DG, Pisani A. Striatal cholinergic dysfunction as a unifying theme in the pathophysiology of dystonia. Prog Neurobiol 2015; 127-128: 91–107. [PubMed: 25697043]

Farley FW, Soriano P, Steffen LS, Dymecki SM. Widespread recombinase expression using FLPeR (flipper) mice. Genesis 2000; 28(3-4): 106–10. [PubMed: 11105051]

Fernagut PO, Diguet E, Stefanova N, Biran M, Wenning GK, Canioni P, et al. Subacute systemic 3-nitropropionic acid intoxication induces a distinct motor disorder in adult C57Bl/6 mice: behavioural and histopathological characterisation. Neuroscience 2002; 114(4): 1005–17. [PubMed: 12379255]

Frederick N, Shah P, Didonna A, Langley M, Kanthasamy A, Opal P. Loss of the dystonia gene Thap1 leads to transcriptional deficits that converge on common pathogenic pathways in dystonic syndromes. Human molecular genetics 2019; 28(8).

Goodchild RE, Kim CE, Dauer WT. Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. Neuron 2005; 48(6): 923–32. [PubMed: 16364897]

Grundmann K, Glockle N, Martella G, Sciamanna G, Hauser TK, Yu L, et al. Generation of a novel rodent model for DYT1 dystonia. Neurobiol Dis 2012; 47(1): 61–74. [PubMed: 22472189]

Grundmann K, Reischmann B, Vanhoutte G, Hubener J, Teismann P, Hauser TK, et al. Overexpression of human wildtype torsinA and human DeltaGAG torsinA in a transgenic mouse model causes phenotypic abnormalities. Neurobiol Dis 2007; 27(2): 190–206. [PubMed: 17601741]

Guzzi G, Della TA, Chirchiglia D, Volpentesta G, Lavano A. Critical reappraisal of DBS targeting for movement disorders. Journal of neurosurgical sciences 2016; 60(2).

Harata NC. Addressing variant pathogenicity: the TorsinA (TOR1A) gene as a model. Hum Mutat 2014; 35(9): v.

Isik E, Aykut A, Atik T, Cogulu O, Ozkinay F. Biallelic TOR1A mutations cause severe arthrogryposis: A case requiring reverse phenotyping. Eur J Med Genet 2019; 62(9): 103544. [PubMed: 30244176]

Kariminejad A, Dahl-Halvarsson M, Ravenscroft G, Afroozan F, Keshavarz E, Goullee H, et al. TOR1A variants cause a severe arthrogryposis with developmental delay, strabismus and tremor. Brain 2017; 140(11): 2851–9. [PubMed: 29053766]

Kharkwal G, Brami-Cherrier K, Lizardi-Ortiz JE, Nelson AB, Ramos M, Del Barrio D, et al. Parkinsonism Driven by Antipsychotics Originates from Dopaminergic Control of Striatal Cholinergic Interneurons. Neuron 2016; 91(1): 67–78. [PubMed: 27387649]

Klein C, Brin M, Kramer P, Sena-Esteves M, de Leon D, Doheny D, et al. Association of a missense change in the D2 dopamine receptor with myoclonus dystonia. Proceedings of the National Academy of Sciences of the United States of America 1999; 96(9).

Klein C, Ozelius LJ. Dystonia: clinical features, genetics, and treatment. Curr Opin Neurol 2002; 15(4): 491–7. [PubMed: 12151848]

Konakova M, Pulst SM. Dystonia-associated forms of torsinA are deficient in ATPase activity. J Mol Neurosci 2005; 25(1): 105–17. [PubMed: 15781971]

Le Moine C, Tison F, Bloch B. D2 dopamine receptor gene expression by cholinergic neurons in the rat striatum. Neuroscience letters 1990; 117(3).

Lemos J, Friend D, Kaplan A, Shin J, Rubinstein M, Kravitz A, et al. Enhanced GABA Transmission Drives Bradykinesia Following Loss of Dopamine D2 Receptor Signaling. Neuron 2016; 90(4).

Leung JC, Klein C, Friedman J, Vieregge P, Jacobs H, Doheny D, et al. Novel mutation in the TOR1A (DYT1) gene in atypical early onset dystonia and polymorphisms in dystonia and early onset parkinsonism. Neurogenetics 2001; 3(3): 133–43. [PubMed: 11523564]

Lim S, Kang U, McGehee D. Striatal cholinergic interneuron regulation and circuit effects. Frontiers in synaptic neuroscience 2014; 6.

Lyu S, Xing H, DeAndrade MP, Liu Y, Perez PD, Yokoi F, et al. The role of BTBD9 in striatum and restless legs syndrome. eNeuro 2019: 0277–19.

Maltese M, Martella G, Madeo G, Fagiolo I, Tassone A, Ponterio G, et al. Anticholinergic drugs rescue synaptic plasticity in DYT1 dystonia: role of M1 muscarinic receptors. Mov Disord 2014; 29(13): 1655–65. [PubMed: 25195914]

Maltese M, Stanic J, Tassone A, Sciamanna G, Ponterio G, Vanni V, et al. Early structural and functional plasticity alterations in a susceptibility period of DYT1 dystonia mouse striatum. eLife 2018; 7.

Martella G, Maltese M, Nistico R, Schirinzi T, Madeo G, Sciamanna G, et al. Regional specificity of synaptic plasticity deficits in a knock-in mouse model of DYT1 dystonia. Neurobiol Dis 2014; 65: 124–32. [PubMed: 24503369]

Maurice N, Mercer J, Chan CS, Hernandez-Lopez S, Held J, Tkatch T, et al. D2 dopamine receptor-mediated modulation of voltage-dependent Na+ channels reduces autonomous activity in striatal cholinergic interneurons. J Neurosci 2004; 24(46): 10289–301. [PubMed: 15548642]

Napolitano F, Pasqualetti M, Usiello A, Santini E, Pacini G, Sciamanna G, et al. Dopamine D2 receptor dysfunction is rescued by adenosine A2A receptor antagonism in a model of DYT1 dystonia. Neurobiol Dis 2010; 38(3): 434–45. [PubMed: 20227500]

Opal P, Tintner R, Jankovic J, Leung J, Breakefield XO, Friedman J, et al. Intrafamilial phenotypic variability of the DYT1 dystonia: from asymptomatic TOR1A gene carrier status to dystonic storm. Mov Disord 2002; 17(2): 339–45. [PubMed: 11921121]

Oswald M, Oorschot D, Schulz J, Lipski J, Reynolds J. IH current generates the afterhyperpolarisation following activation of subthreshold cortical synaptic inputs to striatal cholinergic interneurons. The Journal of physiology 2009; 587(Pt 24).

Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997; 17(1): 40–8. [PubMed: 9288096]

Page ME, Bao L, Andre P, Pelta-Heller J, Sluzas E, Gonzalez-Alegre P, et al. Cell-autonomous alteration of dopaminergic transmission by wild type and mutant (DeltaE) TorsinA in transgenic mice. Neurobiol Dis 2010; 39(3): 318–26. [PubMed: 20460154]

Panov F, Gologorsky Y, Connors G, Tagliati M, Miravite J, Alterman RL. Deep brain stimulation in DYT1 dystonia: a 10-year experience. Neurosurgery 2013; 73(1): 86–93; discussion [PubMed: 23615098]

Perlmutter JS, Stambuk MK, Markham J, Black KJ, McGee-Minnich L, Jankovic J, et al. Decreased [18F]spiperone binding in putamen in idiopathic focal dystonia. J Neurosci 1997; 17(2): 843–50. [PubMed: 8987805]

Pham P, Frei KP, Woo W, Truong DD. Molecular defects of the dystonia-causing torsinA mutation. Neuroreport 2006; 17(16): 1725–8. [PubMed: 17047461]

Pisani A, Bernardi G, Ding J, Surmeier DJ. Re-emergence of striatal cholinergic interneurons in movement disorders. Trends Neurosci 2007; 30(10): 545–53. [PubMed: 17904652]

Reichert SC, Gonzalez-Alegre P, Scharer GH. Biallelic TOR1A variants in an infant with severe arthrogryposis. Neurol Genet 2017; 3(3): e154. [PubMed: 28516161]

Richter F, Gerstenberger J, Bauer A, Liang C, Richter A. Sensorimotor tests unmask a phenotype in the DYT1 knock-in mouse model of dystonia. Behavioural brain research 2017; 317.

Richter F, Richter A. Genetic animal models of dystonia: common features and diversities. Prog Neurobiol 2014; 121: 91–113. [PubMed: 25034123]

Rittiner JE, Caffall ZF, Hernandez-Martinez R, Sanderson SM, Pearson JL, Tsukayama KK, et al. Functional Genomic Analyses of Mendelian and Sporadic Disease Identify Impaired eIF2alpha Signaling as a Generalizable Mechanism for Dystonia. Neuron 2016; 92(6): 1238–51. [PubMed: 27939583]

Ritz K, Groen JL, Kruisdijk JJ, Baas F, Koelman JH, Tijssen MA. Screening for dystonia genes DYT1, 11 and 16 in patients with writer's cramp. Mov Disord 2009; 24(9): 1390–2. [PubMed: 19441135]

Rooney K, Sewell R. Evaluation of selective actions of dopamine D-1 and D-2 receptor agonists and antagonists on opioid antinociception. European journal of pharmacology 1989; 168(3).

Rossi J, Balthasar N, Olson D, Scott M, Berglund E, Lee CE, et al. Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metab 2011; 13(2): 195–204. [PubMed: 21284986]

Sauer B, Henderson N. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. Proceedings of the National Academy of Sciences of the United States of America 1988; 85(14).

Scarduzio M, Zimmerman CN, Jaunarajs KL, Wang Q, Standaert DG, McMahon LL. Strength of cholinergic tone dictates the polarity of dopamine D2 receptor modulation of striatal cholinergic interneuron excitability in DYT1 dystonia. Exp Neurol 2017; 295: 162–75. [PubMed: 28587876]

Sciamanna G, Hollis R, Ball C, Martella G, Tassone A, Marshall A, et al. Cholinergic dysregulation produced by selective inactivation of the dystonia-associated protein torsinA. Neurobiol Dis 2012; 47(3): 416–27. [PubMed: 22579992]

Sciamanna G, Ponterio G, Vanni V, Laricchiuta D, Martella G, Bonsi P, et al. Optogenetic Activation of Striatopallidal Neurons Reveals Altered HCN Gating in DYT1 Dystonia. Cell reports 2020; 31(7).

Sciamanna G, Tassone A, Martella G, Mandolesi G, Puglisi F, Cuomo D, et al. Developmental Profile of the Aberrant Dopamine D2 Receptor Response in Striatal Cholinergic Interneurons in DYT1 Dystonia. PLoS One; 2011.

Shana MA, Gabriel CL, Timothy JON, Alexxai VK, David ML. Dopamine D2 receptor signaling on iMSNs is required for initiation and vigor of learned actions. Neuropsychopharmacology 2020: 1–11.

Sharma N, Baxter MG, Petravicz J, Bragg DC, Schienda A, Standaert DG, et al. Impaired motor learning in mice expressing torsinA with the DYT1 dystonia mutation. J Neurosci 2005; 25(22): 5351–5. [PubMed: 15930383]

Shashidharan P, Kramer BC, Walker RH, Olanow CW, Brin MF. Immunohistochemical localization and distribution of torsinA in normal human and rat brain. Brain Res 2000; 853(2): 197–206. [PubMed: 10640617]

Shen W, Flajolet M, Greengard P, Surmeier D. Dichotomous dopaminergic control of striatal synaptic plasticity. Science (New York, NY) 2008; 321(5890).

Siegert S, Bahn E, Kramer ML, Schulz-Schaeffer WJ, Hewett JW, Breakefield XO, et al. TorsinA expression is detectable in human infants as young as 4 weeks old. Brain Res Dev Brain Res 2005; 157(1): 19–26. [PubMed: 15939081]

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]

Stanley J, Lincoln R, Brown T, McDonald L, Dawson G, Reynolds D. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. Journal of psychopharmacology (Oxford, England) 2005; 19(3).

Stoof JC, Drukarch B, de Boer P, Westerink BH, Groenewegen HJ. Regulation of the activity of striatal cholinergic neurons by dopamine. Neuroscience 1992; 47(4): 755–70. [PubMed: 1579210]

Sun B, Chen S, Zhan S, Le W, Krahl SE. Subthalamic nucleus stimulation for primary dystonia and tardive dystonia. Acta Neurochir Suppl 2007; 97(Pt 2): 207–14. [PubMed: 17691306]

Trinh M, Klann E. Translational control by eIF2α kinases in long-lasting synaptic plasticity and long-term memory. Neurobiology of learning and memory 2013; 105.

van der Weijden M, Rodriguez-Contreras D, Delnooz C, Robinson B, Condon A, Kielhold M, et al. A Gain-of-Function Variant in Dopamine D2 Receptor and Progressive Chorea and Dystonia Phenotype. Movement disorders : official journal of the Movement Disorder Society 2020.

Vasudevan A, Breakefield XO, Bhide PG. Developmental patterns of torsinA and torsinB expression. Brain Res 2006; 1073-1074: 139–45. [PubMed: 16458269]

Verma A, Kulkarni S. Modulatory role of D-1 and D-2 dopamine receptor subtypes in nociception in mice. Journal of psychopharmacology (Oxford, England) 1993; 7(3).

Voelkl B, Altman N, Forsman A, Forstmeier W, Gurevitch J, Jaric I, et al. Reproducibility of animal research in light of biological variation. Nature reviews Neuroscience 2020; 21(7).

Vulinovic F, Lohmann K, Rakovic A, Capetian P, Alvarez-Fischer D, Schmidt A, et al. Unraveling cellular phenotypes of novel TorsinA/TOR1A mutations. Hum Mutat 2014; 35(9): 1114–22. [PubMed: 24931141]

Wall N, De La Parra M, Callaway E, Kreitzer A. Differential innervation of direct- and indirect-pathway striatal projection neurons. Neuron 2013; 79(2).

Wang H, Morales M. Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. The European journal of neuroscience 2009; 29(2).

Wang S, Lai X, Deng Y, Song Y. Correlation between mouse age and human age in anti-tumor research: Significance and method establishment. Life sciences 2020; 242.

Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, et al. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 2006; 50(3): 443–52. [PubMed: 16675398]

Wu Y, Ni L, Fan R, Yao M. Meta-Regression Analysis of the Long-Term Effects of Pallidal and Subthalamic Deep Brain Stimulation for the Treatment of Isolated Dystonia. World neurosurgery 2019; 129.

Xiao J, Gong S, Zhao Y, LeDoux MS. Developmental expression of rat torsinA transcript and protein. Brain Res Dev Brain Res 2004; 152(1): 47–60. [PubMed: 15283994]

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]

Yokoi F, Dang MT, Liu J, Gandre JR, Kwon K, Yuen R, et al. Decreased dopamine receptor 1 activity and impaired motor-skill transfer in Dyt1 DeltaGAG heterozygous knock-in mice. Behav Brain Res 2015; 279: 202–10. [PubMed: 25451552]

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]

Yokoi F, Jiang F, Dexter K, Salvato B, Li Y. Improved survival and overt “dystonic” symptoms in a torsinA hypofunction mouse model. Behavioural brain research 2020a; 381.

Yokoi F, Oleas J, Xing H, Liu Y, Dexter KM, Misztal C, et al. Decreased number of striatal cholinergic interneurons and motor deficits in dopamine receptor 2-expressing-cell-specific Dyt1 conditional knockout mice. Neurobiol Dis 2020b; 134: 104638. [PubMed: 31618684]

Yu H, Kuo H, Huang H, Huang L, Tain Y, Chen C, et al. Glyceraldehyde-3-phosphate dehydrogenase is a reliable internal control in Western blot analysis of leukocyte subpopulations from children. Analytical biochemistry 2011; 413(1).

Zarrindast M, Moghaddampour E. Opposing influences of D-1 and D-2 dopamine receptors activation on morphine-induced antinociception. Archives internationales de pharmacodynamie et de therapie 1989; 300.

Zhang L, Yokoi F, Parsons DS, Standaert DG, Li Y. Alteration of striatal dopaminergic neurotransmission in a mouse model of DYT11 myoclonus-dystonia. PLoS One 2012; 7(3): e33669. [PubMed: 22438980]

Zhao C, Brown RS, Chase AR, Eisele MR, Schlieker C. Regulation of Torsin ATPases by LAP1 and LULL1. Proc Natl Acad Sci U S A 2013; 110(17): E1545–54. [PubMed: 23569223]

Zhao Y, DeCuypere M, LeDoux MS. Abnormal motor function and dopamine neurotransmission in DYT1 DeltaGAG transgenic mice. Exp Neurol 2008; 210(2): 719–30. [PubMed: 18299128]

Zirn B, Grundmann K, Huppke P, Puthenparampil J, Wolburg H, Riess O, et al. Novel TOR1A mutation p.Arg288Gln in early-onset dystonia (DYT1). J Neurol Neurosurg Psychiatry 2008; 79(12): 1327–30. [PubMed: 18477710]

Highlights

1. Lack or reduction of D2R on ChIs led to an age-dependent beam-walking deficit.
2. Lack or reduction of D2R on ChIs mimicked the motor deficit of *Dyt1* KI mice.
3. Lack or reduction of D2R on ChIs led to the increased spontaneous firing of ChIs.
4. Lack or reduction of D2R on ChIs in WT mice attenuated quinpirole response.
5. Loss of D2R on medium spiny neurons in WT mice led to a rotarod deficit.

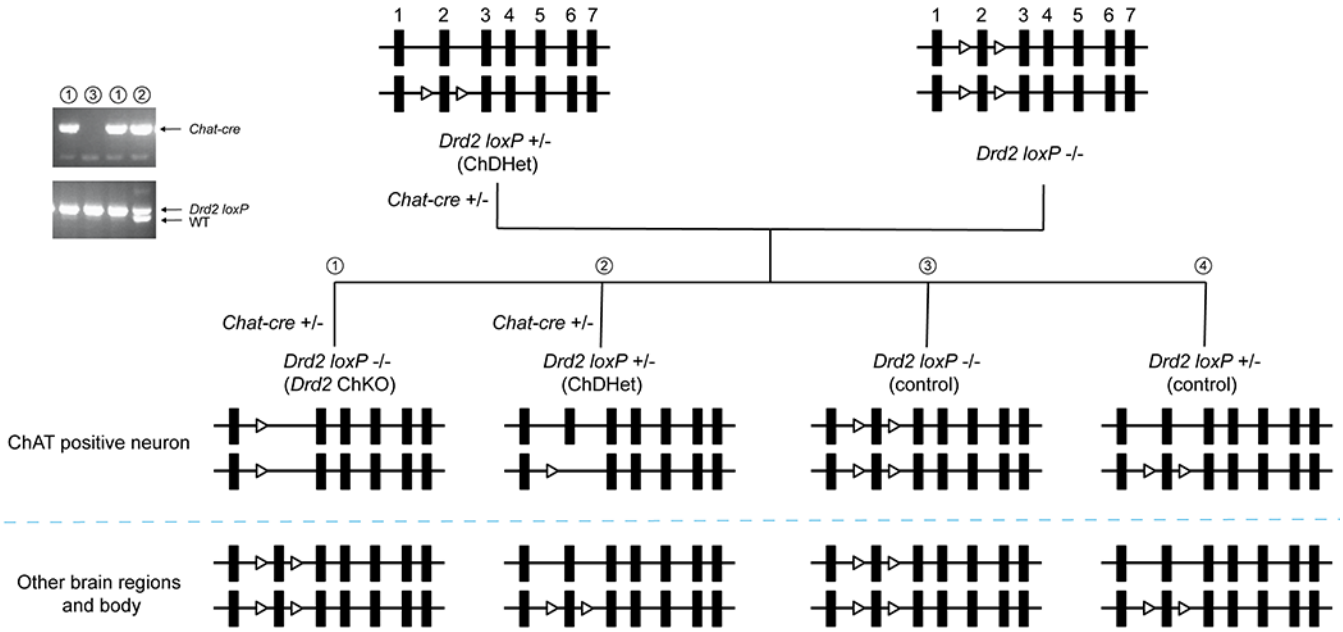

Figure 1.

Schematic diagram of the generation of *Drd2* ChKO mice. *Chat-cre*+/−*Drd2 loxP*+/− (ChDHet) mice were crossed with *Drd2 loxP*−/− mice to generate ① *Chat-cre*+/−*Drd2 loxP* −/− (*Drd2* ChKO), ② ChDHet, ③ *Drd2 loxP*−/−, and ④ *Drd2 loxP*+/− mice. PCR-based tail DNA genotyping of *Chat-cre* and *Drd2 loxP* loci (top left).

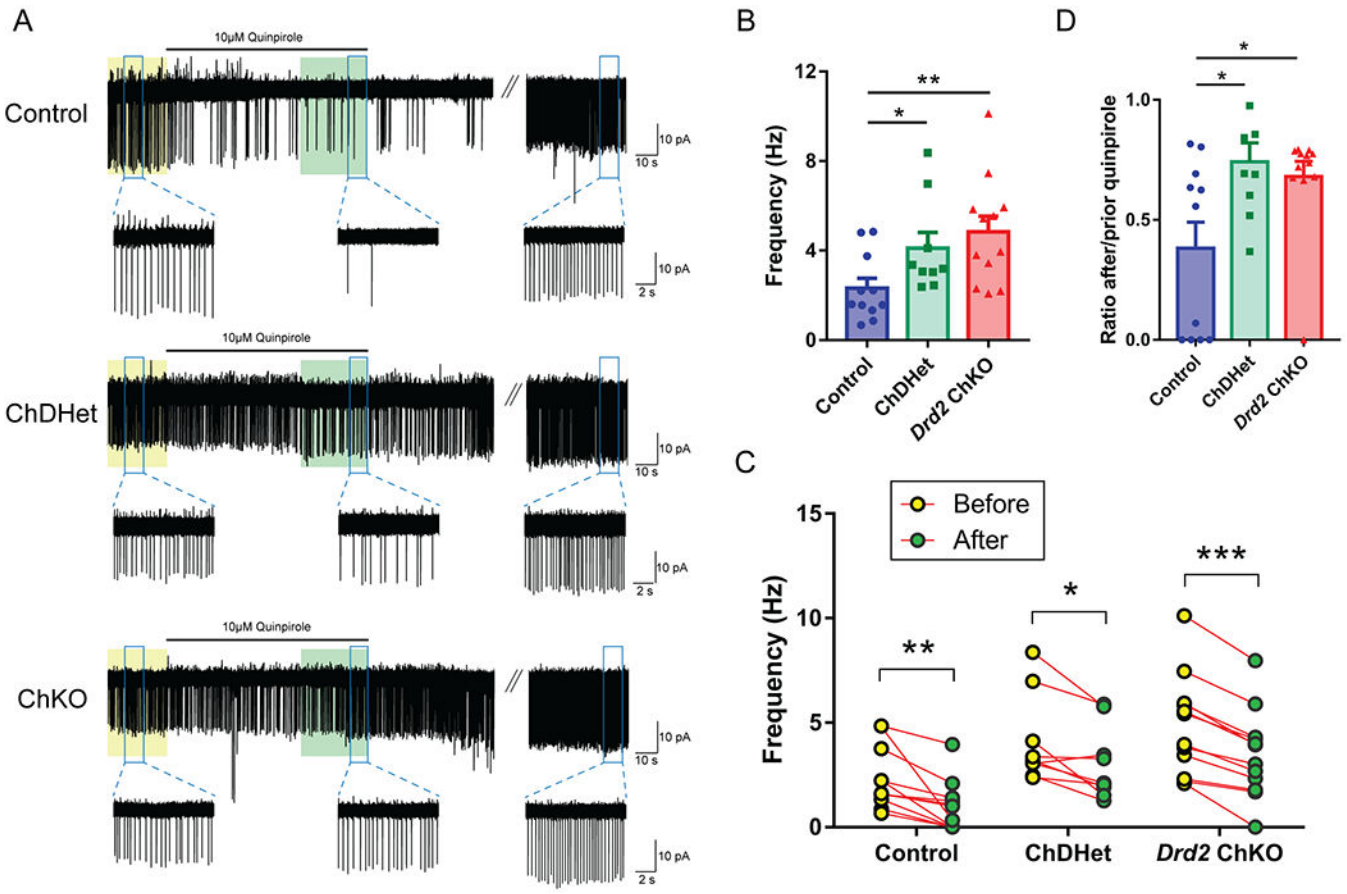

Figure 2.

Electrophysiological characterization of D2R function in *Drd2* ChKO mice. (**A**) Representative recording traces before and after the bath application of the D2R agonist quinpirole (10 μM, 90 seconds). The yellow area indicates the 30 seconds used to determine spontaneous firing property before drug application. The green area indicates 30 seconds used to determine the frequency after 60 seconds of drug application. (**B**) Increased spontaneous firing frequency of ChIs in *Drd2* ChKO and ChDHet compared to control mice before quinpirole treatment. (**C**) All genotypes showed a reduced firing rate after the quinpirole application. (**D**) *Drd2* ChKO and ChDHet mice showed significantly increased frequency ratios of after/before quinpirole over the control mice. Vertical bars represent means ± standard errors. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

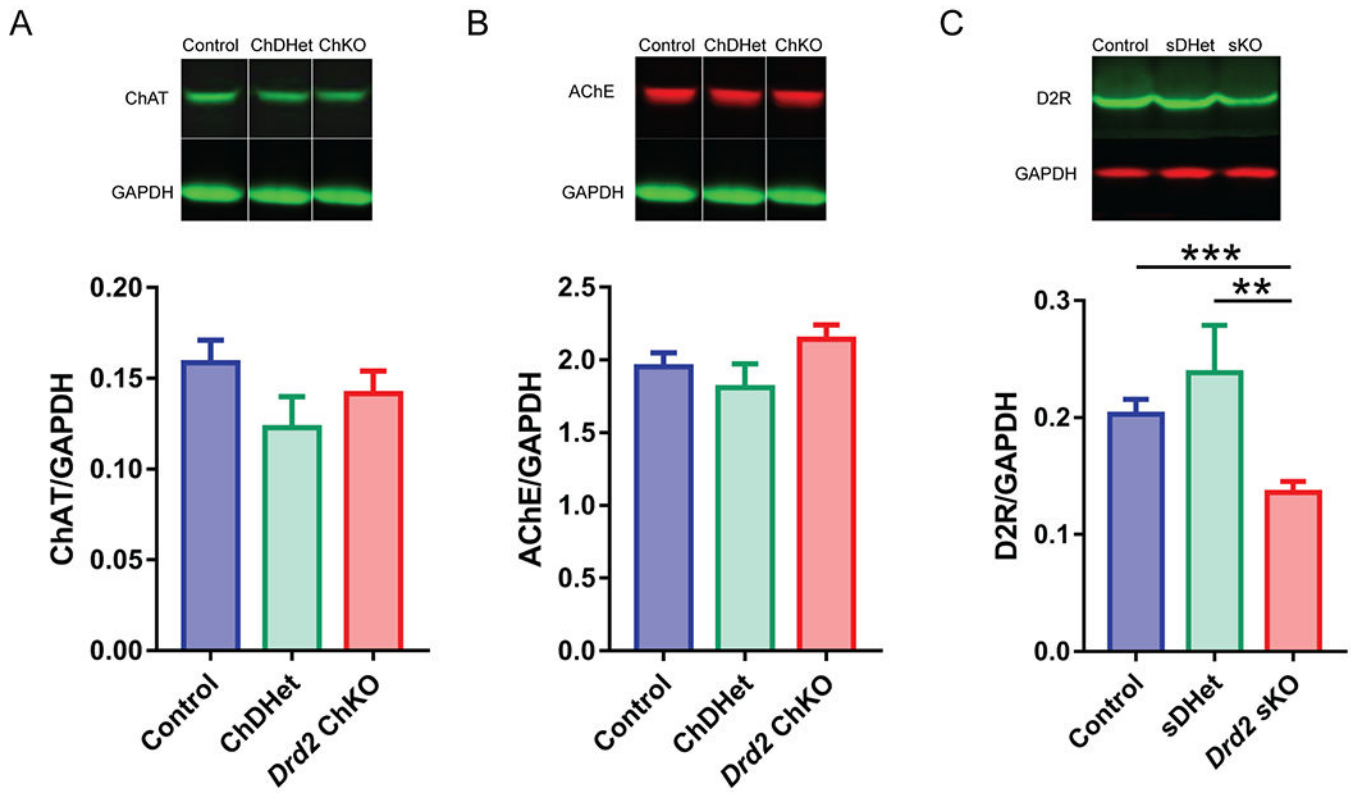

Figure 3.

Striatal protein levels in *Drd2* ChKO and *Drd2* sKO mice. Representative western blot images and their quantifications of striatal ChAT (**A**), AChE (**B**), and their loading controls (GAPDH) in *Drd2* ChKO mice. Representative western blot images and their quantifications of striatal D2R (**C**) and their loading controls (GAPDH) in *Drd2* sKO. Vertical bars represent means ± standard errors.

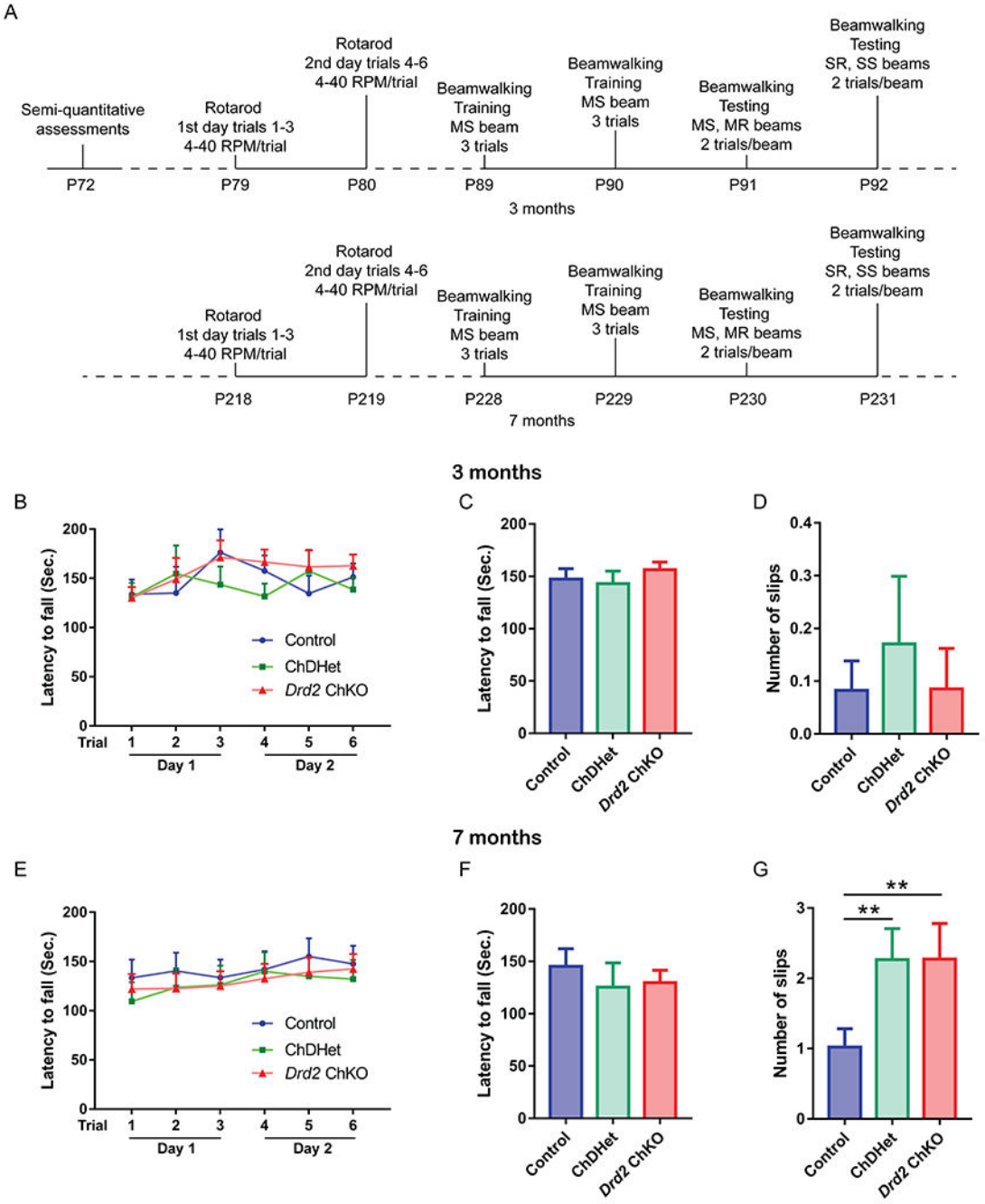

Figure 4.

Impaired motor coordination and balance in *Drd2* ChKO and ChDHet mice. (**A**) Timeline of the behavior tests. Lack of motor deficits in *Drd2* ChKO and ChDHet mice at 3 months of age on the accelerated rotarod test (**B**-**C**) and the beam-walking test (**D**). Lack of motor deficits in *Drd2* ChKO and ChDHet mice at 7 months of age in the rotarod test. 1-6: trial number. 1-3 trials on the first day and 4-6 trials on the second day. (**E**-**F**). *Drd2* ChKO and ChDHet mice displayed excessive slips on the beam-walking test (**G**). $^{**}p \leq 0.01$.

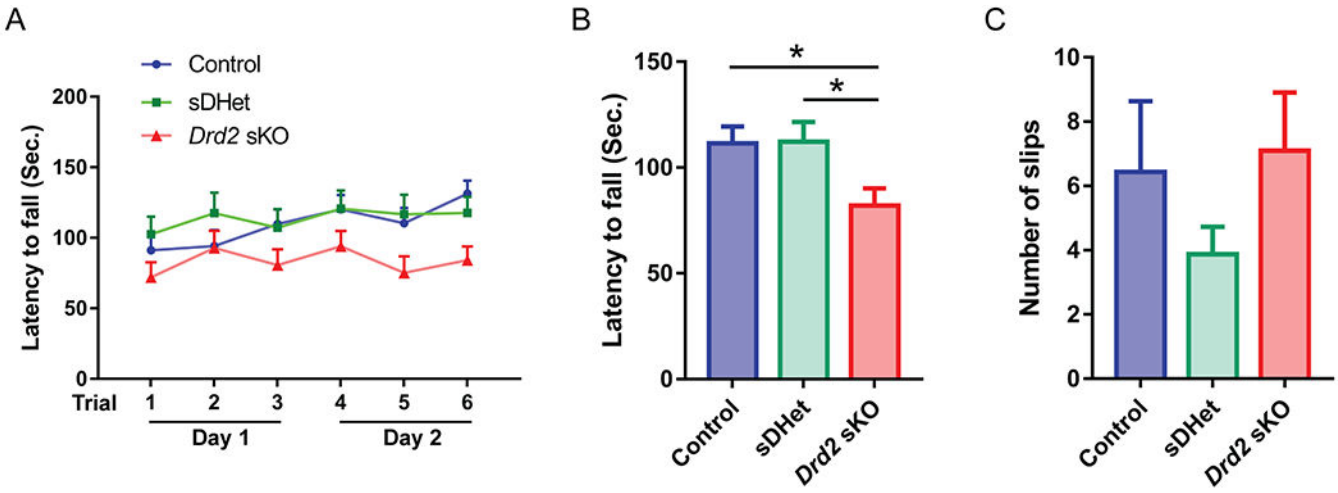

Figure 5.

Impaired motor coordination and balance in *Drd2* sKO mice. (**A**-**B**) *Drd2* sKO mice displayed reduced latency to fall in the rotarod test compared with control and sDHet mice. 1-6: trial number. 1-3 trials on the first day and 4-6 trials on the second day. (**C**) Lack of motor deficits in the beam-walking test in *Drd2* sKO mice. *$p \leq 0.05$.

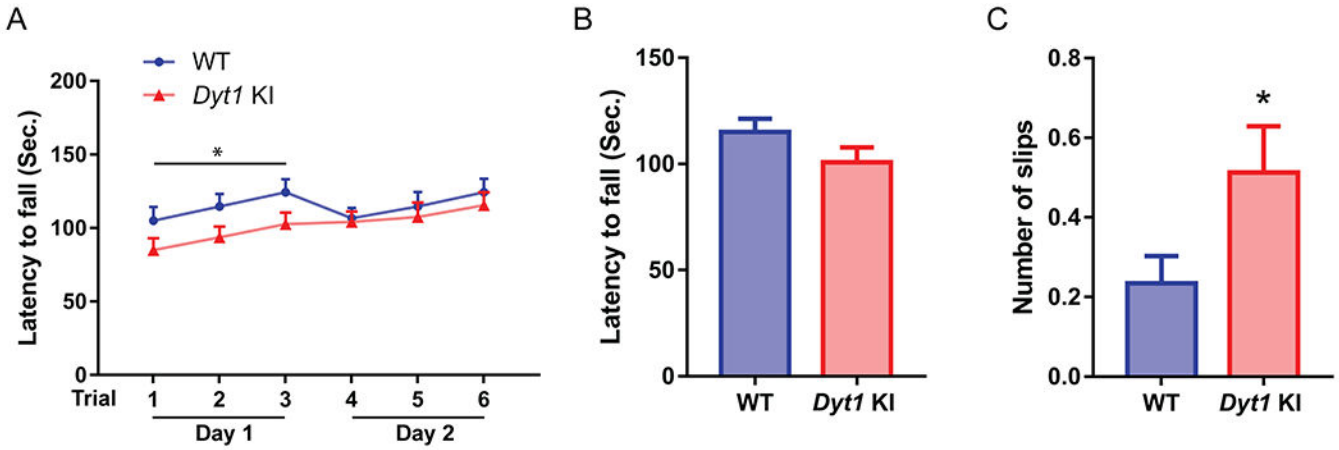

Figure 6.

Impaired motor coordination and balance in *Dyt1* KI mice. (**A**-**B**) *Dyt1* KI mice showed motor deficits on the first day but not across all six trials on the accelerated rotarod test. 1-6: trial number. 1-3 trials on the first day and 4-6 trials on the second day. (**C**) *Dyt1* KI mice displayed excessive slips on the beam-walking test. $*p \leq 0.05$.