Identification of Complex Motor Abnormalities in a Mouse Model of Dystonia

by

Krista Kernodle

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Doctoral Committee:

Professor William T. Dauer, Co-Chair
Assistant Professor Daniel K. Leventhal, Co-Chair
Professor Roger Albin
Professor Kent Berridge
Associate Professor Cynthia Chestek

Krista Kernodle

kkrista@umich.edu

ORCID iD: 0000-0003-4610-0931

Dedication

This dissertation is dedicated to my grandmother, Cherie Kernodle, for her constant love and support. We miss you very much.

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Table of Contents

Dedication ii

Acknowledgements iii

List of Figures vii

Abstract viii

CHAPTER 1: Introduction 1

DYT1 dystonia 3
Focal and Task-Specific Dystonia 6
Pathophysiology of Dystonia 8
Reduced intracortical inhibition. *9*
Maladaptive plasticity. *11*
Abnormal sensorimotor integration. *17*
Models of dystonia 20
Behavioral tasks in rodent models of dystonia 27
References 29

CHAPTER 2: Development of Task-Specific Abnormal Movements in a Mouse Model of Dystonia 45

Abstract 45
Introduction 46
Results 48
Discussion 50
Methods 52
Figures 58
References 62

CHAPTER 3: A Dystonia Mouse Model with Motor and Sequencing Deficits Paralleling Human Disease 64

Abstract 64
Introduction 65
Results 67
Discussion 71
Methods 74
Figures 82
References 87

CHAPTER 4: Research Synthesis 92

Summary of findings 92

How do task-specific abnormal movements develop? 93
Behavioral assays in mouse models of dystonia 97
Concluding Remarks 98
References 100

List of Figures

Figure 2-1: Dlx-CKO mice have normal motor learning in a manual skilled reaching task 58
Figure 2-2: Dlx-CKO mice develop abnormal movements 59
Figure 2-3: Abnormal movements are reduced after administration of THP 60
Figure 2-4: Abnormal movements in Dlx-CKO mice did not replicate in an automated skilled reaching task 61
Figure 3-1: Dlx-CKO mice are impaired in automated skilled reaching 82
Figure 3-2: Dlx-CKO mice exhibit nonspecific deficits in pasta handling 83
Figure 3-3: Grooming structure in control mice 84
Figure 3-4: Dlx-CKO mice perform similar amounts of grooming, but in more variable patterns, than controls 85
Figure 3-5: Dlx-CKO mice have similar phase transitions during syntactic chains to controls ... 86

Abstract

Dystonias are a heterogenous group of often disabling movement disorders characterized by uncontrollable simultaneous contraction of agonist and antagonist muscles. Secondary dystonias are often caused by injuries to the central nervous system such as stroke, trauma, or Parkinson Disease. Primary dystonia, on the other hand, presents idiopathically without clear structural lesions or other neurological symptoms. Symptoms may be present in one body region, as in focal dystonia, or include large regions of the body, as in generalized dystonia. Existing therapies for dystonia are limited and often cause undesirable side effects or require surgery.

A significant barrier to improving therapies for dystonia is a lack of understanding of its pathophysiology. Both genetic and extragenetic factors are believed to contribute, as evidenced by endophenotypes in both symptomatic and non-symptomatic carriers of dystonia-causing genes. These endophenotypes include loss of intracortical inhibition, maladaptive plasticity, and abnormal sensorimotor integration. However, most mouse behavioral assays are implemented independently of cortex. It is therefore important to identify dystonia mouse models in which motor cortex-dependent behaviors are altered in ways relevant to human disease.

I implemented several behavioral paradigms with the goal of characterizing cortically-dependent behaviors in the recently developed Dlx-CKO mouse model of dystonia. I found that Dlx-CKO mice develop abnormal movements during a manual skilled reaching task and that

these abnormal movements respond to antimuscarinics, which are used to treat human dystonia. Additional testing on an automated skilled reaching task failed to replicate these results, suggesting that task demands may influence development of abnormal movements. Automated skilled reaching also revealed that Dlx-CKO mice exhibit a primary motor deficit. This finding is supported by results in a pasta handling task showing that Dlx-CKO mice exhibit more frequent pasta drops. Lastly, grooming was used to assess innate motor sequence performance independently of cortex. Dlx-CKO mice exhibited normal syntactic chain sequences, but reduced chain initiation. This result parallels abnormalities in motor sequencing identified in genetic carriers of DYT1.

This work establishes cortically-dependent motor abnormalities in a genetic mouse model of dystonia. These results support the use of a wider variety of behavioral assays to characterize dystonia models. The motor abnormalities identified in this work may facilitate determination of the interplay between genetic and extragenetic factors in the development of dystonia, and allow identification of physiologic changes that lead directly to impaired motor function.

CHAPTER 1: Introduction

Dystonias are a heterogenous group of often disabling movement disorders characterized by uncontrollable simultaneous contraction of agonist and antagonist muscles. This results in abnormal postures and repetitive, often twisting, movements. These abnormal movements are typically patterned but can be tremulous. Dystonia can manifest focally in one body region, or present in a multifocal, segmental, or generalized pattern. Hemidystonia, affecting one half of the body, may also occur. The most commonly affected areas of the body include the neck, eyelids, jaw or tongue, vocal cords, and hand or forearm. Dystonic movements may be present constantly, intermittently, or situationally, as in musician's or other task-specific dystonias. Abnormal movements often display variability in relation to voluntary actions or external triggers and can be static or progressive, evolving over time.

Dystonias are most often caused by injury to the central nervous system, such as stroke, trauma, or Parkinson Disease. These are termed "secondary dystonias" and are usually accompanied by other neurological symptoms (e.g., seizures). Dystonias developing without clear structural lesions or other neurological symptoms are termed "primary dystonia".

Adult-onset idiopathic dystonia is the most common type of primary dystonia. Typically, dystonias that arise in adults begin in the neck or extremities and remain focal or task specific. Dystonias that appear in childhood (3-12 years of age) or adolescence (13-20 years of age) are

more likely to have a discoverable cause. For example, dystonias caused by an inherited metabolic disorder generally have symptom onset in the first year of age, whereas DOPA-responsive dystonias emerge between ages 6 and 14. Childhood onset dystonias may begin focally but often generalize to involve multiple body regions. Two childhood onset dystonias with a defined genetic etiology are DYT1 and DYT6 dystonia, which are monogenic autosomal-dominant traits with partial penetrance (Albanese et al., 2013; Ozelius et al., 1997).

Treatments for dystonia are limited both by disease etiology and treatment side effects. DOPA-responsive dystonia improves with dopamine replacement therapy using L-DOPA (Segawa, 2011). Focal dystonias may be treated with botulinum toxin injected into the affected muscle (Jankovic, 2013; Tsui et al., 1986). Most forms of dystonia respond to anticholinergic medications, such as trihexyphenidyl (Burke et al., 1986; Fahn, 1983; Jankovic, 2013). Unfortunately, anticholinergic medications have limited effectiveness and produce several undesirable side effects, including drowsiness, confusion, and memory difficulties.

A significant barrier to improving treatments for dystonia is a limited understanding of the underlying neural mechanisms that cause symptoms. Surgical interventions are available in limited cases for treatment of severe dystonia. Patients whose symptoms result in significant disability that cannot be managed by medications are good candidates for deep brain stimulation (DBS). DBS alters local neuronal activity, although the precise mechanisms underlying its efficacy remain unclear. DBS within the globus pallidus interna (GPi) or the subthalamic nucleus (STN) are the most effective surgical interventions for treating dystonia (Eltahawy, Feinstein, et al., 2004; Eltahawy, Saint-Cyr, et al., 2004). Patients with medically refractory primary generalized dystonia most commonly receive DBS, although patients with cervical dystonia (the most common primary focal dystonia) may also benefit. Dystonias associated with

neurodegeneration may also benefit from DBS. Most other types of secondary dystonias are not candidates for DBS, or respond minimally (Ostrem & Starr, 2008).

DYT1 dystonia

The most common inherited childhood-onset primary generalized dystonia is DYT1 dystonia, caused by a mutation in the gene encoding the protein torsinA (Ozelius et al., 1997). Due to its relatively high prevalence and early identification, DYT1 has received intensive laboratory investigation. Given shared neurophysiologic features across primary dystonias (see *Pathophysiology of Dystonia*) it is hoped that understanding the neurobiology of DYT1 will provide insight on shared pathogenesis with other forms of primary dystonia.

DYT1 dystonia is caused by an in-frame GAG deletion (ΔE) in the gene encoding torsinA (*TOR1A*), resulting in the loss of a pair of glutamic acid residues (Ozelius et al., 1997). TorsinA is a member of the AAA (ATPases Associated with diverse cellular activities) protein family, which are implicated in protein quality control and localized to the nuclear membrane (reviewed in Dauer, 2014). TorsinA is imported into the endoplasmic reticulum (ER) where the signal sequence is cleaved (Z. Liu et al., 2003). Mutated torsinA (ΔE *TOR1A*) is similarly imported into the ER lumen, however it then redistributes into the perinuclear space of the nuclear envelope (NE) and causes NE-derived blebs (Gonzalez-Alegre & Paulson, 2004; Goodchild et al., 2005; Goodchild & Dauer, 2004; Naismith et al., 2004). Genetic experiments in various mouse models indicate that ΔE is a loss-of-function mutation (Dang et al., 2005; Goodchild et al., 2005). For example, deletion of torsinA causes similar cell biological and organismal phenotypes as the torsinA ΔE allele (Liang et al., 2014a).

DYT1 dystonia is incompletely penetrant, manifesting in approximately one-third of mutation carriers. The majority of carriers that manifest disease are affected by their teenage

years, with carriers who do not exhibit dystonia by their early twenties typically remaining asymptomatic for life. The existence of a non-manifesting population of DYT1 carriers has prompted investigation into factors influencing penetrance. The only known genetic factor impacting penetrance of DYT1 dystonia is a single nucleotide polymorphism in *TOR1A*, encoding for an amino acid change in torsinA (Risch et al., 2007). However, this polymorphism is rare, and therefore does not explain most of the phenotypic variability in ΔE mutation carriers. One study identified an association between complications of vaginal delivery and DYT1 dystonia (Martino et al., 2013), but environmental determinants of DYT1 phenotypes remain largely unknown.

Neuroimaging studies have revealed sensorimotor networks that correlate with penetrance. F-fluorodeoxyglucose (FDG) positron emission tomography (PET) allows imaging of topographic metabolism patterns in the brain *in vivo*. FDG PET studies in manifesting and non-manifesting carriers of DYT1 and DYT6 mutations identified genotype-specific changes in the posterior putamen/globus pallidus, cerebellum, and supplementary motor area (SMA) (Carbon, Su, et al., 2004; Carbon & Eidelberg, 2009). Carriers across both dystonia genotypes displayed increased FDG uptake in the pre-SMA and parietal association regions, suggesting underlying network abnormalities in clinically heterogenous manifestations of dystonia. While DYT1 carriers displayed increased FDG uptake in the putamen, globus pallidus, and cerebellum at rest, carriers of DYT6 displayed decreased FDG uptake in the striatum, cerebellum, and upper brainstem extending into the thalamus. In DYT1 patients, increased FDG uptake in pre-SMA and parietal association cortices as well as decreased FDG uptake in cerebellum, brainstem, and ventral thalamus distinguished manifesting from non-manifesting carriers (Carbon & Eidelberg, 2009). In both DYT1 and DYT6, decreased FDG uptake in the brainstem and ventral thalamus

suggest basal ganglia pathology, as both of these areas are targets for inhibitory basal ganglia efferents. These results suggest that clinical penetrance of dystonia is associated with abnormal sensorimotor integration and basal ganglia function regardless of genotype.

To elucidate further network differences in manifesting and non-manifesting carriers, $H_2{}^{15}O$ PET imaging, measuring cerebral blood flow, was implemented during sequence learning tasks (Carbon et al., 2008, 2011; Carbon & Eidelberg, 2009; Ghilardi et al., 2003). Participants were asked to perform paced reaching movements from a central starting point to one of eight radial targets with their dominant hand. Targets presented in a predictable counterclockwise order were used as the control task. In the motor sequence learning task (MSEQ), targets were presented in an unknown repeating order and subjects were instructed to learn the sequence. To control for the possible interference of dystonic movements, participants also performed a visual sequence learning task (VSEQ) (Carbon et al., 2011; Ghilardi et al., 2003).

Both manifesting and non-manifesting DYT1 carriers displayed similar deficits in MSEQ and VSEQ tasks compared to controls, while no DYT6 carriers displayed sequencing/learning deficits (Carbon et al., 2011). Additionally, manifesting DYT1 carriers had greater variability in movement time than non-manifesting carriers, indicating greater movement irregularities in manifesting carriers (Carbon & Eidelberg, 2009). Results from PET scans during MSEQ and VSEQ tasks showed both manifesting and non-manifesting DYT1 carriers exhibited greater learning-related cerebellar activation during both tasks (Carbon et al., 2008, 2011). Furthermore, manifesting DYT1 carriers had increased activity in the right lateral pre-motor cortex and in the right inferior parietal cortex relative to non-manifesting DYT1 carriers. The deficits in VSEQ task for manifesting and non-manifesting DYT1 carriers suggests an underlying abnormality in sequence learning, independent of motor control.

Deficits in motor sequence learning as well as specific abnormalities in pre-SMA, parietal cortex (involved in interpretation and integration of sensory information), and cerebellum have been identified as endophenotypes specific to DYT1 dystonia (Carbon et al., 2011). Differences in underlying neural activation during sequence learning tasks between manifesting and non-manifesting DYT1 carriers provide possible neural correlates for the development of dystonic behaviors. However, the path from torsinA loss of function to these neural correlates and the development of dystonic movements remains elusive. Studies in humans are generally restricted to non-invasive methods, limiting the techniques available to disentangle this complicated neural circuitry without the use of animal models.

Focal and Task-Specific Dystonia

Focal dystonias affect an isolated body part and can arise from injury as a secondary dystonia, or as a primary dystonia. Generally, primary focal dystonias are adult-onset and begin in the neck or upper limbs, and may spread to other body parts as the disease progresses. Dystonic movements may be present constantly, intermittently, or situationally, as in musician's or other task-specific dystonias. Task-specific dystonias typically develop following repetitive performance of skilled movements, and subsequently manifest selectively during such movements. Task-specific dystonia develops in adulthood, typically between ages 30 and 60 and is more common among men. Although initially task-specific dystonia may only be triggered during a single task and affect isolated body parts, it may spread to be present during other tasks or to previously unaffected body regions, becoming multi-focal or segmental.

Both genetic and environmental factors may lead to task-specific dystonia (Schmidt et al., 2013). Genetic influences are suggested by its higher prevalence among men and a positive family history of movement disorders in many affected patients. A family history of dystonia is

present in 10-25% of patients that develop task-specific dystonia (Schmidt et al., 2009; Stojanović et al., 1995; Waddy et al., 1991), although a consistent genetic defect has not been identified. Several genetic abnormalities linked to other forms of dystonia have been identified in patients with task specific dystonia, such as DYT6, DYT7, and DYT13 (Bhidayasiri et al., 2005). The gene mutation that causes DYT1 may also cause focal or task-specific dystonia (Friedman et al., 2000; Gasser et al., 1996, 1998; Leube et al., 1999; Opal et al., 2002). In general, however, the DYT1 mutation is uncommon in these patient populations (Friedman et al., 2000; Gasser et al., 1996). Additionally, the *ARSG* gene, encoding arylsulfatase G, has been linked to task-specific dystonia susceptibility (Lohmann et al., 2014; Nibbeling et al., 2015). *ARSG* belongs to the family of sulfatase genes involved in cell signaling, protein degradation, and hormone biosynthesis (Sardiello et al., 2005). Dystonia has been described as a feature of neuronal ceroid lipofuscinosis, caused by a homozygous mutation in the canine *ARSG* homologue (Abitbol et al., 2010). It is possible that mutations of *ARSG* found in task-specific dystonia patients contribute to disease pathogenesis. However, task-specific dystonia patients with a clear genetic explanation account for only a small proportion of the total patient population, indicating that genetics are not sufficient to explain the development of dystonia.

Environmental factors include the repetition of highly dexterous tasks, with the type of task performed influencing symptom onset. Tasks requiring high accuracy, speed, and dexterity (e.g., playing a violin) correlate with increased incidence of dystonia (Altenmüller et al., 2014, 2015; Altenmüller & Jabusch, 2010). Additionally, fatigue, overuse and injury may predispose people to task-specific dystonia. However, it remains unclear how these environmental factors lead to task-specific dystonia.

The unclear etiology of task-specific dystonia has prevented the development of effective treatments. Pharmaceutical interventions, such as baclofen or benzodiazepines (both GABA agonists), are commonly used but very rarely restore normal motor function. Injections of botulinum toxin into the affected muscle may provide relief. However, muscle targeting can be difficult, and patients often develop weakness in the injected area. Most other forms of treatment focus on sensorimotor retraining by splinting the affected body part during task performance to disrupt the dystonic movements. This is posited to prevent abnormal sensorimotor feedback and strengthen normal sensorimotor programs, while weakening those created by performance of dystonic movements.

Pathophysiology of Dystonia

There is currently no adequate neural model to fully account for phenotypic and endophenotypic manifestations of dystonia. However, neuroimaging studies have identified changes in connectivity and metabolic activity in the basal ganglia and cortex of different monogenic and focal dystonia patient populations (Argyelan et al., 2009; Blood et al., 2004, 2012; Carbon, Su, et al., 2004; Delmaire et al., 2007; Draganski et al., 2003; Egger et al., 2007; Garraux et al., 2004). Diffusion tensor imaging and tract tracing in DYT1 patients revealed abnormal structural integrity of the white matter in the sensorimotor cortex, brainstem, and connections between the brainstem and cerebellum (Carbon, Kingsley, et al., 2004). Metabolic abnormalities in pre-SMA and parietal cortex, associated with sensorimotor integration, are associated with clinical penetrance of hereditary dystonia, regardless of genotype (Carbon, Su, et al., 2004). These abnormalities provide possible neural endophenotypes representing disease susceptibility.

The existence of dystonia endophenotypes supports the "two-hit" hypothesis of dystonia pathogenesis: that genetically susceptible individuals can compensate for subclinical physiologic abnormalities, but when stressed by an extragenetic factor (e.g., injury or overtraining), compensatory mechanisms are overwhelmed. This results in maladaptive plasticity, leading to the development of dystonic movements. This hypothesis arose in the context of task-specific dystonia, where overtraining on highly skilled tasks provokes the development of dystonic movements. Subsequent observations support a more general link between limb use and the development of dystonia (Roze et al., 2009), whereby loss of intracortical inhibition and abnormal plasticity lead to dystonia (Quartarone & Hallett, 2013). In this section, I will discuss the evidence for reduced intracortical inhibition, maladaptive plasticity, and abnormal sensorimotor integration in dystonia pathophysiology.

Reduced intracortical inhibition. Loss of inhibition has been reported at several levels of the CNS involved in motor control, including cortex, basal ganglia, brainstem, and spinal cord (Hallett, 2004). Transcranial magnetic stimulation (TMS) experiments provide evidence for abnormal inhibition in the cortex. TMS induces changes in neural excitability using a magnetic field to assess inhibitory circuits in the cortex and cerebellum. In paired-pulse TMS, a conditioning stimulus is followed by a testing stimulus with varying interstimulus intervals. When interstimulus intervals are small (1-4 ms), the motor evoked potential of the conditioned stimulus is inhibited by the test stimulus. This is termed short interval intracortical inhibition (SICI). Long interstimulus intervals (50-500 ms) produce a similar effect, as stimulation occurs during the cortical silent period. This protocol is termed long interval intracortical inhibition (LICI). At intermediate interstimulus intervals (10-15 ms), motor evoked responses are

facilitated in a process known as intracortical facilitation (ICF) (Frey et al., 2021; Van den Bos et al., 2018).

The first TMS experiments conducted in patients with focal hand dystonia found reduced SICI in patients compared to healthy controls. This was interpreted as reduced excitability of inhibitory intracortical networks (Ridding et al., 1995). Importantly, this abnormal cortical inhibition was identified bilaterally in patients with unilateral dystonic symptoms. This suggests that this abnormality is insufficient to cause dystonia by itself, and that it is not a consequence of the dystonic movements (i.e., reverse causation). Additional studies with TMS found abnormal cortical inhibition was present at rest, prior to (Gilio et al., 2003), and during (R. Chen, Classen, et al., 1997; R. Chen, Wassermann, et al., 1997; Stinear & Byblow, 2004) voluntary movement in focal hand dystonia. In genetic dystonia, similar reductions were described in manifesting but not non-manifesting carriers of the DYT1 mutation (Edwards et al., 2006). Imaging results have confirmed that GABAergic signaling in the cortex and cerebellum is abnormal in dystonia (Gallea et al., 2018; Levy & Hallett, 2002). Combined, these results provide evidence that loss of intracortical inhibition, likely as a result of reduced GABAergic signaling, is an endophenotypic trait in manifesting dystonia patients.

One hypothesis for how a loss of intracortical inhibition contributes to the development of dystonic movements is through a loss of "surround inhibition". In surround inhibition, voluntary movement results in excitation of cortical circuits related to the desired motor command and inhibition of cortical circuits related to undesired motor commands (Sohn & Hallett, 2004). An imbalance in the cortical inhibitory system leads to the loss of surround inhibition, creating an "overflow" of neuronal excitation and loss of selectivity, resulting in the development of dystonic movements. SICI is thought to be a measure of surround inhibition

where GABAergic interneurons of the cortex are recruited by the collateral axons of pyramidal neurons during the conditioning stimulus, and inhibit the motor evoked potentials normally produced by the test stimulus. In this way, loss of surround inhibition has been identified in patients with focal hand dystonia. In one experiment, movements were performed for a period of time to allow examination of changes in surround inhibition during movement initiation compared to tonic contraction (Beck et al., 2008). Healthy controls showed strong surround inhibition during movement initiation that was absent during tonic contractions. Patients with focal hand dystonia displayed deficits of surround inhibition during movement initiation, with similar SICI before and during movement.

Impairments of dexterity in dystonia patients are thought to reflect the loss of surround inhibition. For example, pianists with task-specific hand dystonia lose independent control of their fingers during key-strike tasks (Furuya et al., 2015, 2018; Furuya & Altenmüller, 2013). Disorganized somatotopic representations in the sensorimotor cortex have consistently been identified and also support a loss of surround inhibition (Mantel et al., 2016; Tyč et al., 2012; Uehara et al., 2019). However, several studies have shown reduced brain activity during motor tasks in dystonia patients (Haslinger et al., 2005; Islam et al., 2009; Oga et al., 2002), contradicting the "loss of surround inhibition" hypothesis. Controversy remains as to whether loss of surround inhibition is related to disease penetrance or an epiphenomenon resulting from dystonia.

Maladaptive plasticity. Neuroplasticity refers to the neural processes underlying learning and the adaptability of the brain to external stimuli. Long-term potentiation (LTP) and long-term depression (LTD) describe the strengthening or weakening of synaptic connections in slice physiology. It is believed that these cellular mechanisms underly the processes of learning and

adaptation in humans and animals. Repetitive TMS (rTMS) produces changes in cortical excitability that outlast the duration of stimulation and is therefore believed to induce and measure neural plasticity in the cortex. rTMS with low-frequency stimulation results in cortical inhibition, suggesting induction of LTD-like changes in neural excitability, whereas high-frequency stimulation results in cortical excitation and is interpreted as LTP-like changes

Maladaptive plasticity is thought to occur in dystonia as a result of genetics, injury, or a breakdown of normal plasticity due to overtraining, as in task-specific dystonia. Patients with dystonia display longer lasting changes in both LTD-like and LTP-like cortical excitability induced with rTMS (Edwards et al., 2006; Quartarone et al., 2003; Ruge et al., 2011; Tamura et al., 2009; Tisch et al., 2007). These results support the hypothesis that maladaptive cortical plasticity contributes to dystonia pathogenesis. However, contradicting results have been published (Sadnicka et al., 2014). This experiment used paired associative stimulation (PAS), which repeatedly and concurrently pairs electrical stimulation of a peripheral nerve with TMS of the motor cortex and is believed to be a measure of cortical plasticity. Previously identified abnormalities of plasticity in dystonic patients could not be replicated with PAS (Sadnicka et al., 2014). They demonstrate that measures obtained from PAS have high individual variability, even in healthy controls, likely contributing to this result.

Other brain regions may contribute to maladaptive plasticity in cortex, most notably the basal ganglia. The basal ganglia (BG) comprise the striatum (caudate, putamen, and nucleus accumbens), subthalamic nucleus (STN), globus pallidus (divided into the internal segment, GPi; and the external, GPe), and substantia nigra (pars compacta, SNc; pars reticulata, SNr). The striatum is an input nucleus of the BG, receiving glutamatergic innervation from the cortex. Striatal afferents are processed through interactions with cholinergic and GABAergic

interneurons as well as collateral interaction from GABAergic medium spiny neurons (MSNs), which are the main striatal efferent. The striatum projects to globus pallidus, SNr, and SNc, receiving reciprocal dopamine projections from SNc onto MSNs. Striatal efferents project to GPi and SNr via the monosynaptic "direct" pathway and via GPe and STN in the multisynaptic "indirect" pathway. STN is another BG input nucleus, receiving innervation from cortex. The cortico-subthalamic projections make up the "hyper-direct" pathway, activation of which is thought to be involved in limiting motor response or the quick cancellation of ongoing movement (Wichmann, 2018). The main output structures of the basal ganglia, GPi and SNr, are inhibitory to thalamic nuclei, superior colliculus, and the pedunculopontine area of the brainstem. These pathways form the corticostriatal loop, as the thalamus then projects back to cortex.

There is strong evidence for basal ganglia dysfunction in dystonia. Damage to the basal ganglia, especially striatum, can cause secondary dystonia. This may occur with loss of dopamine caused by damage to SNc (as in Parkinson Disease) or direct striatal injury. Additionally, DBS targeting GPi and STN are effective treatments for medically intractable dystonia. However, not all motor symptoms of dystonia are immediately alleviated; quick "phasic" dystonic movements respond earlier and more robustly to DBS than slower "tonic" postures (Chung & Huh, 2016). This supports the hypothesis that some dystonic movements result from plastic changes within or downstream of the basal ganglia.

Models of basal ganglia function suggest that abnormal BG output is a result of an imbalance between the direct and indirect pathways. In this model, the direct and indirect pathways exhibit opposing influences on GPi and SNr. Activation of the direct pathway via D1-receptor expressing striatal projection neurons facilitates movement via excitatory connections to

SNr/GPi. On the other hand, while activation of the indirect pathway via D2-receptor expressing striatal projection neurons suppresses movement via inhibitory connections to SNr/GPi (Albin et al., 1989; DeLong, 1990). As the main output nuclei of the BG, SNr/GPi provide inhibitory control over its thalamic nuclei targets and thalamic projections to cortex are excitatory.

In this model, abnormal function of the indirect pathway would lead to a loss of inhibition at SNr/GPi. This may be coupled with overexcitation of SNr/GPi via the direct pathway. This leads to a loss of inhibition over thalamus and overexcitation in cortex. The result is manifestation of abnormal movements seen in Dystonia. However, there is strong evidence that this model is inadequate (for full review, see Magnusson & Leventhal, 2021). For example, pallidotomy in this model should result in decreased thalamic inhibition, resulting in increased movement. This is true in patients with Parkinson Disease, a hypokinetic disorder, but pallidotomy also improves symptoms of dystonia, which is hyperkinetic (Baron et al., 2000; Bastian et al., 2003; Lozano et al., 1997; Vitek & Bakay, 1997). This directly contradicts current models of BG function, where pallidotomy in dystonia would worsen symptoms. Also in this model, loss of GPi results in loss of inhibition of thalamus, and thus increased movement speed. However, damage to or inactivation of GPi results in slower movements in previously healthy subjects (Desmurget & Turner, 2010; Mink & Thach, 1991). Despite evidence opposing this hypothesis of BG function, an adequate replacement has not been proposed.

Intraoperative deep brain recordings of humans with dystonia demonstrate reduced single unit firing rates and burstier firing patterns compared to healthy nonhuman primates or patients with Parkinson Disease (Alam et al., 2016; McClelland et al., 2016; Starr et al., 2005; Tang et al., 2007; Vitek et al., 1999; Zhuang et al., 2004). Low pallidal firing rates in dystonia patients are inversely correlated with the severity of the dystonia (Starr et al., 2005). Increased power in

the low-frequency alpha (4-12 Hz) band of local field potentials (LFPs) in GPi was also demonstrated in dystonic patients, with correlations between low-frequency activity and EMG activity during simultaneous recordings from affected muscles (C. C. Chen et al., 2006; X. Liu et al., 2008; Neumann et al., 2012; Silberstein et al., 2003; Zhu et al., 2018). In patients with DBS, low-frequency activity was decreased when the stimulator was turned on compared to when it was turned off (Barow et al., 2014). Additionally, LFP activity was recorded bilaterally from the STN of patients with Parkinson Disease and from the GPi of patients with dystonia at rest or during passive, active, and ballistic movements. The amplitude of alpha frequency oscillations in GPi and STN contralateral to the moving limb was significantly higher in ballistic fast movements compared with all other conditions, suggesting ballistic fast movements synchronize BG activity in the alpha band, at least in dystonia patients (Singh et al., 2011). These results collectively suggest that a mechanism of action for DBS is to suppress synchronized alpha band BG activity, at least for the "phasic" symptoms of dystonia. It seems likely that "tonic" dystonia improves as a result of neural plasticity in response to DBS.

Abnormal dopamine function is implicated in the development of dystonic movements by the existence of DOPA-responsive dystonia and patient response to dopamine modulation in other diseases. For example, dopamine blocking drugs can cause acute dystonic reactions in young people and tardive dystonia in older people (Rupniak et al., 1986). Additionally, patients with Parkinson Disease often have dystonic dyskinesia, caused by long-term use of dopamine replacement therapies. The uptake of PET ligands that bind D2Rs is decreased in the striatum of patients with various forms of idiopathic dystonia and even non-manifesting carriers of DYT1 and DYT6 dystonia (Asanuma et al., 2005; Berger et al., 2007; Carbon et al., 2009; Hierholzer et al., 1994; Horstink et al., 1997; Perlmutter et al., 1997). This suggests excessive dopaminergic

tone, leading to lower availability for radioligand biding, or alternatively that there are fewer D2Rs expressed. These results support abnormal dopaminergic function as an endophenotypic trait across clinically heterogenous manifestations of dystonia. However, radioligands have significant limitations in selectivity. They do not distinguish between pre- and post-synaptic dopaminergic receptors, preventing cell-type specific localization of altered physiology. Additionally, most radioligands can be displaced by endogenous dopamine, thus decreased radioligand binding could represent the presence of increased endogenous dopamine. Experiments using the highly selective D2R radioligand N-methyl-benperidol (NMB) did not identify reduced striatal D2R biding in isolated idiopathic focal dystonia. NMB is highly selective for D2Rs and not displaced by endogenous dopamine. These conflicting results may be explained by interactions of less selective radioligands with D3 receptors (D3R), which are autoreceptors expressed on presynaptic nigrostriatal fibers. Reductions in D3R, rather than D2R, would then explain the decreased radioligand binding identified in earlier studies. However experiments directly testing this have not yet been published.

Measurements of dopamine in the striatum of patients has revealed conflicting results. Post-mortem striatal dopamine levels have been found to be normal (Furukawa et al., 2000) or reduced (Hornykiewicz et al., 1988). PET imaging using raclopride, a selective D2R/D3R antagonist, is used to examine changes in receptor availability caused by alterations in striatal dopamine concentration. When patients with focal or task-specific dystonia performed motor tasks involving the affected body part, raclopride displacement was reduced compared to healthy controls. This suggests increased endogenous dopamine release in patients. However, motor tasks that involved unaffected body parts of the same patients resulted in increased raclopride displacement compared to controls, suggesting decreased endogenous dopamine. Changes in

striatal D2R may also be organized somatotopically, localized within affected brain regions to the topographic representation of the affected body part. (Black et al., 2014).

Changes in dopamine concentrations and D2R availability may be a result of reciprocal modulation between dopamine and acetylcholine. Mouse models of dystonia suggest that changes in dopamine and D2R ligand binding may be a result of a cholinergic mediated "paradoxical excitation," where D2R activation lowers the firing threshold, leading to increased excitation rather than inhibition (Pisani et al., 2006; see *Models of Dystonia*). In addition to paradoxical excitation, mouse models of dystonia are consistently associated with a loss of LTD and low frequency stimulation induced synaptic depotentiation, as well as increased magnitude of LTP. It is believed that paradoxical excitation is responsible for altered bidirectional corticostriatal synaptic plasticity and may provide a neural basis for abnormal motor learning and performance in human dystonia.

Striatal cholinergic abnormalities are implicated in human dystonia pathophysiology. Antimuscarinic medications are at least partially effective in treating most forms of dystonia. Additionally, young DYT1 patients display decreased vesicular acetylcholine transporter (VAChT) expression is in the striatum and cerebellum, whereas old DYT1 patients had similar VAChT expression levels to age matched controls (Mazere et al., 2021). These results provide support for abnormal ACh signaling at the time of disease onset and progression. However, direct evidence that striatal cholinergic abnormalities cause dystonia is lacking.

Abnormal sensorimotor integration. Sensorimotor integration abnormalities are exemplified by the existence of a maneuver (sensory trick) performed by dystonic patients to alleviate motor symptoms (Brüggemann, 2021; Conte et al., 2018; Pandey et al., 2017; Patel et al., 2014). Patients with dystonia also experience distortion of sensory modalities and 'phantom'

movements or postural sensations and may describe pain or discomfort of the affected body part, especially in cervical dystonia (Tinazzi et al., 2003).

Abnormalities of spatial discrimination have been identified in patients with focal dystonia. The grating orientation task (GOT) is used to measure spatial discrimination. In this task, the fingertips of both hands are tested to identify the smallest grating ridge width for which orientation can be accurately distinguished. This task is sensitive to age and depends on lateral inhibition mediated by cortical GABAergic interneurons (Hicks & Dykes, 1983; Johnson, 2001). In patients with focal dystonia, the grating spatial period is longer than in healthy controls suggesting an increase in tactile sensory sensitivity (Molloy et al., 2003). This was also true in non-manifesting relatives of focal dystonia patients. However, in the same study, GOT was normal in patients with DYT1 dystonia, despite these patients displaying hand dystonia. Focal hand dystonia patients displayed normal GOT after administration of botulinum toxin (Walsh & Hutchinson, 2007), although these results have not been replicated (Conte et al., 2019). These results suggest that tactile sensory deficits are present as an endophenotype in focal dystonia. That GOT normalizes after botulinum toxin injections suggests that abnormalities of GOT may result from abnormal sensory feedback from the affected body part rather than be a precursor to disease development.

Increased somatosensory temporal discrimination has been identified as an endophenotype in focal and generalized idiopathic dystonias. The somatosensory temporal discrimination threshold (STDT) is the shortest interval between two tactile stimuli delivered to the same body part at which an individual can recognize the stimuli as temporally distinct (Conte et al., 2019). Studies using TMS demonstrated that the primary somatosensory cortex exhibits strong influence over STDT in healthy individuals (Rocchi et al., 2016). Patients with dystonia

have increased STDT both in affected and unaffected body parts. There is also evidence of increased STDT in family members of patients with cervical dystonia (Bradley et al., 2009; Fiorio et al., 2007; Kimmich et al., 2014). Interestingly, two studies have explored changes in STDT between different types of dystonia and found that there were no significant differences (Bradley et al., 2012; Scontrini et al., 2009). Together, these results provide strong evidence that abnormalities in STDT exist prior to disease manifestation and may be involved in dystonia pathogenesis.

Patients with dystonia display altered perception of arm movement during proprioceptive sensory processing. Proprioceptive processing can be measured from the tonic vibration reflex (TVR) elicited through vibration of muscles or tendons, which activates muscle spindles and γ-motor neurons. When the vibrated arm is immobilized, the vibratory stimulus evokes an illusion of movement, which is accompanied by activation of sensorimotor areas of the brain (Conte et al., 2019). Abnormal perception of movement is reported in patients with focal dystonias, most notably cervical dystonia (Grünewald et al., 1997; Yoneda et al., 2000). In some patients with cervical dystonia, vibration of the neck muscles induces dystonic postures (Karnath et al., 2000; Lekhel et al., 1997). In writer's cramp, a task-specific focal hand dystonia, TVR induced abnormal postures. However, injections of lidocaine into the dystonic muscles alleviated dystonic movements during TVR (Marinelli et al., 2011; Pelosin et al., 2009). Together, these results suggest that a failure to integrate proprioceptive information with motor output may result in dystonic movements. Despite significant evidence of sensorimotor abnormalities in dystonia, direct evidence for which of these is caused by or a result of dystonia is unknown.

Models of dystonia

Our ability to study the pathogenesis of dystonia in humans has both technical and ethical limitations. Humans are typically diagnosed after symptom onset, limiting our ability to study dystonia as it develops. It is therefore unclear if differences between dystonia patients and controls cause dystonia, result from dystonia, or result from its treatment. Additionally, data collection is generally limited to non-invasive techniques, such as transcranial magnetic stimulation (TMS) or neural imaging (e.g., fMRI and PET). TMS has low penetrance, addressing mostly questions of cortical physiology. Imaging techniques have spatiotemporal limitations, measuring not more than 1 cubic millimeter with a lag of 2 to 3 seconds. During deep brain stimulation (DBS) surgery, single neuron and local field potential (LFP) recordings are available. These surgeries are invasive and only performed when symptoms are severe and non-responsive to medical treatment. Recordings are only obtained from DBS target regions, generally GPi or STN, and patients may only perform limited tasks. Therefore, valid animal models are needed to study dystonia pathophysiology in detail.

Models of Task-Specific Dystonia. Without a clear genetic mutation or neuropathological signature to recapitulate, the development of experimental models to study task-specific dystonia has been slow. Nonhuman primate models were developed using behavioral paradigms requiring repetitive tasks, such as grasping or unnatural hand positioning, which caused repetitive strain injury (Blake et al., 2002; Byl et al., 1996, 1997). Results from these models show that repetitive training can lead to abnormal movements reminiscent of dystonia, and demonstrate enlargement or overlapping of hand receptive fields in primary sensory cortex. This reorganization occurred in symptomatic but not asymptomatic monkeys, suggesting that reorganization of the sensory homunculus is a feature of dystonia. However, these tasks also caused peripheral tissue damage,

complicating interpretation of the results. A task with sufficient repetitive and alternating movements to degrade the topographical representations of the hand and/or degrade motor control without peripheral tissue damage could not be established in non-human primates (Byl, 2007). With the decline in nonhuman primate research and increased patient neuroimaging data, further work on this model was halted. A single rat model has been suggested to model dystonia through chronic repetitive strain injury induced by skilled reaching (Barbe et al., 2003; Barr & Barbe, 2002, 2004). However, these rats do not develop overt dystonic movements. Instead, they develop alternative reaching strategies, making relevance to task-specific dystonia unclear.

Models of DYT1 Dystonia. Our ability to study the pathogenesis of DYT1 dystonia is restricted to models that allow for genetic manipulation, such as Caenorhabditis elegans, drosophila, and rodent models. Although zebrafish have a DYT1 gene homologue, it is non-essential for early motor system development. This limits its use for studying human dystonia, as there is strong evidence for abnormal torsinA function during CNS development and maturation (Li et al., 2021).

Invertebrate models of dystonia are used to investigate questions of genetic and cellular dysfunction caused by mutated torsinA. There are several drosophila models that are used to explore torsinA gene regulation and function. Results from these studies demonstrate a role for torsinA in lipid metabolism. Caenorhabditis elegans are similarly used to explore torsinA function molecularly. Results from these studies demonstrate abnormal nuclear pore biology (VanGompel et al., 2015) that is conserved across species. However, the majority of these models do not produce abnormal behavioral results. Those that do, such as changes in locomotion in drosophila expressing ΔE torsinA, have no clear relationship to human dystonia.

Rodents can perform a wide variety of motor and behavioral tasks with parallels to human tasks, making them good models for studying dystonia. Early models of DYT1 dystonia relied on expression of human wildtype or ΔE TOR1A transgenes in rodents (hWT & hMut, respectively). The results from these studies vary considerably. Some identified nuclear envelope abnormalities and reduced motor learning on the accelerating rotarod task (Grundmann et al., 2007, 2012; Shashidharan et al., 2005), while others showed subtle differences in beam walking and gait abnormalities (Grundmann et al., 2012; Sharma et al., 2005; Zhao et al., 2008). These models presented both with (Grundmann et al., 2012; Shashidharan et al., 2005; Zhao et al., 2008) and without (Grundmann et al., 2007; Sharma et al., 2005) overt abnormal movements.

These inconsistencies in physiologic and behavioral data are likely due to varied transgene expression across studies, with many displaying very modest expression. This may be explained by the use of different promoters for transgene expression and could explain the nominal differences seen between groups on various beam walking and gait analyses (Shashidharan et al., 2005; Zhao et al., 2008). It is also likely that transgene insertion sites, copy number, and pattern of expression differ across these models. Additionally, behavioral experiments require large sample sizes due to intrinsic variability in many of these assays. The sample sizes in these studies were generally small with limited controls.

Mouse models with manipulations of endogenous mouse torsin1a (*tor1a*) have more consistent phenotypes. Global models either inactivating *tor1a* (*tor1a*$^{-/-}$) or in which the ΔE mutation has been introduced in the endogenous mouse *tor1a* gene (*tor1a*$^{-/\Delta E}$ & *tor1a*$^{\Delta E/\Delta E}$) exhibit perinatal lethality (Dang et al., 2005, 2006; Goodchild et al., 2005). These data support the ΔE mutation causes loss-of-function. Examination of CNS at embryonic day 18 (usually the final day of gestation) illustrated normal gross neural development in these mutants. However,

nuclear membrane abnormalities termed “blebs”, were identified (Goodchild et al., 2005). Severity and number of cells affected by blebbing increased in a rostral-caudal gradient throughout the central nervous system, suggesting development after neural migration but during neural maturation. Perinatal lethality of systemic models with inactivated *tor1a* prevented further behavioral characterization.

Heterozygous *tor1a* mice (*tor1a*$^{+/-}$) or ΔE knock-in mice (*tor1a*$^{+/\Delta E}$, mimicking the human genotype) have normal birth and survival rates, allowing for more detailed characterization. However, they exhibit no nuclear membrane abnormalities, overt motor abnormalities, or changes in motor learning (Dang et al., 2006; Goodchild et al., 2005; Tanabe et al., 2012). Subtle deficits in motor coordination have been identified, though (Song et al., 2012). In the absence of abnormal movements, *tor1a*$^{+/\Delta E}$ (*Dyt1*) mice provide a genetic model similar to asymptomatic carriers of the human DYT1 mutation. Indeed, these models display several abnormalities also seen in manifesting and non-manifesting DYT1 carriers, such as reduced striatal dopamine release (Song et al., 2012), reduced D2 receptor binding and protein levels (Bonsi et al., 2019; Dang et al., 2012), and abnormal striatal plasticity (Dang et al., 2012; Martella et al., 2014).

Conditional removal of torsinA has been used to overcome perinatal lethality in the global mutants. Mice in which torsinA function is impaired in precursor cells giving rise to multiple neuronal cell types display overt abnormal twisting movements (Liang et al., 2014b). These mice also exhibit abnormal concentration of torsinA in the NE of cells, which has been observed in primary fibroblasts from DYT1 patients. However, these mice also exhibited considerable neurodegeneration in sensorimotor structures, including cortex, GP, and deep

cerebellar nuclei. Although these brain structures have been identified as relevant to dystonia pathogenesis, there is only limited evidence for neurodegeneration in patients with dystonia.

Conditional removal of *tor1a* from cortex (*Dyt1* cKO) or striatum (*Dyt1* sKO) did not produce nuclear membrane abnormalities, overt abnormal movements, or differences of motor learning measured by the rotarod (Yokoi et al., 2008, 2011). However, *Dyt1* sKO mice had decreased D2 receptor binding despite no measurable changes in monoamine levels of cKO or sKO mice (Yokoi et al., 2011). Hyperactivity was identified in *Dyt1* cKO mice, but gait abnormalities were limited to male *Dyt1* cKO mice (Yokoi et al., 2008). Additionally, both *Dyt1* cKO and *Dyt1* sKO mice had an increase in slips on the beam-walking task, suggesting subtle motor coordination abnormalities. Conditional removal of *tor1a* from cerebellum produced cellular abnormalities in the granular cell layer and Purkinje cells in the cerebellum (Song et al., 2014), however no behavioral characterization was performed with these animals, making it difficult to compare with other models of DYT1 dystonia.

Models of DYT1 dystonia consistently display alterations in striatal physiology. For example, D2 receptors (D2Rs) are widely expressed in the striatum of control animals, yet transgenic hMut mice showed a ~30% reduction in receptor expression despite similar mRNA levels to control and hWT mice (Napolitano et al., 2010). Similarly, *Dyt1* mice display reduced D2R protein levels in western blots (Bonsi et al., 2019) and *Dyt1* sKO mice exhibit reduced radioligand binding to D2R in striatal membrane fractures (Yokoi et al., 2011). In WT mice, activation of D2Rs reduces the activity of cholinergic interneurons. In DYT1 rodent models with genotypes mimicking the human genotype, however, D2R activation led to an increase in cholinergic interneuron firing known as “paradoxical excitation” (Eskow Jaunarajs et al., 2019; Grundmann et al., 2012; Pisani et al., 2006; Sciamanna et al., 2011).

Cholinergic interneurons (ChIs) express high levels of dopamine D2Rs and M2/M4 muscarinic acetylcholine (mACh) autoreceptors. When activated, both of these receptor classes reduce tonic firing in ChIs and subsequent acetylcholine (ACh) release through shared coupling to $G_{i/o}$ signaling pathways (Eskow Jaunarajs et al., 2019; Scarduzio et al., 2017). Additionally, striatal cholinergic tone influences dopaminergic modulation of striatal ACh release, perhaps through functional interactions between D2R and M2/M4 mACh autoreceptors (Scarduzio et al., 2017; Sciamanna, Tassone, et al., 2012). Pharmacological blockade of M2/M4 mACh autoreceptors on ChIs of *Dyt1* mice prevents the paradoxical excitatory effect of D2R activation. Furthermore, in WT mice, increasing the extracellular acetylcholine level or directly activating mAChRs can reverse the polarity of D2R signaling, producing the paradoxical excitation seen in *Dyt1* mice (Scarduzio et al., 2017). These data suggest that abnormal striatal cholinergic tone, resulting in increased ChI firing, plays an important role in the pathophysiology of dystonia.

Several mouse models of dystonia were created based on the consistent findings of striatal cholinergic dysfunction to examine torsinA loss-of-function in specific neuron populations. D2Rs are expressed on several different types of neurons in the striatum. These include indirect pathway MSNs, striatal cholinergic interneurons, a subset of GABAergic interneurons, afferent glutamatergic neurons from cortex and dopaminergic neurons from midbrain. Mice with *tor1a* conditionally removed from D2R-expressing cells (d2KO mice) showed significant reduction of cholinergic neurons in the striatum. Testing on rotarod and beam walking revealed deficits in male d2KO mice, though no overt dystonic symptoms were observed (Yokoi et al., 2020). Cholinergic-specific *tor1a* knock-out (ChKO) mice recapitulated the paradoxical excitation seen in *Dyt1* mice as well as a loss of muscarinic M2/M4 receptor inhibitory function (Sciamanna, Hollis, et al., 2012). Although no overt dystonic symptoms were

observed, deficits in rotarod performance were identified in ChKO mice. In contrast to the d2KO mice, ChKO mice did not display selective neuronal loss of ChIs. More recently, the ChAT-CKO model was developed by conditionally removing torsinA from cholinergic neurons (Pappas et al., 2018). These mice displayed no overt abnormal movements but were tremulous and weak compared to controls. Incredibly, rotarod performance in these mice was normal, in contrast to earlier findings (Sciamanna, Hollis, et al., 2012). Similar to d2KO mice, there was a ~34% reduction in cholinergic interneurons in the dorsal striatum of ChAT-CKO mice compared to controls (Pappas et al., 2018). In both d2KO and ChAT-CKO mice, ChI cell loss was highly selective, suggesting a specific vulnerability to loss of torsinA function in this neuronal population. Differences between the two cholinergic specific models, ChKO and ChAT-CKO, may come from the different methods used to manipulate torsinA expression (Liang et al., 2014b; Yokoi et al., 2008).

In an effort to develop a model with a motor phenotype, torsinA was conditionally removed from progenitor cells giving rise to forebrain GABAergic and cholinergic neurons (Pappas et al., 2015). GABA is the main inhibitory transmitter in the brain, accounting for 10-20% of the total neuronal population. Therefore, selective removal of torsinA from forebrain GABAergic and cholinergic neurons was motivated by the loss of intracortical inhibition identified in human patients as well as cholinergic signaling abnormalities. Although Dlx-CKO mice are born without motor abnormalities, they develop abnormal hind limb clasping and truncal torsion during the tail suspension task as juveniles (postnatal day 14), recapitulating the abnormal movements that develop selectively when torsinA is removed during critical CNS developmental periods (Liang et al., 2014b). When treated with the antimuscarinic scopolamine, Dlx-CKO mice had a significant reduction in limb clasping and twisting, supporting the

predictive validity of this model for DYT1 dystonia. The appearance of these abnormal movements coincides with a selective loss of dorsal striatal cholinergic interneurons, as seen in other models of DYT1 dystonia (Yokoi et al., 2020; Pappas et al., 2018). Behavioral analysis revealed that Dlx-CKO mice did not display motor learning abnormalities on the rotarod but did display deficits of motor coordination in the grid hang test (Pappas et al., 2015). The circuit specificity as well as overt abnormal movements in Dlx-CKO mice make them a strong model to examine the physiologic path from torsinA loss of function to the development of overt abnormal movements.

Behavioral tasks in rodent models of dystonia

The narrow repertoire of rodent behavioral assays used to study genetic models of dystonia limits our ability to understand their translational value. The majority of experiments use rotarod, beam walking tests, and open field to establish behavioral abnormalities in dystonia models. Although these tasks provide simple ways to examine gross motor coordination and motor learning, the relevance of abnormalities in these tasks to human dystonia is unclear. Similarly, the disease relevance of abnormal limb clasping and truncal torsion identified in Dlx-CKO mice exclusively during tail suspension is unclear.

This ambiguity arises from the fact that the behavioral abnormalities identified in human dystonia are not analogous to behaviors tested in rodent models. For example, tasks requiring intensive basal ganglia and/or cerebellar involvement, such as sequence learning and motor adaptation, are associated with human dystonia. However, rotarod and balance beam can only assess gross motor abnormalities and do not require sequence learning or motor adaptation. The serial reaction time (SRT) task is an example of a rodent behavioral task with a direct analogue in humans. In humans, SRT tasks consist of implicit sequence learning during the repeated

presentation of uncued serial ordered stimuli. After participants are exposed the regularly repeating sequence several times, it is replaced with a random sequence. Learning is assessed by how closely participants follow the regular sequence despite presentation of a random sequence. The rodent analogue of this task uses nose-poke hole positioning to construct the repeated and random sequence. Both human and rodent SRT tasks are sensitive to basal ganglia lesions. Currently, no studies using the SRT task in mouse models of dystonia exist..

An additional limitation of behavior in mice is that many behaviors can be implemented without cortical control. For example, mouse grooming behaviors are unaffected by cortical lesions (Berridge, 1989). This makes interpreting loss of cortical inhibition difficult to assess in rodents. However, fine digit control in rodents is cortically dependent (Castro, 1972). Skilled reaching is a behavioral paradigm often used to assess dexterous behaviors in rodents (Basista & Yoshida, 2020). During this task, rodents are trained to reach for and grasp sugar pellets, requiring fine digit and multi-joint coordination. In an experiment using optogenetic inhibition in the skilled reaching task, cortical inhibition prevented initiation of reaching movements or froze execution (Guo et al., 2015). However, untrained forelimb movements were unaffected during cortical inhibition. These results provide strong evidence that skilled reaching depends on motor cortex. Skilled reaching is also sensitive to lesions in the basal ganglia (Lopez-Huerta et al., 2021) and is therefore a behavioral paradigm well suited for assessing motor abnormalities in models of dystonia.

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CHAPTER 2: Development of Task-Specific Abnormal Movements in a Mouse Model of Dystonia

Abstract

Task-specific dystonias are primary dystonias that occur selectively during the performance of highly trained and repetitive tasks. A signature feature of primary dystonia is that there are no other neurological symptoms or CNS damage, hindering the development of animal models to study disease pathogenesis. Genetic and extragenetic factors are believed to contribute to the development of task-specific dystonia, though the mechanisms remain unclear. To date, no genetic models of task-specific dystonia have been developed. The goal of this study is to address the lack of genetic models for task-specific dystonia by challenging the Dlx-CKO mouse model on a single-pellet skilled reaching task. Dlx-CKO mice lack torsinA in GABAergic and cholinergic forebrain neurons, two groups of neurons strongly implicated in dystonia pathophysiology. Dlx-CKO mice display no overt motor abnormalities at baseline, and have similar success rates on a single-pellet skilled reaching task compared to controls. However, Dlx-CKO mice developed stereotyped abnormal movements during skilled reaching that increased in frequency with training. Trihexyphenidyl, an anticholinergic medication used to treat dystonia in humans, reduced abnormal movements during skilled reaching. However, development of abnormal movements was not reproduced in a larger study using an automated skilled reaching task. Manual skilled reaching is the first task to provoke manifestation of abnormal movements without peripheral tissue injury and Dlx-CKO mice may provide a genetic model to examine the pathogenesis of task-specific dystonia.

Introduction

Primary dystonias are a group of movement disorders characterized by excessive muscle activation resulting in abnormal movements and postures. A defining feature of primary dystonia is that there are no other neurological symptoms or CNS damage. Task-specific dystonias are primary dystonias that occur selectively during the performance of highly trained and repetitive tasks (e.g., playing a piano). Initially, task-specific dystonias affect only one body part and are triggered by a specific task. Over time, they may extend to other tasks or previously unaffected body parts.

Both genetic and extragenetic factors are believed to contribute to task-specific dystonia. A positive family history of dystonia is reported in 10-25% of patients that develop task-specific dystonia (Schmidt et al., 2009; Stojanović et al., 1995; Waddy et al., 1991). Additionally, the *ARSG* gene, encoding arylsulfatase G, has been linked to susceptibility (Lohmann et al., 2014; Nibbeling et al., 2015). However, these account for only a small proportion of cases; no gene has been identified consistently across task-specific dystonia populations. Dystonia is more common with repetitive tasks that require high accuracy, speed, and dexterity, suggesting that task demands influence the development of task-specific dystonia (Altenmüller et al., 2014, 2015; Altenmüller & Jabusch, 2010). Additionally, fatigue or overuse and injury can be predisposing factors for the development of task-specific dystonia. It remains unclear how genetics and task parameters interact, leading to the development of dystonic movements.

Several pathophysiologic mechanisms have been proposed to explain task-specific dystonia, including loss of cortical inhibition, maladaptive plasticity, and aberrant sensorimotor integration. Studies using transcranial magnetic stimulation (TMS) have reported excess excitability, reduced intracortical inhibition, and increased intracortical facilitation in the motor

cortex of patients with task-specific dystonia (Hallett, 2004). These abnormalities have been attributed to altered signaling by GABAergic interneurons in the cortex (Gallea et al., 2018; Levy & Hallett, 2002). Maladaptive plasticity is supported by disorganized somatotopic representations of dystonic body parts, which may be expanded or overlap with adjacent body parts. Additionally, a tactile stimulation paradigm revealed abnormal brain activity in the somatosensory cortex (S1) during fMRI, providing support for abnormal sensorimotor integration (Kimmich et al., 2014). However, it remains unclear whether these mechanisms are a requirement for the development of dystonia or a result of the disease.

Without a clear genetic mutation or neuropathological signature to recapitulate, few animal models exist to study task-specific dystonia. Non-human primate models were developed by forced repetition of complex tasks, such as grasping or unnatural hand positioning. Repetitive training on such tasks can lead to the development of dystonic-like movements and abnormal motor and sensory representations in the cortex (Blake et al., 2002; Byl et al., 1996, 1997). However, these tasks also caused peripheral tissue damage, complicating their interpretation. A task with sufficient repetitive movements to degrade the topographical representations of the hand and/or degrade motor control without peripheral tissue damage could not be established in non-human primates (Byl, 2007). Similarly, a rodent model of repetitive strain injury developed dystonic-like movements during a skilled reaching task (Barbe et al., 2003). However, there was no further investigation into this model. To date, there are no genetic models of task-specific dystonia.

The recently developed Dlx-CKO mouse model of dystonia is one of a few genetic models of dystonia that develops abnormal movements. Mutations in torsinA are responsible for DYT1 dystonia, the most common genetic form of dystonia. Dlx-CKO mice lack torsinA in

GABAergic and cholinergic forebrain neurons. This genetic manipulation was inspired by changes in intracortical inhibition and the implication of cholinergic dysfunction in dystonia. Dlx-CKO mice display no overt motor abnormalities at baseline. However, in adolescence (postnatal day 14) they develop limb clasping and truncal torsion during tail suspension. The development of limb clasping correlates with the highly selective loss of dorsal striatal cholinergic interneurons, which are involved in corticostriatal plasticity.

In these experiments, we address the lack of genetic models for task-specific dystonia by challenging the Dlx-CKO mouse model of dystonia on a single-pellet skilled reaching task. Rodent skilled reaching is one of the most common paradigms for studying dexterous motor skill. Mice are not naturally adept at skilled reaching and must learn coordinated forelimb and digit movements to reach for, grasp and consume sugar pellets. Skilled reaching requires coordinated multi-joint and digit movements for successful pellet retrieval, mimicking the demands of tasks that commonly induce task-specific dystonia in humans. Efficient skilled reaching also requires an intact motor cortex. Our results suggest that repetitive task performance leads to task-specific abnormal movements in Dlx-CKO, but not control, mice. We also show that abnormal movements decline after administration of the anticholinergic trihexyphenidyl, providing support for the predictive validity of Dlx-CKO mice for the study of task-specific dystonia. Finally, we attempted to replicate these findings using an automated skilled reaching task. We were unable to replicate the development of abnormal movements in this task, likely due to changes in task demands.

Results

We tested control and Dlx-CKO mice on a manual single-pellet skilled reaching task to challenge the motor system with a cortically-dependent task. Each mouse underwent 25 sessions

of skilled reaching. Trials started with the experimenter placing a pellet on a shelf (Figure 2-1A). Mice could make multiple reaches until the pellet was obtained or knocked from the shelf. New trials were initiated after the mouse removed the pellet from the shelf and stopped reaching.

Dlx-CKO mice display normal motor learning in the manual skilled reaching task. The single-pellet skilled reaching task was used to assess motor learning and dexterous skill performance. Skilled reaching is a cortically-dependent task in which mice are trained to reach for and grasp small sugar pellets from a shelf (Figure 2-1A). Successful pellet retrieval requires accurate paw transport to the pellet followed by precise, coordinated hand and digit movements that are acquired with practice. Learning is assessed by changes in the number of successfully retrieved pellets divided by the total number of trials (success rate). Dlx-CKO mice displayed no difference in success rate from controls at any point in the first 10 days of training (Figure 2-1B). This is consistent with previous results, where Dlx-CKO mice displayed no deficits of motor learning on the rotarod task.

Dlx-CKO mice develop abnormal movements during skilled reaching. In humans, task-specific dystonia develops after repeated training on highly dexterous tasks. Therefore, mice were observed throughout the skilled reaching training period for the appearance of abnormal movements. Stereotyped abnormal movements were observed in Dlx-CKO mice during training and involved both forelimbs moving laterally from midline repeatedly (Figure 2-2A). After 7 days of training, 72% of Dlx-CKO mice developed stereotyped abnormal movements compared to 0% of control mice (Figure 2-2B). These movements were most frequently observed immediately prior to or following an apparently normal reach in Dlx-CKO mice. They were not observed in the home cage or after training on the rotarod task (Pappas et al., 2015). Additionally, the number of trials with abnormal movements increased with training (Figure 2-

2C). These data suggest that dexterous skill repetition leads to the development and worsening of task-specific abnormal movements in Dlx-CKO mice, mirroring human task-specific dystonia.

Anticholinergic treatment ameliorates stereotyped abnormal movement in Dlx-CKO mice. Chronic anticholinergic administrated is a common therapy for DYT1 dystonia. Mice that developed stereotyped abnormal movements during the initial experiment were treated with the antimuscarinic agent trihexyphenidyl (THP) prior to further training on the skilled reaching task (Figure 2-3). THP administration prior to skilled reaching reduced the percent of reaching trials with abnormal movements in Dlx-CKO mice from 13% to 4%. These results support the predictive validity of Dlx-CKO mice for the study of task-specific dystonia.

Stereotyped abnormal movements were not reproduced in an automated skilled reaching task. We attempted to replicate the development of abnormal movements in skilled reaching in an automated version of the task. Automated skilled reaching tasks reduces the variability introduced from experimenters performing the training or differences in attention to training by the same experimenter across sessions. In the automated task, an actuator raises a sugar pellet to reaching height during a trial and lowers, obtaining a new pellet, between trials. Although abnormal movements were present throughout training, they were present in both Dlx-CKO and control mice (Figure 2-4). There was no statistically significant difference between genotypes or across sessions in these animals. Thus, automated skilled reaching failed to replicate the genotype specific abnormal movements identified in the manual skilled reaching task.

Discussion

Our results suggest that Dlx-CKO mice develop stereotyped task-specific abnormal movements that worsen with training under specific task requirements. . We used a version of the single-pellet skilled reaching in which pellets are manually placed on a shelf task to assess

motor learning and dexterous skill performance. There were no differences in motor learning between Dlx-CKO and control mice. However, Dlx-CKO mice developed stereotyped abnormal movements during skilled reaching that increased in frequency with repeated training. Administration of trihexyphenidyl, an anticholinergic, reduced these abnormal movements. These results suggest that Dlx-CKO mice develop task-specific abnormal movements with repeated training in a dexterous task, which may serve as a useful model to study the pathophysiology of task-specific dystonia. However, this result was not reproduced in a larger study using an automated skilled reaching task.

The discrepancy between manual and automated skilled reaching may be a result of task design. In the manual skilled reaching task, mice reached to obtain sugar pellets from a shelf rather than the small pedestal used in the automated task. During the automated task, Dlx-CKO mice knocked the pellet from the pedestal less frequently. Further examination revealed Dlx-CKO mice often miss the pellet entirely during the automated task, suggesting less pellet contact. It is possible that mice trained on the more difficult automated skilled reaching task did not receive enough sensory feedback via pellet contact to induce dystonic-like movements. Experiments designed to test how tactile sensory feedback differs in Dlx-CKO mice compared to controls may elucidate how changes in task demands affect manifestation of abnormal movements. One possible experimental design would be using a pasta reaching matrix, where mice would be able to reach for pasta of different thicknesses and oriented vertically or horizontally, forcing mice to adapt their reach pattern to obtain the pasta. Previous results using this task have found that rats adjust their paw movements using haptic information, suggesting a role for sensorimotor integration (Ballermann et al., 2000).

It is also possible that implementation of the manual task was flawed, with variability introduced by the experimenter. This variability could have been introduced during training or video review, where all videos were reviewed by a single reviewer. Additionally, our skilled reaching task allowed unrestricted movement, complicating experimenter identification of abnormal movements. When training reviewers, we often had to train them to recognize both mouse grooming and abnormal movements, as these two behaviors appear similar. This raises two possibilities: 1) the abnormal movement identified during manual skilled reaching was actually a variant of grooming, or 2) grooming behaviors masked identification of abnormal movements during our automated skilled reaching task. Further experiments are required to establish whether skilled reaching can lead to the development of task-specific abnormal movements. Using a head-fixed apparatus, limiting unrelated motor activity, will likely improve experimenters' ability to identify and characterize any abnormal movements.

In summary, Dlx-CKO mice displayed task-specific abnormal movements during a manual skilled reaching task. Administration of antimuscarinics, used to treat human dystonia, resulted in a reduction of abnormal movements. However, these task-specific abnormal movements were not replicated when Dlx-CKO mice were tested on an automated task, possibly due to changes in task demands. Replication of these results is warranted with larger sample sizes. This is the first model of task-specific dystonia to display abnormal movements without peripheral tissue injury and may provide a genetic model to examine the pathogenesis of task-specific dystonia.

Methods

Mice. All animal procedures were approved by the University of Michigan Institutional Animal Care and use Committee. Numbers of mice included in each experimental group and

analysis are indicated in figure legends. Dlx-CKO mice were generated by crossing Cre^{+} $tor1a^{+/-}$ (Pappas et al., 2015) with $tor1a^{flx/flx}$ mice (Liang et al., 2014), using the breeding strategy described in (Pappas et al., 2015). Mice with genotype $tor1a^{flx/+}$ were used as age and sex matched littermate controls. Male and female (control=18, Dlx-CKO=10) mice were housed in groups of 1-5. All testing was carried out during the dark phase of a normal light/dark cycle. Food restriction was imposed on all animals during the training and testing periods of the skilled reaching and pasta handling tasks for no more than 6 days in a row such that animals' weights were maintained ≥90% of their free-feeding weight. Water was available ad libitum in their home cages. Mice displaying seizures were excluded from experiments (fewer than 1 in 10 mice).

Skilled reaching. Manual Skilled Reaching. Training and testing were carried out in custom-built skilled reaching chambers built according to (Farr & Whishaw, 2002). A reaching slot (10 mm x 7 cm) was cut into the front panel of the chamber 17.5 mm from the floor. A shelf was attached to the front of the chamber with two divots, 1 cm from the front of the chamber and in line with the left and right edge of the reaching slot. Sugar pellets were placed on the divot contralateral to each mouse's paw preference. Videos of each session were recorded at 100 frames-per-second and 1920 x 1080 pixels by a high-definition color digital camera (HBLK-6FT-0309, Panasonic, Kadoma, Japan).

Habituation. The purpose of habituation is to familiarize mice with the reaching chamber and sucrose reward pellets. Habituation lasted for three sessions, each 10 minutes in length. Mice were placed on food restriction and introduced to the sucrose reward pellets in their home cages 24 hours prior to the first day of habituation. On day 1 of habituation, a pile of 10 pellets was

placed in the skilled reaching chamber to encourage exploration. On days 2 and 3 of habituation, mice received 3 sucrose pellets placed at the front of the chamber.

Pre-training. During 'pre-training', mice were evaluated for reaching paw-preference and trained to reach for the sugar pellet. Paw preferencing and training mice to reach for the pellet was performed as in (Bova et al., 2020). Once mice reached for the sugar pellet with their preferred paw 10 times without being baited by the experimenter, they began training. Videos of each session were captured.

Training. After pre-training, mice were trained 5 days per week for a total of 25 sessions. Each session continued until mice successfully obtained 30 pellets or 20 minutes elapsed, whichever came first. Videos were captured of the entire training session.

Automated Skilled Reaching. Training and testing were carried out in the same chambers as in the manual skilled reaching task. Two mirrors were placed on either side of the front of the reaching chamber and angled to allow side views of the paw during reaching. A linear actuator with potentiometer feedback (Actuonix, Saanichton, Canada) was connected to an acrylic pellet delivery rod and mounted in a custom frame below the support box. The pellet delivery rod extended through a funnel mounted to the top of the frame. Before each session, the actuator was positioned 1 cm from the front of the reaching slot so that the delivery rod was aligned with the right or left edge of the slot according to each mouse's paw preference. Videos of the entire session were recorded by a camera mounted in front of the reaching slot.

Trial performance. A custom-built Arduino (Arduino Mega 2560 Rev3, Arduino, Boston, MA) based system controlled the experiment. Each training session began with the pellet delivery rod at the lowest position inside the funnel. When a session began, the pellet delivery rod rose to the bottom of the reaching slot, triggering an LED to indicate the start of a trial. The

delivery rod remained in place for 3 seconds before lowering, triggering the LED to turn off. This began an intertrial interval of ~5 seconds wherein the pellet delivery rod retracted into the funnel to pick up a new pellet, then rose for a new trial.

Habituation. The purpose of habituation is to familiarize mice with the reaching chamber and sucrose reward pellets. Habituation lasted for three sessions, each 20 minutes in length. Mice were placed on food restriction and introduced to the sucrose reward pellets in their home cages 24 hours prior to the first day of habituation. On day 1 of habituation, a pile of 10 pellets was placed in the skilled reaching chamber to encourage exploration. On days 2 and 3 of habituation, mice received no sucrose pellets, and the skilled reaching apparatus was turned on for 5 minutes in each session to familiarize mice with the sound.

Pre-training. During 'pre-training', mice were evaluated for reaching paw-preference and trained to reach for the linear actuator. Paw preference and training mice to reach for the linear actuator was performed as in (Bova et al., 2020). Once mice reached for the delivery rod 10 times without being baited by the experimenter, they began training on the automated task. Experimenters switched on the actuator in a "manual" mode, allowing them to control trial length (the duration the actuator is in "reaching position" at the bottom of the slot). Experimenters slowly decreased trial length to 3 seconds, at which point the actuator was turned to "automatic". Pre-training was complete once mice performed 20 trials in a single session (30 minutes) with the actuator on "automatic". During the "automatic" mode, the delivery rod travels through a funnel of sugar pellets, picking one up, and continues to extend until the pellet is level with the reaching slot. The delivery rod remains extended for 3 seconds, then beings retracting into the funnel to start a new trial. ".

Training. After pre-training, mice began 30-min training sessions with the automated system. Mice were trained for 5 days per week for a total of 21 sessions. Videos were captured of the entire training session.

Skilled reaching analysis. Skilled reaching videos were reviewed for the first 10 sessions with reviewers blinded to the mouse's genotype and day of testing. Reach outcome for each trial was scored by visual inspection as follows: 0 – no pellet presented or other mechanical failure; 1-first attempt success (obtained pellet on initial limb advance); 2 – success (obtained pellet, but not on first attempt); 3 – forelimb advanced, pellet was grasped then dropped in the box; 4 – forelimb advance, but the pellet was knocked off the pedestal ('pellet displaced'); 5 – the mouse reached but the pellet remained on the pedestal ('pellet remained'); 6 – pellet was obtained using its tongue; 7 – the mouse did not perform any reaches; 8 – the mouse used its non-preferred paw to reach; 9 – obtained pellet with use of both paw and tongue. Outcome percentages were calculated by dividing the number of trials of each outcome by the total number of trials per session. Success rate was calculated for each session by dividing the total number of scores 1 and 2 by the total number of trials (sum of scores 1, 2, 3, 4, and 5) (Figure 2-1B).

In addition to reach scores, trials received a score of 0 or 1 for the absence or presence of observed abnormal movements, respectively. Mice that displayed two or more abnormal movements on consecutive training days were considered to have developed abnormal movements (Figure 2-2A).

Administration of Trihexyphenidyl. After completing 25 days of training on the manual skilled reaching task, mice that developed stereotyped abnormal movements were treated with the antimuscarinic, trihexyphenidyl (THP), and trained for an additional 5 days on the manual

task. Mice were administered 0.5 mg/ml THP at doses of 10 mg/kg each day 30 minutes prior to training on the manual skilled reaching task.

Statistics. A linear mixed-effects model (using R *lmer*) was used to evaluate skilled reaching success rates. Random intercepts/effects for each mouse were included, where effect of session varied between mice, and main interaction effects of genotype and session number were evaluated. The proportion of mice affected by abnormal movements was tested using a chi-square test to identify differences between genotypes. A Poisson regression model was implemented (using R *lmer*) to identify differences in abnormal movements (offset by number of trials) in the automated skilled reaching tasks due to the count nature of the data. To test whether THP had an effect on the number of abnormal movements, a Welch's two sample t-test (using R *t.test*) was used to compare the proportion of trials with abnormal movements on the last 5 days of training against 5 days with THP administration.

Figures

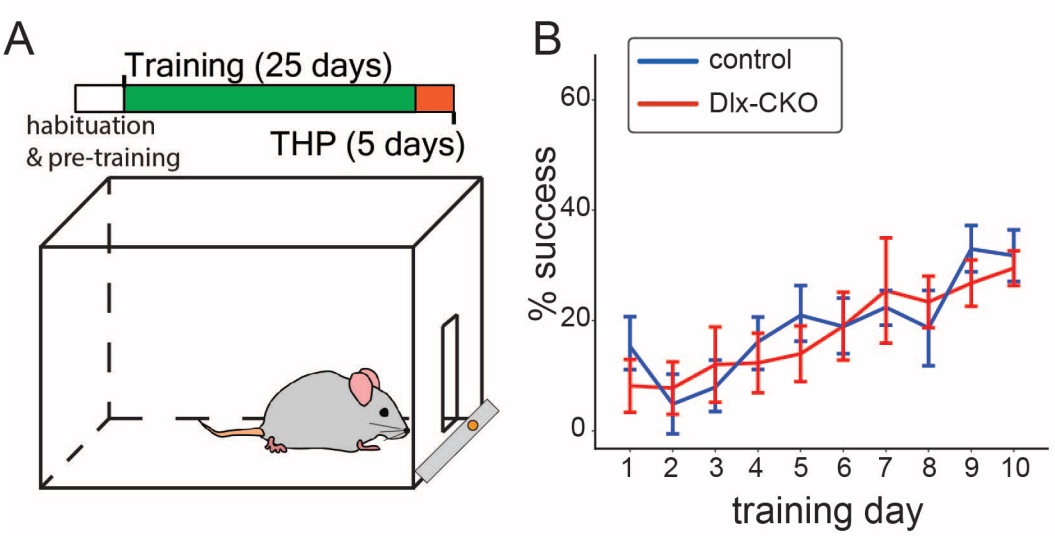

Figure 2-1: Dlx-CKO mice have normal motor learning in a manual skilled reaching task

A) Top: Behavioral timeline for experiments. Bottom: Behavioral chamber used in manual skilled reaching. B) Average "any attempt" success rate. Linear mixed-effects model: effect of genotype: $p>0.94$. (control: n=4, Dlx-CKO: n=6).

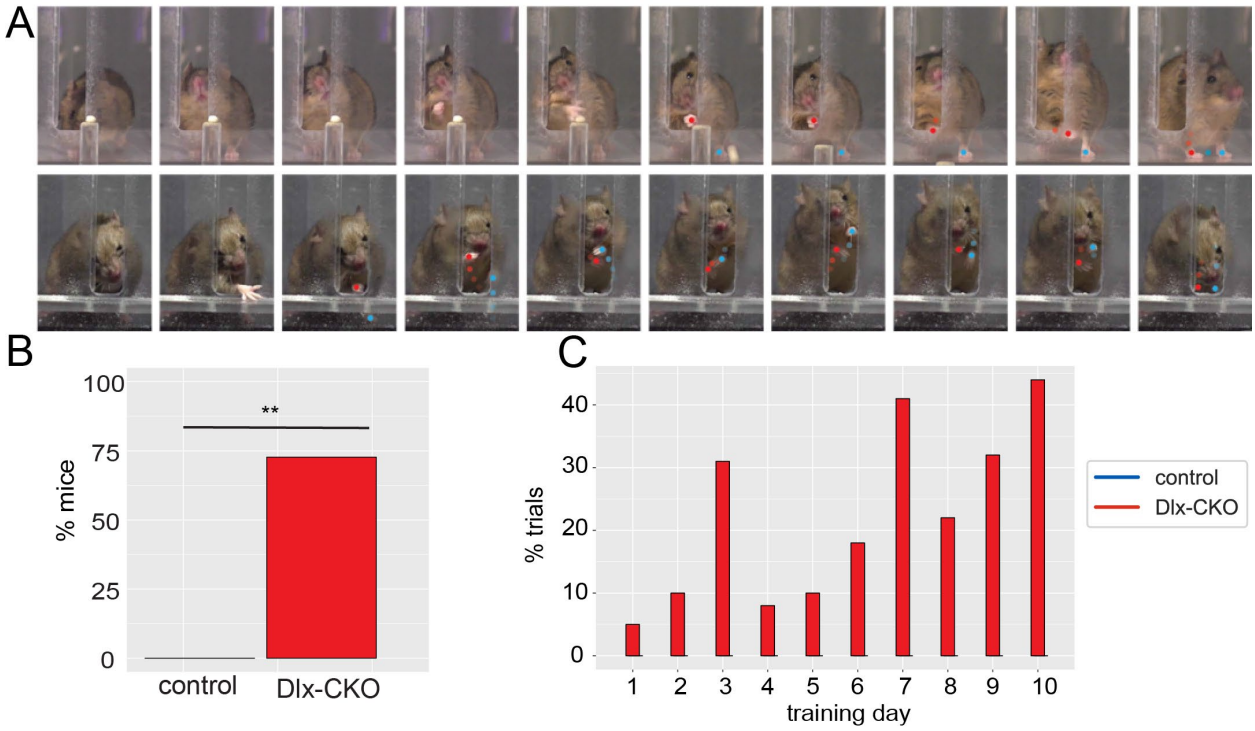

Figure 2-2: Dlx-CKO mice develop abnormal movements

A) Video stills demonstrating normal reaching behavior (top) and the abnormal movement (bottom). Top: A control mouse performs a reach with a normal movement sequence. Bottom: A Dlx-CKO mouse performs a reach followed by the abnormal movement. The dots represent paw movement between stills, showing the location of abnormal movements in front of the torso compared to normal reaching. Red dots correspond with preferred paw movements and blue dots with non-preferred paw movements. B) The proportion of animals in each genotype that developed the abnormal movement. C) The proportion of trials where abnormal movements were observed. Chi-squared test, $p<0.002$. (control: n=4, Dlx-CKO: n=6).

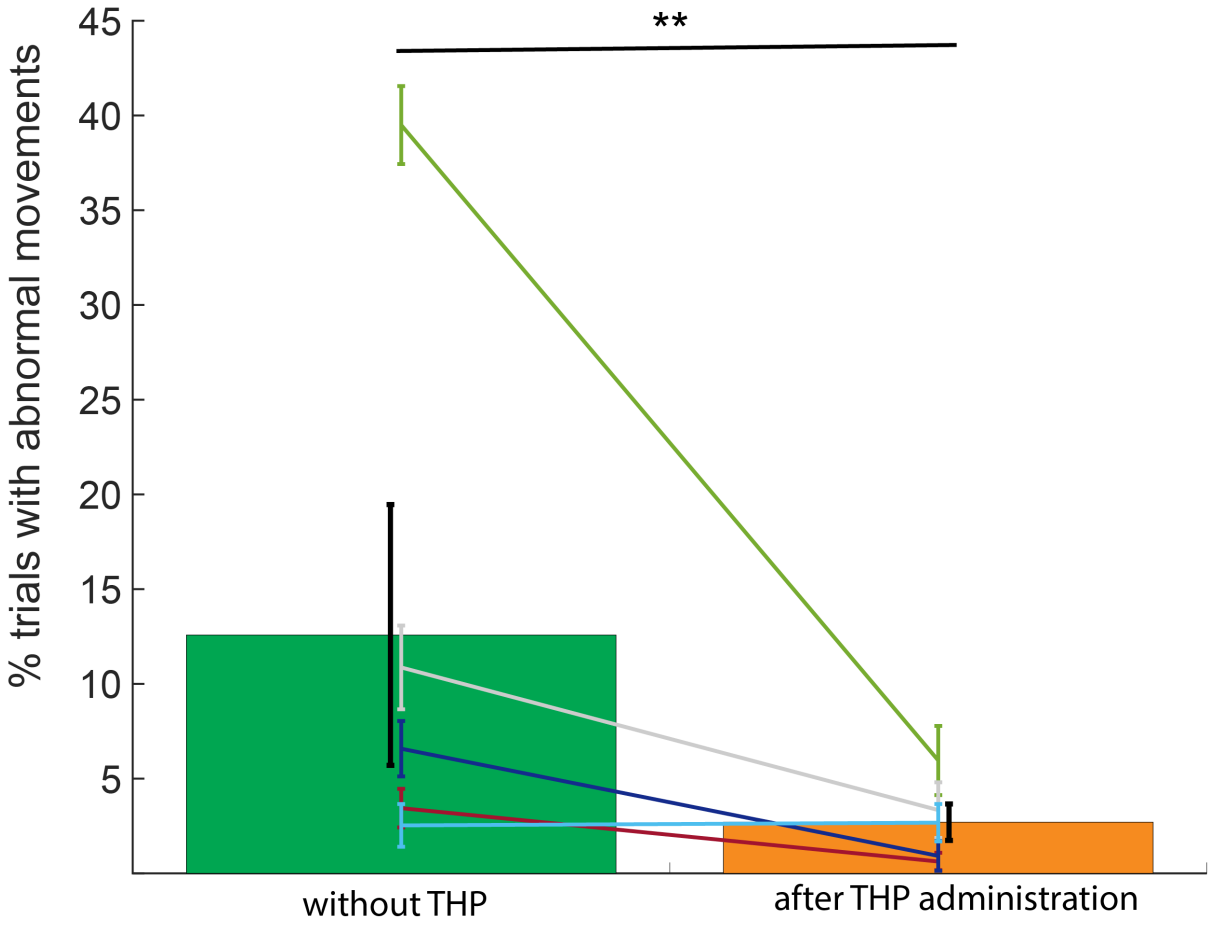

Figure 2-3: Abnormal movements are reduced after administration of THP

Left: Mean percent of trials on which Dlx-CKO mice (n=5) performed an abnormal movement. Right: Mean percent of trials on which the same mice performed an abnormal movement after administration of trihexyphenidyl (THP). Lines represent the mean for individual mice, indicate by color, prior to versus after THP administration. Welch's two sample t-test: $p<0.004$.

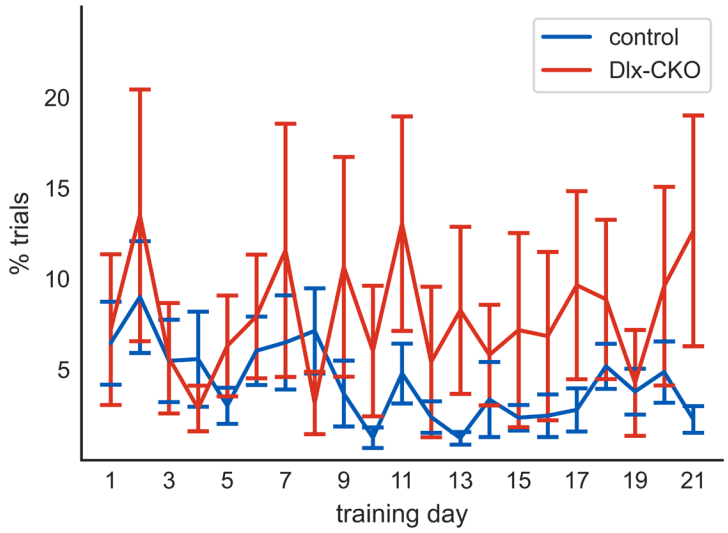

Figure 2-4: Abnormal movements in Dlx-CKO mice did not replicate in an automated skilled reaching task

Fraction of trials in which abnormal movements were observed during the automated skilled reaching task. Linear mixed-effects model: effect of genotype: $F_{1,21}$=1.35, p=0.19; effect of session: $F_{2,20}$=-1.79, p=0.09; interaction between genotype and session: $F_{2,20}$=1.67, p=0.11. (control: n=14, Dlx-CKO: n=9).

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CHAPTER 3: A Dystonia Mouse Model with Motor and Sequencing Deficits Paralleling Human Disease

Co-authored by Allison M. Bakerian, Allison Cropsey, William T. Dauer, and Daniel K. Leventhal

Abstract

The dystonias are a group of movement disorders characterized by involuntary twisting movements and postures. A lack of well characterized behavioral models of dystonia has impeded identification of circuit abnormalities giving rise to the disease. Most mouse behavioral assays are implemented independently of cortex, but cortical dysfunction is implicated in human dystonia. It is therefore important to identify dystonia models in which motor cortex-dependent behaviors are altered in ways relevant to human disease. The goal of this study was to characterize cortically-dependent behaviors in the recently-developed Dlx-CKO mouse model of DYT1 dystonia. Mice performed three behavioral tasks: skilled reaching, pasta handling, and water-elicited grooming. These tests assess, respectively, motor learning, dexterous skill, and innate motor sequencing. Furthermore, the first two tasks depend strongly on motor cortex, while dorsal striatum is critical for normal grooming. Dlx-CKO mice exhibited significantly lower success rates and pellet contacts compared to control mice on skilled reaching and dropped the pasta more frequently during pasta handling. Despite the skilled reaching impairments, Dlx-CKO

mice were capable of learning; with training, they more consistently contacted the target. Grooming patterns of Dlx-CKO mice are more disorganized than in control mice, as evidenced by a higher proportion of non-chain grooming. However, when Dlx-CKO mice engage in syntactic chains, they execute them similarly to control mice. These abnormalities may provide targets for preclinical intervention trials, as well as facilitate determination of the physiologic path from torsinA dysfunction to motor phenotype.

Introduction

The dystonias are an often-disabling group of movement disorders characterized by involuntary twisting movements and postures. A defining feature of primary dystonia is that there are no other neurological symptoms or CNS damage, making it difficult to model in animals. The discovery of specific gene mutations that cause primary dystonia allowed the creation of animal models with high "construct" validity – that is, they closely recapitulate the human genotype. For example, a 3-base pair in-frame deletion ("ΔE") mutation in torsinA causes autosomal dominant early-onset generalized torsion dystonia (Ozelius et al., 1997). However, heterozygous torsinA mice (*tor1a*$^{+/-}$) or ΔE knock-in mice (*tor1a*$^{+/\Delta E}$, mimicking the human genotype) do not exhibit a motor phenotype (Tanabe et al., 2012). Mice in which torsinA is globally deleted (*tor1a*$^{-/-}$) or in which the ΔE mutation has been introduced in the endogenous mouse torsinA gene (*tor1a*$^{-/\Delta E}$ & *tor1a*$^{\Delta E/\Delta E}$) all exhibit perinatal lethality and characteristic subcellular nuclear membrane abnormalities (Dang et al., 2005, 2012; Goodchild et al., 2005). These data demonstrate that the ΔE mutation impairs torsinA function, supporting the use of torsinA loss-of-function models for DYT1 dystonia.

DYT1 mouse models with cell-type or region specific torsinA deletion are viable and exhibit specific motor abnormalities. Mice with torsinA conditionally removed from forebrain

cholinergic and GABAergic neurons ("Dlx-CKO" mice) are born with no apparent motor abnormalities but as juveniles (post-natal day 14) develop abnormal limb clasping during tail suspension. The onset of these movements corresponds with the selective loss of striatal cholinergic interneurons (ChIs) in the dorsal striatum (Pappas et al., 2015). The relationship between hindlimb clasping and dystonia in humans remains unclear, however.

One way to improve the translational value of dystonia models is to expand the repertoire of preclinical assays. Human dystonia is often induced or exacerbated by voluntary movement, perhaps due to "overflow" in sensorimotor cortical regions from desired activation patterns. Cortical inhibition is reduced in humans with dystonia, suggesting that this abnormality may contribute to recruitment of topographically adjacent motor cortical regions during movement (Quartarone et al., 2003; Sohn & Hallett, 2004). These data indicate a central role for cortical dysfunction in dystonia, but most behavioral assays in mice are implemented independently of cortex (Basista & Yoshida, 2020; Kawai et al., 2015). For example, simple lever press tasks and rodent grooming are not affected by cortical lesions (Berridge & Whishaw, 1992; Kawai et al., 2015). On the other hand, dexterous skills requiring finely controlled multi-joint and digit coordination are highly sensitive to cortical lesions (Basista & Yoshida, 2020; Lemke et al., 2019).

Given the cortical abnormalities observed in humans with dystonia, we explored the ability of Dlx-CKO mice to perform cortically-dependent motor tasks. We tested Dlx-CKO mice on three tasks: (1) skilled reaching, (2) pasta handling, and (3) water-elicited grooming. The skilled reaching and pasta handling tasks are both sensitive to cortical lesions (Allred et al., 2008; Lemke et al., 2019; Whishaw & Coles, 1996). However, while skilled reaching requires mice to learn coordinated forelimb and digit movements to reach for, grasp, and consume sugar

pellets, the pasta handling task requires minimal training (Allred et al., 2008). This enables examination of cortically-dependent motor function with and without motor learning requirements. The water-elicited grooming task is a reliable way to induce a highly stereotyped behavior in mice (Kalueff et al., 2007, 2016). Because of its high stereotypy, grooming is sensitive to a variety of experimental manipulations and provides a way to examine "innate" motor sequencing independently of motor cortical function (Berridge & Whishaw, 1992). This battery of tests therefore assesses motor learning, dexterous skill, and innate motor sequencing.

Results

We tested control and Dlx-CKO mice on three tasks designed to assess motor learning (skilled reaching), dexterous skill (skilled reaching and pasta handling), and innate motor sequencing (grooming). Each mouse underwent the same sequence of behavioral assays (Figure 3-1A, Top): three sessions of induced grooming, 21 sessions of skilled reaching, an additional session of induced grooming, and finally 3 sessions of pasta handling.

Skilled reaching deficits suggest a primary motor impairment in Dlx-CKO mice. The single-pellet skilled reaching task was used to examine motor learning as well as dexterous skill performance. Skilled reaching is a cortically-dependent task in which mice are trained to reach for and grasp small sugar pellets from a pedestal (Figure 3-1A, Bottom). Successful pellet retrieval requires accurate paw transport to the pellet followed by precise, coordinated hand and digit movements that are acquired with practice (Lemke et al., 2019). Dlx-CKO mice were consistently less successful obtaining sugar pellets than controls, suggesting a deficit in motor execution, motor learning, or both (Figure 3-1B).

Learning is typically assessed by changes in the number of successfully retrieved pellets divided by the total number of attempts (success rate). However, success rates did not change

with training in our task (Figure 3-1B). This is likely because our skilled reaching task is more difficult than others in which the pellet can slide along a shelf instead of falling off a pedestal. To assess learning in greater detail, we examined the frequency of two distinct failure mechanisms. The first is trials in which the mouse knocked the pellet off the pedestal (Figure 3-1C, Top: "pellet displaced"). This is in contrast to trials in which the mouse makes little or no contact with the pellet, failing to knock it from the pedestal (Figure 3-1C, Bottom: "pellet remains"). As training progressed, Dlx-CKO mice exhibited a lower proportion of "pellet displaced" trials and a higher proportion of "pellet remains" trials (Figure 3-1D). Although there was no statistically significant effect of training day for these data, differences between controls and Dlx-CKO mice narrowed considerably by the last session; this suggests that Dlx-CKO mice are capable of motor learning. The inability to accurately target the pellet suggests that Dlx-CKO mice have a primary impairment in postural control and/or proximal limb movement that interferes with the "transport" phase of skilled reaching (Ueno et al., 2018).

Dlx-CKO mice show deficits of dexterity in pasta handling. During pasta handling, mice hold and manipulate small pieces of pasta for consumption. Similar to skilled reaching, pasta handling requires dexterous motor control and an intact motor cortex (Allred et al., 2008; Whishaw & Coles, 1996). There are two key features of pasta handling that distinguish it from skilled reaching. First, while mice qualitatively improve their pasta handling with repetition, it requires much less practice than skilled reaching. In addition, there is no barrier between the mice and the pasta, reducing the need for accurate proximal limb control. Thus, pasta handling examines fine digit control more so than proximal forelimb control and motor learning.

Dlx-CKO mice dropped the pasta significantly more frequently than control mice, suggesting a primary deficit in fine digit control (Figure 3-2C). Brief pauses during pasta

consumption are common in wildtype mice, and it is during these pauses that Dlx-CKO mice tended to drop the pellets. To further explore failure mechanisms in pasta handling, we looked for qualitative differences in pasta handling that have been associated with specific brain lesions (Whishaw & Coles, 1996). Typically, mice hold the pasta with both paws. A paw near the mouth guides the pasta into the mouth (guide paw), while the other grasps the pasta slightly distal to the guide paw (grasp paw) (Figure 3-2A, Left). As the pasta decreases in length, mice tend to hold the paws symmetrically about the pasta in front of the mouth (Figure 3-2B, Right). Atypical pasta handling behaviors include: any forepaw no contact; pasta long, paws together (Figure 3-2B, Left); pasta short, paws apart (Figure 3-2B, Right); guide/grasp switch; and drops (see descriptions of these patterns in *Pasta handling analysis*) (Whishaw et al., 1997, 1998; Whishaw & Coles, 1996). Specific atypical handling patterns are associated with specific cortical lesions (Allred et al., 2008; Whishaw et al., 1997). However, control and Dlx-CKO mice exhibited similar frequencies of atypical handling patterns (Figure 3-2B). We are therefore unable to localize abnormalities that cause impaired manual dexterity in Dlx-CKO mice.

Grooming sequences are disrupted in Dlx-CKO mice. Skilled reaching and pasta handling are sensitive to cortical lesions but may also be impaired with subcortical lesions. We therefore also characterized grooming behavior, which is insensitive to cortical lesions but sensitive to striatal lesions (Berridge & Whishaw, 1992). Grooming is an innate behavior with a patterned, sequential organization that starts at the nose and progresses across the body in a cephalocaudal pattern (Berridge & Fentress, 1987). About 10-15% of grooming is composed of highly stereotyped and ordered movements called a syntactic chain (Kalueff et al., 2007). Syntactic chains have distinct phases that follow an expected progression from 1 to 4 and are usually embedded in more flexible non-chain grooming bouts (Figure 3-3). Adherence to chain

syntax is low in models with altered cerebellar and striatal physiology, with shorter more frequent non-chain bouts being common (Berridge, 1989; Cromwell & Berridge, 1996).

We performed grooming assessments before and after skilled reaching training to test whether skill learning would affect grooming performance. However, there was no effect of session (pre-skilled reaching versus post-skilled reaching) on grooming outcomes between groups (see *2.9. Statistics*). Therefore, all analyses are collapsed across sessions. Dlx-CKO and control mice performed similar amounts of grooming, but grooming structure was less organized in Dlx-CKO mice. There was not a significant difference in the time spent grooming (in total or separated into chain and non-chain grooming) or total number of bouts initiated between groups (Figs. 4A,B). However, Dlx-CKO mice initiated fewer syntactic chains, defined as phase 1 grooming that progresses to phase 2 or 3 (Cromwell & Berridge, 1996) (Figure 3-4B). Dlx-CKO mice performed similar total (chain plus non-chain) amounts of unilateral and bilateral strokes compared to control mice, but fewer ellipses (Figure 3-4F). Consistent with previous results, neither control nor Dlx-CKO mice performed multiple, fast elliptical strokes outside of syntactic grooming (Cromwell & Berridge, 1996). Thus, Dlx-CKO mice progressed normally through syntactic chains once they were initiated. (Figures 3-4E and 3-5). Finally, there was no effect of genotype on the duration of non-chain or chain grooming bouts (linear regression, effect of genotype on duration of: non-chain bouts: $t(12)=0.029$, $p=0.98$; syntactic chains: $t(12)=-1.44$, $p=0.18$). The distributions of the durations of non-chain and chain grooming bouts were similar between groups, providing further evidence that individual grooming bouts of the same type (chain vs non-chain) were similar between Dlx-CKO and control mice (Figure 3-4C,D). Taken together, these findings indicate that the temporal structure of grooming is more variable in Dlx-CKO mice, as more of their grooming was spent in unstructured non-chain bouts.

Discussion

Our results suggest that Dlx-CKO mice have primary impairments in coordination and manual dexterity. We used a battery of tests designed to assess motor learning (skilled reaching), dexterous skill (skilled reaching and pasta handling), and innate motor sequencing (grooming). Dlx-CKO mice had significantly lower success rates and pellet contacts compared to control mice on the skilled reaching task, and dropped the pasta more frequently during pasta handling. Additionally, our grooming experiment identified deficits in sequence initiation, but not sequence progression. These results are reminiscent of endophenotypes seen in human dystonia and may provide a mechanism to dissect the physiologic path from torsinA dysfunction to motor phenotype.

There are several potential explanations for skilled reaching impairments in Dlx-CKO mice. Our results suggest that Dlx-CKO mice have at least a primary motor deficit, and possibly a sensory deficit. Rodents use their whiskers to identify the reaching slot (Parmiani et al., 2018), and a combination of olfaction and prior experience to localize the pellet (Parmiani et al., 2021; Whishaw & Tomie, 1989). However, the reach itself is ballistic with little or no online adjustment. Motor cortex is essential for ballistic movements during the transport phase of reaching, while sensory information influences grasping and food release (Ballermann et al., 2000; Ueno et al., 2018). Dlx-CKO mice frequently missed the pellet entirely (Figure 3-1D), and therefore had limited opportunities to use somatosensory feedback to adjust reaches. Coupled with the increase in drops during pasta handling, our results are more consistent with primary motor rather than sensory deficits. However, we cannot completely exclude the latter. Future experiments designed to explore sensory deficits independent of skilled reaching in Dlx-CKO mice may provide additional clarity.

Neither control nor Dlx-CKO mice exhibited statistically significant increases in their success rates in our task, preventing us from identify motor learning deficits based on success rates. However, Dlx-CKO and control mice did increase the number of "pellet displaced" trials with training, suggesting that they were adapting their reaching strategies (Figure 3-1D). The stable success rates may have been related to task design. In many versions of skilled reaching, mice retrieve pellets from a shelf allowing them to slide the pellet into the chamber. Because our task used a pedestal, the mice had to both located and securely grasp the pellet to prevent it from falling between the pedestal and the front of the chamber. Whether the mice would have improved their grasping to increase success rate with further training is uncertain. Regardless, the change in the rate of pellet displacement shows strongly suggests the engagement of motor learning. This is largely consistent with previous results on the accelerating rotarod, in which Dlx-CKO mice showed normal improvement (Pappas et al., 2015). On the rotarod, however, there was no difference between Dlx-CKO mice and controls in baseline performance, suggesting that skilled reaching is a more sensitive assay of motor impairment (at least for Dlx-CKO mice).

Chain grooming is strongly dependent on basal ganglia function. Striatal lesions disrupt syntactic chain completion and syntax without affecting syntactic chain initiation rates (Berridge & Whishaw, 1992). This pattern is different from our results, wherein Dlx-CKO mice maintained syntactic chain completion rates and syntax but decreased syntactic chain initiations (Figure 3-4B). These results suggest the striatal control of chain syntax remains largely intact for Dlx-CKO mice, at least once a chain is initiated. Why syntactic chain initiation was reduced remains unclear. While changes in dorsal striatal activity tend to signal transitions between chain grooming phases (but not non-chain grooming phases), SNr activity tends to signal the start of a

chain (Aldridge & Berridge, 1998; Meyer-Luehmann et al., 2002). Basal ganglia output is abnormal in dystonia, with low frequency bursty, oscillatory firing patterns (Barow et al., 2014; Silberstein et al., 2003; Starr et al., 2005), raising the possibility that disrupted SNr output interferes with chain initiation in Dlx-CKO mice. Direct SNr recordings will be needed to test this hypothesis. If nigral firing patterns are indeed abnormal in Dlx-CKO mice, the next step would be to identify the upstream cause of these changes. Striatal cholinergic dysfunction has been observed in Dlx-CKO mice (Pappas et al., 2015), though the precise mechanisms through which this could lead to abnormal basal ganglia output are unknown.

Progress on understanding the pathophysiology of dystonia has been limited by the availability of mouse models with clear phenotypes (see Pappas et al., 2014 for review). Mouse models mimicking the human DYT1 genotype (heterozygous *tor1a*$^{\Delta E/+}$) or over expressing human mutant torsinA display motor deficits in the beam walking task but inconsistent performance on the rotarod task (Dang et al., 2005; Grundmann et al., 2007, 2012; Sharma et al., 2005). Inconsistent results have also been found in mouse models with brain region specific torsinA knock-out, with some displaying deficits in beam walk but normal rotarod performance (Dang et al., 2006; Pappas et al., 2015; Yokoi et al., 2008, 2011), and yet others with normal beam walk but reduced rotarod performance (Sciamanna et al., 2012). These inconsistencies complicate understanding the relevance to human dystonia. Here, we have shown that Dlx-CKO mice have specific motor deficits with parallels in human dystonia. Impaired manual dexterity has been identified in musicians' dystonia (in pianists), isolated cervical dystonia, and manifesting (but not asymptomatic) DYT1 carriers (Balas et al., 2006; Furuya et al., 2015; Oktayoglu et al., 2020). It is not known if impaired dexterity in humans with dystonia responds to treatment (e.g., DBS or anticholinergics), or if other DYT1 models also have impaired manual

dexterity. Nonetheless, these clear abnormalities in skilled reaching and pasta handling could be targets for preclinical therapeutic trials and be used to establish physiology-phenotype correlations. Similarly, DYT1 carriers have abnormalities in motor sequencing, though this is true of both manifesting and non-manifesting carriers (Carbon et al., 2011). The abnormal sequence initiation in grooming patterns that we observed therefore parallels motor sequence deficits in human dystonia and may provide a mechanism to dissect the physiology underlying this endophenotype.

In conclusion, Dlx-CKO mice display primary motor deficits and motor sequencing abnormalities with parallels in human DYT1 dystonia. These abnormalities may provide targets for preclinical intervention trials, as well as facilitate determination of the physiologic path from torsinA dysfunction to motor phenotype.

Methods

Mice. All animal procedures were approved by the University of Michigan Institutional Animal Care and use Committee. Numbers of mice included in each experimental group and analysis are indicated in figure legends. Dlx-CKO mice were generated by crossing Cre^{+} $tor1a^{+/-}$ (Pappas et al., 2015) with $tor1a^{flx/flx}$ mice (Liang et al., 2014), using the breeding strategy described in (Pappas et al., 2015). Mice with genotype $tor1a^{flx/+}$ were used as age and sex matched littermate controls. Male (control=5, Dlx-CKO=5) and female (control=9, Dlx-CKO=4) mice were housed in groups of 2-3 on a reverse light/dark cycle for at least two weeks prior to experiments. All testing was carried out during the dark phase. Food restriction was imposed on all animals during the training and testing periods of the skilled reaching and pasta handling tasks for no more than 6 days in a row such that animals' weights were maintained ≥90% of their

free-feeding weight. Water was available ad libitum in their home cages. A small percentage of Dlx-CKO mice had seizures at an early age and died before experiments began.

Grooming. Mice were placed in an acrylic cylinder (15 cm diameter x 20 cm height). A camera was mounted to the platform and focused on the cylinder. Two mirrors were positioned on the left and right to allow clear views of the mouse from multiple angles. Videos of the entire session were recorded. Before each trial, mice were lightly sprayed with water on the face and whiskers, then placed into the cylinder. Mice were allowed to move freely, and grooming behaviors were spontaneous. Trials lasted for a total of 15 minutes, with two trials constituting a session. Each mouse performed three sessions of grooming, distributed over 3 weeks, prior to beginning the skilled reaching task. Mice performed one additional session of grooming after the final training session in the skilled reaching task and prior to beginning the pasta handling task.

Skilled reaching. Automated reaching system. Training and testing were carried out in custom-built skilled reaching chambers built according to (Farr & Whishaw, 2002). A reaching slot (10 mm x 7 cm) was cut into the front panel of the chamber 17.5 mm from the floor. Two mirrors were placed on either side of the front of the reaching chamber and angled to allow side views of the paw during reaching. A linear actuator with potentiometer feedback (Actuonix, Saanichton, Canada) was connected to an acrylic pellet delivery rod and mounted in a custom frame below the support box. The pellet delivery rod extended through a funnel mounted to the top of the frame. Before each session, the actuator was positioned 1 cm from the front of the reaching slot so that the delivery rod was aligned with the right or left edge of the slot according to each mouse's paw preference. Videos of the entire session were recorded by a camera mounted in front of the reaching slot.

Trial performance. A custom-built Arduino (Arduino Mega 2560 Rev3, Arduino, Boston, MA) based system controlled the experiment. Each training session began with the pellet delivery rod at the lowest position inside the funnel. When a session began, the pellet delivery rod rose to the bottom of the reaching slot, triggering an LED to indicate the start of a trial. The delivery rod remained in place for 3 seconds before lowering, triggering the LED to turn off. This began an intertrial interval of ~5 seconds wherein the pellet delivery rod retracted into the funnel to pick up a new pellet, then rose for a new trial.

Habituation. The purpose of habituation is to familiarize mice with the reaching chamber and sucrose reward pellets. Habituation lasted for three sessions, each 20 minutes in length. Mice were placed on food restriction and introduced to the sucrose reward pellets in their home cages 24 hours prior to the first day of habituation. On day 1 of habituation, a pile of 10 pellets was placed in the skilled reaching chamber to encourage exploration. On days 2 and 3 of habituation, mice received no sucrose pellets, and the skilled reaching apparatus was turned on for 5 minutes in each session to familiarize mice with the sound.

Pre-training. During 'pre-training', mice were evaluated for reaching paw-preference and trained to reach for the linear actuator. Paw preference and training mice to reach for the linear actuator was performed as in (Bova et al., 2020). Once mice reached for the delivery rod 10 times without being baited by the experimenter, they began training on the automated task. Experimenters used a "manual" mode to control trial length (duration the actuator is in "reaching position" – at the bottom of the slot). Experimenters slowly decreased trial length to 3 seconds, at which point the actuator was set to "automatic". Pre-training was complete once mice performed 20 trials in a single session (30 minutes) with the actuator on "automatic".

Training. After pre-training, mice began 30-min training sessions with the automated system. Mice were trained for 5 days per week for a total of 21 sessions. Videos were captured of the entire training session.

Pasta handling. After skilled reaching was complete and the final session of grooming administered, mice began the pasta handling task. Mice were placed in a small transparent box (4 in x 4 in x 20 in) with a mirror covering the entire base of the box. A camera was positioned facing down at the mirror to allow views of the forelimbs, paws, and mouth from below the mouse during eating. The task was administered as in Tennant et al., 2010. Single trials were administered by placing a piece of capellini pasta 2.6 cm long onto the floor inside the box, positioned to optimize viewing of the mouse during eating. Trials began once experimenters' hands were removed from the box and continued until the piece of pasta had been consumed. Up to 4 trials were performed per session, with a maximum session duration of 20 minutes. Videos of the entire session were recorded.

Video recording. All videos were recorded at 100 frames-per-second and 1920 x 1080 pixels by a high-definition color digital camera (HBLK-6FT-0309, Panasonic, Kadoma, Japan).

Grooming sequence analysis. Behavioral analysis consisted of frame-by-frame video scoring to assess bout and syntactic chain onset. A single bout is continuous grooming without long pauses. A pause occurs when the mouse stops grooming briefly (<6 s) but quickly resumes, without performing locomotor activity (Kalueff et al., 2007). Behaviors performed throughout bouts were assigned numerical values: 0 – no grooming present; 1 – small, elliptical strokes about the mouth and nose (Phase 1); 2 – asynchronous, unilateral strokes increasing in amplitude from vibrissae to the eyes and occasionally the ears (Phase 2); 3 – synchronous, bilateral strokes involving both forepaws from the vibrissae to the eyes and ears (Phase 3); 4 – Licking of the

torso or haunches (Phase 4); 5 – licking of the forepaws that does not include elliptical strokes. Syntactic chain onset was defined as initiation of Phase 1 grooming that progressed to Phase 2 or Phase 3. All other grooming was defined as non-chain. A complete chain progressed through Phase 1, Phase 2 and/or Phase 3, and Phase 4 (Berridge & Fentress, 1987). Phase transitions performed within syntactic chains were grouped into typical and atypical transitions. Typical transitions consist of those from phases 1→2, 2→3, 3→4, and 4→0 (here 0 represents no grooming). All other transitions were considered atypical and can be broken into three groups: skips (e.g., 2→4), reverses (e.g., 4→3), and premature termination (e.g., 3→0) (Kalueff et al., 2007). Frame number was recorded for bout initiation, bout end, syntactic chain initiation, and syntactic chain end.

Time spent grooming for each session was calculated by dividing total time grooming (sum of all individual bout and syntactic chain durations) by the total trial time. Time spent performing non-chain grooming was calculated by subtracting the time spent performing syntactic chains from their respective bouts (isolating non-chain grooming time), then dividing by the total trial time. Time spent performing chain grooming for each session was calculated by dividing the sum of all individual chain durations by the total trial time (Figure 3-4A). Initiations per minute of grooming were calculated for each session by taking the number of non-chain bouts initiated, syntactic chains initiated, or both (total) and dividing it by the time spent grooming in minutes (Figure 3-4B). Chain duration was measured from onset of Phase 1 through the end of Phase 4 (Berridge & Whishaw, 1992). Non-chain grooming bout duration was calculated by subtracting the duration of any syntactic chains within a grooming bout from the total bout duration. Distributions of bout durations were compared to determine if the durations of individual bouts differed between experimental groups. Non-chain bout durations were

grouped into bins of 5 s; syntactic chain durations were grouped into bins of 3 s (Fig 4C,D). Chain completion rates were calculated per session by dividing the number of complete chains by the number of chains initiated (Figure 3- 4E). The frequency of different grooming strokes was calculated by summing all instances of a single phase performed by trial (Figure 3-4F).

Skilled reaching analysis. Skilled reaching videos were segmented into individual videos for each trial and assigned random codes so that scorers were blinded to the mouse's genotype and day of testing. Reach outcome for each trial was scored by visual inspection as follows: 0 – no pellet presented or other mechanical failure; 1- first attempt success (obtained pellet on initial limb advance); 2 – success (obtained pellet, but not on first attempt); 3 – forelimb advanced, pellet was grasped then dropped in the box; 4 – forelimb advance, but the pellet was knocked off the pedestal ('pellet displaced'); 5 – the mouse reached but the pellet remained on the pedestal ('pellet remained'); 6 – pellet was obtained using its tongue; 7 – the mouse did not perform any reaches; 8 – the mouse used its non-preferred paw to reach; 9 – obtained pellet with use of both paw and tongue. Outcome percentages were calculated by dividing the number of trials of each outcome by the total number of trials per session. For comparison of failure mechanisms in skilled reaching, 'unsuccessful' trials were defined as trials where a reach was performed but no reward pellet obtained (scores 3, 4, and 5).

Success rate was calculated for each session by dividing the total number of scores 1 and 2 by the total number of trials (sum of scores 1, 2, 3, 4, and 5) (Figure 3-1B). The rates of 'pellet displaced' and 'pellet remains' trials were calculated by dividing the number of scores 4 or 5, respectively, by the total number of unsuccessful trials (sum of scores 3, 4, and 5) (Figure 3-1D).

Pasta handling analysis. Behavioral analysis consisted of frame-by-frame assessment of handling patterns. Typically, mice hold the pasta with both paws. A paw near the mouth moves

the pasta into the mouth (guide paw), while the other grasps the pasta slightly distal to the guide paw (grasp paw). As the pasta decreases in length, mice tend to hold the paws symmetrically about the pasta in front of the mouth. Atypical pasta handling behaviors described by Whishaw and colleagues (Whishaw et al., 1997, 1998; Whishaw & Coles, 1996) were counted during each trial: *any forepaw no contact* – one of the paws does not contact the pasta during eating (other than during forepaw adjustments); *pasta long, paws together* – the paws are held symmetrically about the pasta before the pasta is half the original length (Figure 3-2B, Left); *pasta short, paws apart* – the mouse fails to move the paws into a symmetrical position after the pasta is less than half the original length (Figure 3-2B, Right); *guide/grasp switch* – the guide and grasp paws switch during eating; *mouth pulling* – the mouth is used to pull the pasta through the paws; and *drops* – the pasta is dropped after eating is initiated. Trials wherein the mouse faced away from the camera, obscuring paw movements for >3 s were excluded from analysis.

Statistics. To test whether skilled reaching had an effect on grooming, a Welch's two sample t-test (using R *t.test*) was used to compare grooming outcomes before and after skilled reaching. Given the similarity in grooming outcomes pre- and post-reaching (total time spent grooming: $t(14)=0.086$, $p=0.93$; time spent non-chain grooming: $t(14)=0.088$, $p=0.93$; time spent chain grooming: $t(11)=0.042$, $p=0.97$), further analyses combined all 4 sessions of grooming. A linear regression model (using R *glm*) was used to evaluate time spent grooming with genotype as the independent variable. The Kruskall-Wallis test by ranks (using R kruskal.*test*) was used to examine differences of genotype on grooming initiation rates (Figure 3-4B) as well as grooming stroke type (Figure 3-4F). A Poisson regression model was implemented (using R *lmer*) for chain completion (offset by number of chains; Figure 3-4E), transition type (offset by number of

chains; Figure 3-5), atypical transition type (offset by the number of atypical transitions; Supplemental Figure 3-1B) due to the count nature of the data.

Linear mixed-effects models were used to evaluate success and failure rates in skilled reaching. We implemented linear mixed-effects models (using R *lmer*) with random intercepts/effects for each mouse (where effect of session varied between mice) and main interaction effects of genotype and session number. Linear mixed-effects models included averages for all 21 sessions of training for all mice. A linear mixed-effects model with the fixed effect of session number and a random effect for the interaction between genotype and session number was used to identify differences between groups on specific training days.

A Poisson regression model was implemented to examine genotypic differences on atypical handling patterns in pasta handling. Reduced sample sizes (control=3, Dlx-CKO=4) in pasta handling are due to lack of participation or positioning of the mouse during pasta handling, preventing analysis.

Figures

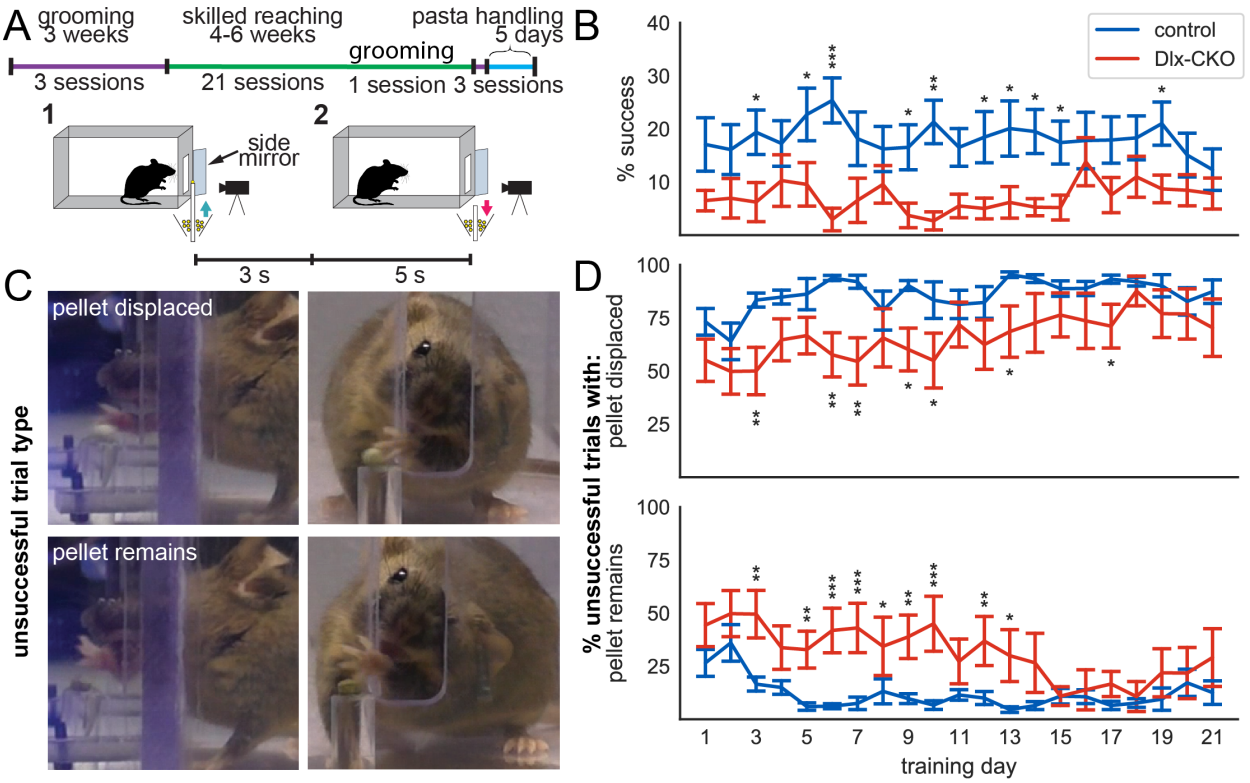

Figure 3-1: Dlx-CKO mice are impaired in automated skilled reaching

A) Top: Timeline for a complete set of experiments on a single mouse. Bottom: Timeline for a single skilled reaching trial. (1) – the pedestal rises, bringing a sugar pellet into position to allow reaching, where it stays for 3 seconds before (2) – the pedestal descends to retrieve a new pellet for the next trial. B) Average "any attempt" success rate. Linear mixed-effects model: effect of genotype: $F_{1,21}$=-2.56, p=0.018; effect of session: $F_{1,21}$=-0.653, p=0.52; interaction between genotype and session: $F_{2,20}$=0.862, p=0.40. Asterisks indicate a significant difference between experimental groups in specific sessions. C) Still image of a control mouse at maximum paw extension during an unsuccessful trial in which the paw contacted the pellet, displacing it (Top) and in which the paw missed the pellet, allowing it to remain on the pedestal (Bottom). Arrows indicate distance between paw and pellet. D) Fraction of unsuccessful trials during which mice displaced (Top; "pellet displaced") or did not displace (Bottom; "pellet remained") the pellet. Asterisks indicate a significant difference between groups in specific sessions. Linear mixed-effects model for "pellet displaced" trials: effect of genotype: $F_{2,20}$=-3.69, p=0.0014; effect of session: $F_{1,21}$=1.80, p=0.087; interaction between genotype and session: $F_{1,21}$=1.26, p=0.22. Linear mixed-effects model for "pellet remains" trials: effect of genotype: $F_{1,21}$=4.15, p=0.00046; effect of session: $F_{1,21}$=-1.73, p=0.099; interaction between genotype and session: $F_{1,21}$=-1.97, p=0.062. For individual session in panels B and D, * indicates p< 0.05; ** indicates p<0.01; *** indicates p<0.001. (n=23; control=14, Dlx-CKO=9).

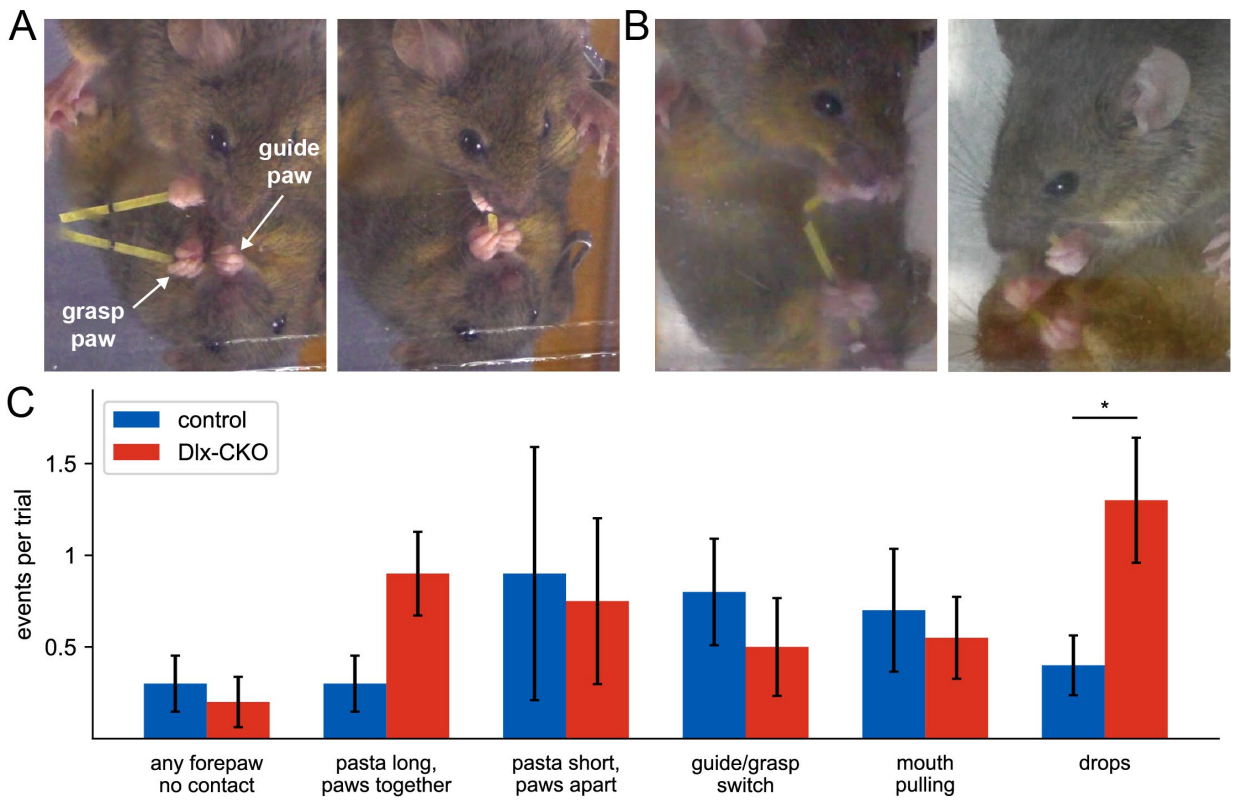

Figure 3-2: Dlx-CKO mice exhibit nonspecific deficits in pasta handling

A) Typical grasp patterns during pasta handling – "pasta long, paws apart" (Left); and "pasta short, paws together" (Right). B) Atypical grasp patterns – "pasta long, paws together" (Left) and "pasta short, paws apart" (Right). C) Average number of atypical behaviors per trial (a trial is handling a single piece of pasta). See *Pasta handling analysis* for description of behaviors. Poisson regression model for experimental group differences (significance indicated with asterisk): any forepaw, no contact (z=-0.53, p=0.60); pasta long, paws together (z=1.76, p=0.078); pasta short, paws apart (z=-0.43, p=0.67); guide/grasp switch (z=-0.99, p=0.32); mouth pulling (z=-0.50, p=0.62); drops (z=2.20, p=0.028). (control=3, Dlx-CKO=4).

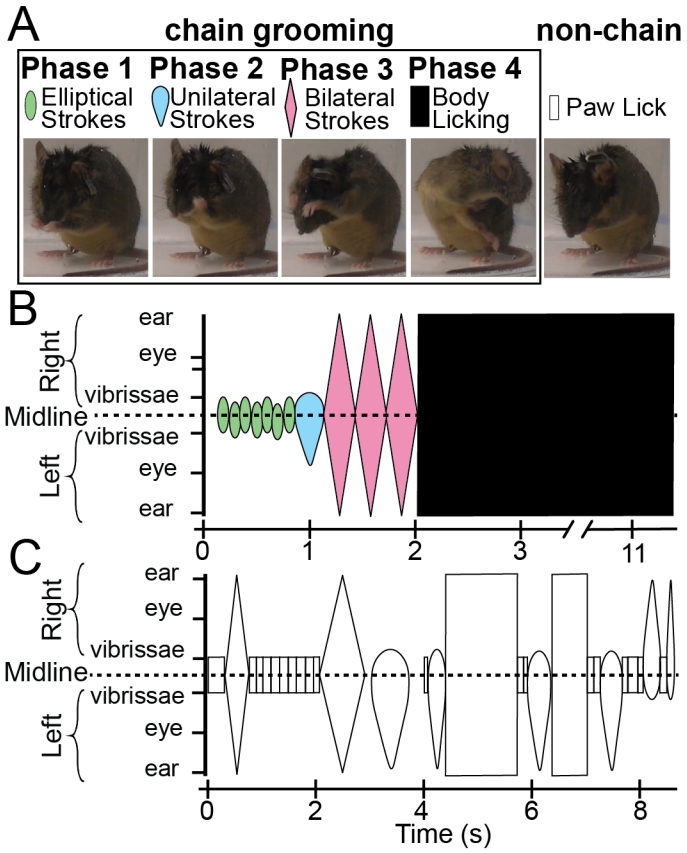

Figure 3-3: Grooming structure in control mice

A) Mouse grooming consists of syntactic chains, made up of stereotyped strokes, which are usually embedded in longer and less stereotyped non-chain grooming bouts. Stroke types are similar in chain and non-chain grooming with the exception of paw licks, which do not occur in chain grooming. Shapes correspond to forelimb stroke types, and colors correspond to syntactic chain phases. *Elliptical strokes* consist of small bilateral strokes near the nose. *Unilateral strokes* consist of larger elliptical motions from the vibrissae to the snout that occur asynchronously. *Bilateral strokes* are similar to unilateral strokes, but occur synchronously in both arms. *Body licking* consists of body twisting so the mouse can clean its haunches. *Paw licks* consist of paw licking without small elliptical strokes. B) Choreography of a complete syntactic chain. Individual symbols indicate the stroke type, and their size represents the amplitude of the movement with respect to facial landmarks. C) Choreography of a non-chain grooming bout. Transitions between stroke types occur at a slower pace and in a less predictable order.

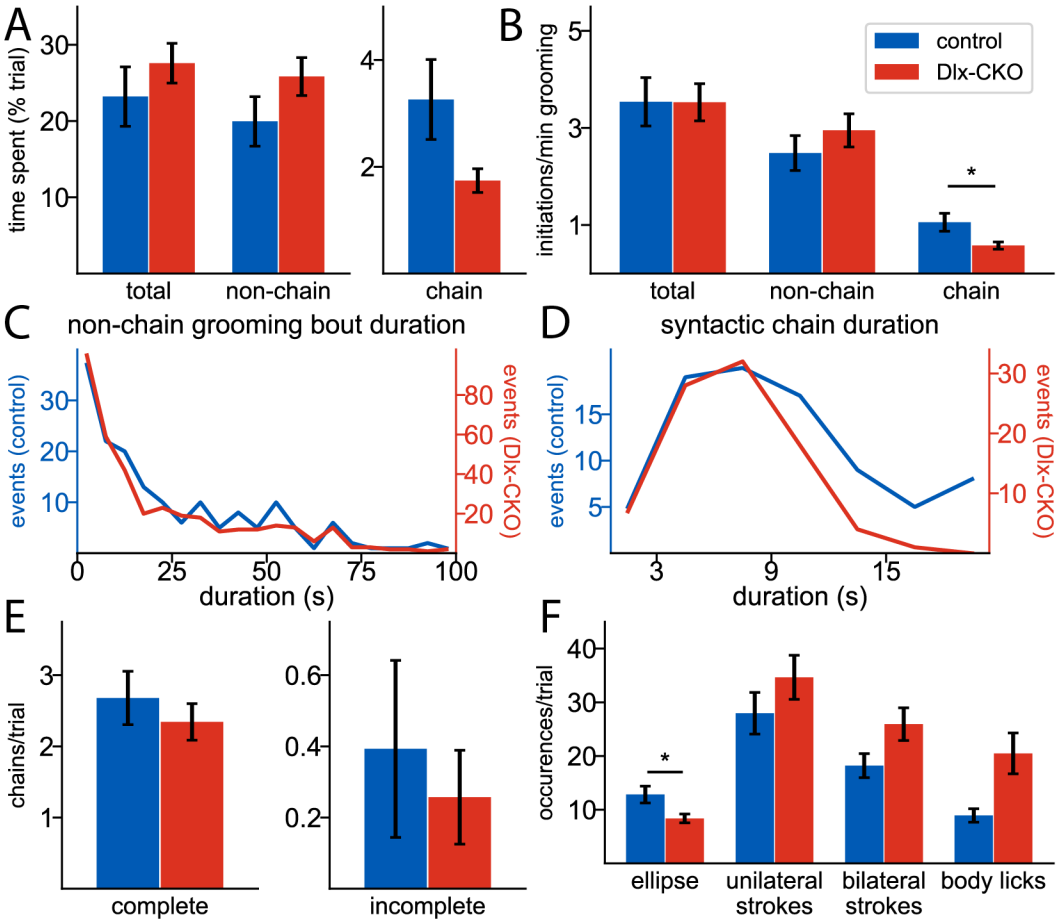

Figure 3-4: Dlx-CKO mice perform similar amounts of grooming, but in more variable patterns, than controls

A) Time spent grooming in total, in non-chain bouts, and in chain bouts. Linear Regression, effect of genotype: total time spent grooming: t(12)=0.053, p=0.96; time spent non-chain grooming t(12)=1.40, p=0.19; time spent chain grooming: t(12)=-1.93, p=0.079. B) Number of bout initiations per minute of grooming in total, for non-chain grooming, and for chain grooming. Kruskal-Wallis test, effect of genotype: non-chain bout initiation: chi-squared=2.49, df=1, p=0.11; syntactic chain initiation: chi-squared=4.83, df=1, p=0.028. C & D) Distribution of non-chain (C) and syntactic chain (D) grooming bout durations. The left and right axes represent counts for control and Dlx-CKO mice, respectively. Axes were scaled to maximum counts to facilitate comparison. E) Number of complete and incomplete syntactic chains per trial. Poisson Regression, effect of genotype on: number complete syntactic chains: z(12)=0.49, p=0.63; number incomplete syntactic chains: z(12)=-1.05, p=0.29. F) Frequency of performance for each grooming stroke type. Kruskall-Wallis test, effect of genotype: phase 1: chi-squared=4.16, df=1, p=0.041; phase 2: chi-squared=0.99, df=1, p=0.32; phase 3: chi-squared=2.26, df=1, p=0.13; phase 4: chi-squared=1.86, df=1, p=0.17. (control=5, Dlx-CKO=8).

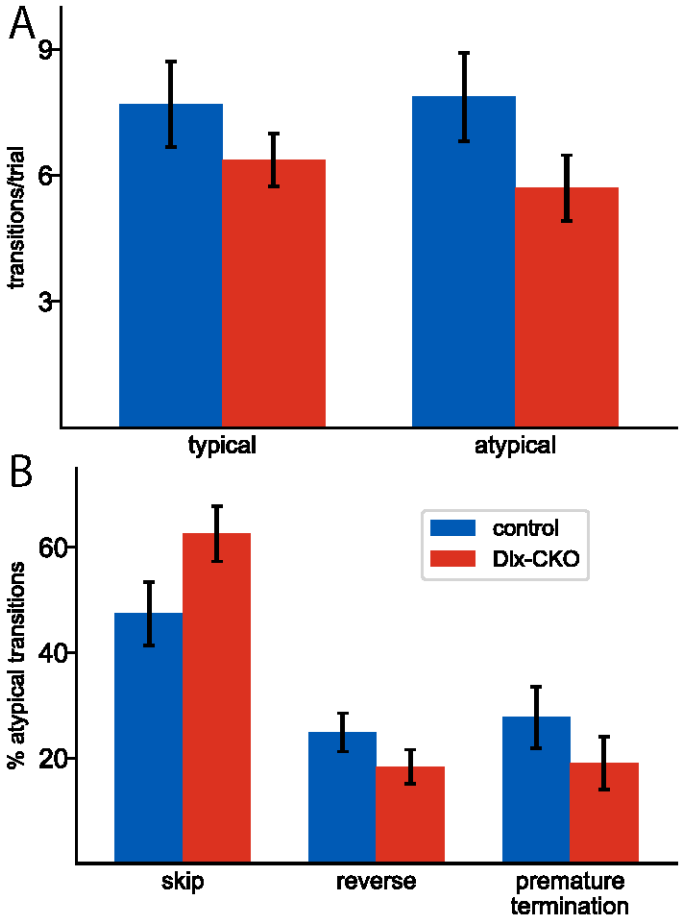

Figure 3-5: Dlx-CKO mice have similar phase transitions during syntactic chains to controls

A) Transitions between phases of grooming. Typical transitions consist of those from phases 1→2, 2→3, 3→4, and 4→0 (0 represents no grooming). All other transitions were considered atypical. Poisson Regression, effect of genotype: typical transitions: $z(12)=-0.61$, $p=0.54$; atypical transitions: $z(12)=0.29$. B) Distribution of atypical transition by type. Atypical transitions are broken into three groups: skips (e.g., 2→4), reverses (e.g., 4→3), and premature termination (e.g., 3→0). Poisson Regression, effect of genotype: skip: $z(12)=1.18$, $p=0.24$; reverse: $z(12)=-0.56$, $p=0.57$; premature termination: $z(12)=-1.50$, $p=0.13$. (control=5, Dlx-CKO=8).

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CHAPTER 4: Research Synthesis

Summary of findings

Identifying the pathophysiologic causes of dystonia is an important step towards improving its treatment. Unfortunately, our understanding of dystonia pathophysiology has been limited by the lack of animal models with clear motor abnormalities that could be correlated with physiologic changes. By assaying skills that require coordinated forelimb control and/or motor sequencing, I demonstrated clear motor abnormalities in a mouse model of DYT1 dystonia. In Chapter 2, I showed that the Dlx-CKO mouse model of dystonia develops stereotyped abnormal movements after repeated training on a manual single pellet skilled reaching task. These abnormal movements decreased after application of the antimuscarinic trihexyphenidyl, which is used to treat human dystonia. This suggests abnormal movements that develop as a result of training on skilled reaching have predictive validity for dystonia. Additionally, this is the first genetic model of dystonia demonstrating the development of task-specific abnormal movements after repeated training on a dexterous task. We were unable to replicate the development of abnormal movements in a follow up experiment with an automated skilled reaching task. This suggests task demands may influence the development of abnormal movements. However, replication of the development of abnormal movements remains essential.

In Chapter 3, I implemented three behavioral tasks to assess motor learning, dexterous skill, and innate motor sequencing. Dexterous skill and motor learning were assessed on an automated version of the single pellet skilled reaching task. Success rates on this task were lower than in the manual task and did not improve over time, likely due to changes in task demands.

However, an examination of failure mechanisms in the automated skilled reaching task revealed that Dlx-CKO mice removed the pellet from the pedestal less frequently than control mice throughout training. This suggests that Dlx-CKO mice have a primary motor or sensory deficit resulting in inaccurate paw transport and failure to contact the sugar pellet during reaches. To minimize any confounds from abnormal motor learning, Dlx-CKO mice were also tested on the pasta handling task. Dlx-CKO mice more frequently dropped the pasta than controls, suggesting abnormal dexterous control of the paw during this task. Finally, both before and after skilled reaching, Dlx-CKO mice performed a water-elicited grooming task. The Dlx-CKO initiated fewer syntactic chains during grooming than control mice but performed similar amounts of overall grooming. This suggests that temporal structure of grooming is more variable in Dlx-CKO mice, as more of their grooming was spent in unstructured non-chain bouts.

How do task-specific abnormal movements develop?

We showed that Dlx-CKO mice develop stereotyped and task-specific abnormal movements after training on a manual skilled reaching task (Chapter 2). These results are similar to those of earlier models of task-specific dystonia, where training on repetitive reaching or grasping tasks induced dystonic-like movements in both nonhuman primates and rats (Byl, 2007; Byl et al., 1996, 1997). However, our task did not induce peripheral tissue injury and therefore more closely models humans with primary task-specific dystonias. Additionally, Dlx-CKO mice have circuit abnormalities in GABAergic and cholinergic neurons of the forebrain, which are cell populations identified as key in the pathogenesis of dystonia (Pappas et al., 2015). However, we were unable to replicate our findings in an automated skilled reaching task.

One possible explanation for this failure in replication is that changes in task demands, notably increased task difficulty, prevented the development of abnormal movements. In our

automated task, sugar pellets were delivered on a small pedestal rather than a shelf as in the manual task. This likely required different reaching strategies than those performed by mice in the manual skilled reaching task. In previous models of task-specific dystonia, animals that adopt different strategies for task implementation have different risks for developing task-specific abnormal movements. For example, in a nonhuman primate model of task specific dystonia, subjects repeatedly grasped and pulled a lever. Primates that changed strategies over time to include movement of larger muscle groups (e.g., arm and shoulder) did not develop task-specific motor abnormalities, while primates that maintained localized strategies of the hand did (Byl et al., 1997). It is conceivable that increased task difficulty in the automated task caused mice to execute different reaching strategies, thus preventing the development of abnormal movements.

Lack of sensory feedback to Dlx-CKO mice during the automated task may have prevented the development of abnormal movements. Analysis of failure mechanisms in the automated task revealed that Dlx-CKO mice knocked the pellet from the pedestal less frequently than control mice, suggesting that they made less pellet contact (Chapter 3). Experiments using the tonic vibration reflex (TVR) in humans with task-specific dystonia demonstrate that sensory feedback can induce abnormal postures (Karnath et al., 2000; Lekhel et al., 1997; Marinelli et al., 2011; Pelosin et al., 2009). This suggests that sensory feedback is critical to the manifestation of dystonic movements in task-specific dystonia patients. The reduced sensory feedback experienced by Dlx-CKO mice may not have recruited sensorimotor circuits responsible for the development of abnormal movements. Tasks requiring more direct sensorimotor integration are likely to provide further insight into this hypothesis. The pasta reaching matrix is one possible task that enables manipulation of the sensory qualities of the reaching target. For example, mice may be trained to reach for pasta at a single orientation and then tested on differing orientations.

This may be a measure of proprioception. Alternatively, some pieces of pasta can be replaced with rods of differing diameters and materials, requiring mice to discriminate between different sensory modalities to successfully obtain a pasta piece. In either case, the speed at which mice adjust their reaching and grasping strategies may be used to assess group differences. I would expect Dlx-CKO mice to perform poorly in a test with pasta pieces presented at differing orientations, based on the failure to transport the paw accurately during skilled reaching. However, it is possible that increased sensory feedback by exchanging some pasta pieces for rods may allow Dlx-CKO mice to retain success rates similar to control mice. This task may also trigger the requires sensorimotor network pathways that are necessary for the development of task-specific abnormal movements.

The interpretation for DYT1 dystonia is less straightforward. Patients with DYT1 dystonia experience abnormal movements at baseline, although they are worsened by voluntary movement. Additionally, movements are not isolated to one region of the body but rather are more generalized. This differs significantly from task-specific dystonia, which is usually focal and develops after repeated training on highly skilled tasks. However, the manifestation of DYT1 dystonia depends on processes that occur during CNS maturation. In particular, maturation of the cholinergic interneurons and abnormal cholinergic signaling (Li, Kim, et al., 2021). TorsinA loss of function in adulthood does not result in the overt abnormal motor phenotypes, whereas prenatal loss of function does (Li, Levin, et al., 2021). Dlx-CKO mice experience selective neurodegeneration in dorsal striatal cholinergic interneurons at postnatal day 14, coinciding with the onset of limb clasping during the tail suspension task (Pappas et al., 2015). Remaining cholinergic interneurons exhibit abnormal morphology and physiology. Cholinergic interneurons in striatum are implicated in the integration of sensory information into

striatal circuitry (Reig & Silberberg, 2014) as well as voluntary movement (Bonsi et al., 2011). This abnormal cholinergic signaling may produce broad changes in sensorimotor integration, resulting in manifestations of abnormal movements. Experiments to test this hypothesis could utilize tamoxifen-induced Cre to conditionally remove torsinA in mature forebrain GABAergic and cholinergic neurons (similar to Dlx-CKO mice). Coupled with skilled reaching, tamoxifen could be introduced at varying time points in CNS development (e.g., prenatal, adolescence, adulthood). Mice would then undergo training on a skilled reaching task, perhaps head-fixed to isolate potential abnormal movements. It is possible that loss of torsinA in adulthood may still result in development of abnormal movements during or impairments in skilled reaching. This would support the hypothesis for striatal cholinergic involvement in the manifestation of dystonic movements. Alternatively, if abnormal movements are not exhibited except with prenatal torsinA loss of function, this would provide further evidence for the developmental nature of dystonia.

One hypothesis to explain the development of abnormal movements in Dlx-CKO mice during skilled reaching is a loss of surround inhibition. In this hypothesis, loss of surround inhibition in motor cortex leads to recruitment of proximal antagonistic muscles. How surround inhibition is generated, however, remains unclear. Surround inhibition has not been investigated in Dlx-CKO mice. However, circuit abnormalities in the GABAergic and cholinergic forebrain neurons make this a plausible hypothesis. This hypothesis could be further tested by directly measuring intracortical inhibition and cortical surround inhibition in slice preparations or *in vivo*. In measurements of cortical surround inhibition, results would show activity in cortical areas of muscles or body regions that often move together and inhibition of cortical areas corresponding to unrelated and adjacent muscle groups. In this way, loss of cortical surround inhibition would

be measured by increased activity in the areas corresponding to unrelated and adjacent muscle groups of the stimulated cortical area.

Behavioral assays in mouse models of dystonia

The majority of mouse models of dystonia do not develop overt abnormal movements. Several of these models do exhibit acetylcholine-mediated changes in dopaminergic signaling and receptor availability (Eskow Jaunarajs et al., 2019; Martella et al., 2014; Pisani et al., 2006; Sciamanna et al., 2011, 2012; see Chapter 1: *Rodent models of dystonia*). However, limited behavioral tasks and inconsistent results have made understanding the relevance of these models to human dystonia difficult. The most commonly used behavioral tasks in rodents include rotarod, beam walk, and the open-field test. These tasks require gross motor control and do not assess sequence learning. Additionally, much of mouse behavior can be performed without use of cortex (Basista & Yoshida, 2020; Berridge & Whishaw, 1992; Kawai et al., 2015), complicating our ability to identify endophenotypes in the cortex of these models.

In Chapter 3, I addressed the limited application of behavioral assays to include cortically dependent behaviors. I demonstrate that Dlx-CKO mice have lower success rates on the single pellet skilled reaching task as a result of a failure in paw transport to the pellet. While neither group improved their success rates with training, Dlx-CKO mice had a larger proportion of trials where the pellet remained on the actuator after reach performance. This indicates that Dlx-CKO mice were unable to effectively transport their paw to the sugar pellet, suggesting a primary motor or sensory deficit.

Our results are more likely explained by a primary motor deficit in Dlx-CKO mice rather than a sensory deficit. During skilled reaching rodents use their whiskers to identify the reaching slot and a combination of olfaction and prior experience to localize the pellet (Parmiani et al.,

2018, 2021; Whishaw & Tomie, 1989). It is plausible that abnormal sensory feedback from this process could result in the failure to correctly locate the pellet. However, the more likely cause is a failure of motor cortex to perform the ballistic components of the reach. Reach-to-grasp movements are ballistic with little or no online adjustment. Motor cortex is essential for ballistic movements during the transport and grasping phases of reaching, while sensory information influences grasping and food release (Ballermann et al., 2000; Ueno et al., 2018). Dlx-CKO mice displayed a failure in paw transport and likely reduced sensory feedback during the skilled reaching task. Therefore, abnormalities in the transport phase are more likely to coincide with motor cortex abnormalities.

Decreased sequence initiation in Dlx-CKO mouse grooming parallels abnormal movement initiation in humans with dystonia. Our results in Chapter 3 demonstrate that Dlx-CKO mice perform similar overall amounts of grooming, but less of that time is spent performing syntactic chains. Additionally, when syntactic chains were initiated, their sequence was similar to that of controls. These results suggest that Dlx-CKO mice have abnormal innate motor sequencing. Similarly, DYT1 carriers have abnormalities in motor sequencing, though this is true for both manifesting and non-manifesting carriers (Carbon et al., 2011). The abnormal sequence initiation in grooming patterns that we observed therefore parallels motor sequence deficits in human dystonia. This is the first behavioral endophenotype identified in a genetic mouse model of dystonia and may provide a model in which to dissect DYT1 pathophysiology.

Concluding Remarks

The work presented in this dissertation establishes that a novel mouse model of DYT1 dystonia has specific motor abnormalities that parallel those seen in human dystonia. These abnormal movements may result from abnormal cholinergic signaling resulting from loss of

cholinergic interneurons in the striatum. We show that repeated training on a manual skilled reaching task can lead Dlx-CKO mice to develop abnormal movements, however motor learning was intact. In a more difficult automated skilled reaching task, abnormal movements did not develop and Dlx-CKO mice displayed reduced success rates compared to controls. Reduced success rates were a result of failure to effectively transport the paw to the pellet. Combined with increases in the number of drops during pasta handling, these results suggest that Dlx-CKO mice have a primary motor deficit. We also demonstrated that Dlx-CKO mice display abnormal sequence initiation, paralleling motor sequence deficits identified in genetic carriers of DYT1 dystonia. These results have generated several hypotheses that can be tested utilizing slightly altered version of the skilled reaching task, such as head-fixed skilled reaching or the pasta reaching matrix. These studies will continue to build upon the validity of these models to identify dystonia pathophysiology, an essential step towards developing more effective therapies for dystonia.

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