DYT1 dystonia is an inherited movement disorder caused by a trinucleotide deletion (ΔGAG) in the \textit{DYT1} (\textit{TOR1A}) gene, which codes for the torsinA protein. Dr. Yuqing Li’s laboratory previously reported the characterization of a DYT1 dystonia mouse model, a knock-in carrying ΔGAG in \textit{Dyt1} (KI), which displays a motor learning deficit of motor skill transfer. We report here that this motor learning deficit was reversed with an anticholinergic drug, trihexyphenidyl (THP), a drug commonly used to treat movement problems in dystonia patients. We further show a potential substrate for the pathophysiology, a reduction in D2 receptors in the striatum in KI mice, which may abolish LTD induction in the striatum. KI mice are partially resistant to FPL64176, an agonist of L-type calcium channels involved in LTD induction. These data suggest that altered communication between cholinergic interneurons and medium spiny neurons may be responsible for the LTD deficit. We also show that despite not having visible twisting and repetitive movements similar to dystonia, KI mice show sustained muscle contractions as assessed by electromyographic analysis. Finally, we addressed whether the torsinA$^{AE}$ mutation is acting as a loss-of-function or a gain-of-function, or both, using electrophysiological recordings in hippocampal slices. Three mutant mouse lines were tested: heterozygous knock-out mice (+/Δ),
conditional homozygous knock-out mice (cKO), and torsinA$^{\Delta E}$ KI mice. We discovered that both +/Δ and cKO mice show enhanced LTP in the CA1 region of the hippocampus, while no change was observed in the KI mice. In contrast, KI mice showed significantly enhanced paired pulse ratios (PPRs), which were absent in +/Δ and cKO mice. In addition, KI mice revealed a decrease in frequency, but not amplitude or kinetics, of spontaneous excitatory post-synaptic currents in CA1 neurons, while these were not altered in +/Δ and cKO mice. The differences in synaptic alterations between the torsinA$^{\Delta E}$ KI and both the heterozygous and conditional homozogous knock-out of torsinA, lend support to a gain-of-function of torsinA$^{\Delta E}$, the mutation which underlies human dystonia.

**Keywords:** DYT1, TOR1A, dystonia, torsinA, gain-of-function, pre-synaptic
DEDICATION

This thesis is dedicated to Shay Hyman a dearly missed personal friend, exceptional scientist, and colleague.
ACKNOWLEDGEMENTS

I would like to thank God and my Lord Jesus Christ for His giving me my talents and natural abilities in order to complete this work. Thanks to my mentor, Yuqing Li, for his exceptional mentorship without whom I would not have completed this thesis. Thanks to Ning Peng, Michael Wyss, Jing Wang, Susan Campbell, David Sweatt, Miki Juno, Mark deAndrade, and Fumiaki Yokoi for the many technical assistance and discussions. Thank you to Mark deAndrade and Trent Steidinger for their personal friendship, and to my family for their love.
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INTRODUCTION

What is dystonia?

Dystonia is defined clinically as sustained muscle contractions, that often involve both agonist and antagonist muscles, causing twisting and repetitive movements or abnormal postures (Fahn, 1988). Dystonia can be classified by the means in which the disorder develops, the part of the body that is affected, or the age-of-onset of symptoms. Primary dystonia arises spontaneously and often has a hereditary component. Conversely, secondary dystonia is a result of environmental factors or insults that affect the brain, such as traumatic brain injury or stroke. Generalized dystonia has symptoms that affect most or all of the body, while focal dystonia has symptoms that affect only a specific region of the body. Early-onset dystonia symptoms appear in childhood while late-onset dystonia symptoms appear in adulthood. The most common primary, generalized, early-onset form of dystonia is Oppenheim’s dystonia (DYT1 dystonia). Symptoms of DYT1 dystonia usually appear in childhood or adolescence, first affecting one limb, then progressing to other limbs. In many cases, all musculoskeletal components of the body become affected, with the exception of the cranial muscles, which are mostly spared (Bressman et al., 2000). More than 300,000 people in North America have primary dystonia and many affected individuals can be seriously disabled and confined to a wheelchair.
**DYT1/TOR1A mutations cause primary dystonia**

To date, there are at least 20 forms of primary, monogenic dystonia, but only half have been linked to a specific gene (Breakefield et al., 2008, Camargos et al., 2008, Fuchs et al., 2009, Muller, 2009). In particular, DYT1 dystonia is inherited in an autosomal dominant fashion and has a reduced penetrance of 30% to 40% (Fahn, 1991). The gene shown to cause DYT1 dystonia is *DYT1/TOR1A*, which codes for the torsinA protein. The most common mutation in human DYT1 dystonia is a three base pair deletion (∆GAG) in the coding region of the *DYT1/TOR1A* gene (Breakefield et al., 2001). This deletion removes a glutamic acid residue from the C-terminal coding region of the torsinA protein, making a mutant form of the torsinA protein referred to as mutant torsinA or torsinA^ΔE^ (Ozelius et al., 1997). The ∆GAG mutation is related not only to generalized dystonia, but also to some forms of focal or multifocal dystonia (Maniak et al., 2003, Sibbing et al., 2003). The torsinA protein is a AAA+ protein (ATPase Associated with a variety of cellular Activities), which may function as a chaperone in the endoplasmic reticulum (see below).

**The function of wild-type and mutant torsinA**

Although the genetics of DYT1 dystonia have been well studied, the consequences of the ∆GAG mutation on torsinA function are unclear. That is, it is uncertain whether torsinA^ΔE^ is a loss-of-function or a gain-of-function mutation, or both. Recent studies using various genetic animal models suggest at least a partial loss-of-function of torsinA^ΔE^ contributes to the pathology in DYT1 dystonia (Goodchild and Dauer, 2005,
Dang et al., 2006, Yokoi et al., 2008). Biochemical and cellular studies have also suggested functional alterations of torsinA\(^{\Delta E}\) cause the pathology in dystonia (Hewett et al., 2000, Liu et al., 2003, Cao et al., 2005, Pham et al., 2006, Naismith et al., 2009, Vander Heyden et al., 2009). In contrast to the above studies, other biochemical and cellular studies provide evidence for a gain-of-function of mutant torsinA\(^{\Delta E}\). For example, in *Drosophila melanogaster* and various mammalian cell lines, overexpression of torsinA\(^{\Delta E}\) results in a redistribution of torsinA\(^{\Delta E}\) to the nuclear envelope (Hewett et al., 2000, Kustedjo et al., 2000, Kustedjo et al., 2003, Gonzalez-Alegre and Paulson, 2004, Goodchild and Dauer, 2004, Koh et al., 2004, Naismith et al., 2004). Redistributed torsinA\(^{\Delta E}\) has been reported to recruit wild-type torsinA from the endoplasmic reticulum (Torres et al., 2004, Gonzalez-Alegre et al., 2005) and may lead to a loss-of-function of wild-type torsinA. Other studies in cell culture have found that torsinA\(^{\Delta E}\), but not wild-type torsinA, interacts with proteins involved in dopamine synthesis and storage (Misbahuddin et al., 2005, O'Farrell et al., 2009).

The lack of studies that directly compare knockdown or knock-out of wild-type torsinA with torsinA\(^{\Delta E}\) make it difficult to interpret these apparently different results. Uncertainty about the ultimate consequence of torsinA\(^{\Delta E}\), whether it causes the pathophysiology through a loss-of-function, a gain-of-function, or both, prevents significant advancement in the development of more effective therapeutics for DYT1 dystonia patients, since different treatment strategies would be selected depending on how torsinA\(^{\Delta E}\) causes pathophysiology. For example, with a loss-of-function mutation, the proper treatment strategy is to restore wild-type torsinA while with a gain-of-function mutation; the proper treatment strategy is to reduce expression of torsinA\(^{\Delta E}\).
In addition to the pathophysiology, the cellular function of wild-type torsinA is not well understood, even though multiple functions have been associated with wild-type torsinA. TorsinA is a member of the AAA+ family of proteins (Ozelius et al., 1997), and is thought to function primarily as a chaperone protein. This is supported by both localization and functional studies in vitro and in vivo. Wild-type torsinA is predominantly localized in the ER and nucleus in neurons (Shashidharan et al., 2000, Konakova and Pulst, 2001, Walker et al., 2001, Walker et al., 2002, Augood et al., 2003, Rostasy et al., 2003, Xiao et al., 2004). The chaperone-like function of wild-type torsinA has been reported in vitro, but with no apparent functional alterations in torsinA^AE (Burdette et al., 2010). In addition, wild-type torsinA has been shown to prevent accumulation of α-synuclein in mammalian cell cultures and polyglutamine-repeat proteins in Caenorhabditis elegans when overexpressed (McLean et al., 2002, Caldwell et al., 2003). In patient fibroblasts, wild-type torsinA promotes protein processing through the secretory pathway, while down regulation of wild-type torsinA or expression of torsinA^AE interferes with protein processing (Hewett et al., 2007, Hewett et al., 2008).

A recent study showed that wild-type torsinA and torsinA^AE interact with snapin and that wild-type torsinA and torsinA^AE promoted vesicular recycling dynamics in vitro (Granata et al., 2008). Wild-type torsinA and torsinA^AE were shown to co-immunoprecipitate with snapin from both PC12 and SHSY-5Y cells. (Granata et al., 2008). Snapin was originally identified as a SNAP25 (synaptosomal-associated protein of 25 kDa) binding protein (Ilardi et al., 1999). Snapin has since been shown to modulate regulated exocytosis. Specifically, there is an increase in the release probability and rate of depression during high-frequency stimulation in cultured cortical neurons from snapin
knock-out mice (Pan et al., 2009). A decrease in calcium-dependent neurosecretion in cultured chromaffin cells from snapin knock-out mice has also been reported ((Tian et al., 2005, Pan et al., 2009). In addition, overexpression of wild-type torsinA or torsinA<sup>AE</sup> in SHSY-5Y cells negatively affects endocytosis without affecting exocytosis. In summary, torsinA has protein motifs that implicate it as a chaperone, and is proposed to function in either ER-secretory or endosomal membrane trafficking.

**Brain regions implicated in dystonia**

Although the pathophysiology of dystonia is still largely a mystery, the basal ganglia have been a focus of study. In addition, since there is a physiological connection between the cerebellum output and the input of the basal ganglia, the cerebellum may also be involved in the pathophysiology of dystonia (Hoshi et al., 2005). Indeed, reduced cerebellothalamic corticofugal connectivity in DYT1 dystonia patients has been observed by functional Magnetic Resonance Imaging (Argyelan et al., 2009). The basal ganglia include the striatum (caudate and putamen), the subthalamic nucleus, the globus pallidus (interna and externa), and the substantia nigra. The striatum is the first recipient of most of the inputs to the basal ganglia. It receives excitatory input from all the cerebral cortex, except the primary visual and auditory cortices, and also modulatory inputs from intralaminar thalamic and substantia nigra nuclei. Projections from the striatum to the globus pallidus interna (GPi) use γ-amino-butyric acid (GABA) as an inhibitory neurotransmitter and form two pathways, a direct pathway to the GPi and an indirect pathway to the GPi via globus pallidus pars externa (GPe). The direct pathway mainly expresses D1 receptor (D1R) receptors and is likely involved in the facilitation of wanted movement. The indi-
rect pathway mainly expresses D2 receptors (D2R) and is thought to suppress unwanted movement. Well coordinated interaction between direct and indirect pathways leads to the smooth and precise movement of the body. The projections from GPi are the major outputs of the basal ganglia controlling limb movements, use GABA as an inhibitory neurotransmitter, and form a major projection to the thalamus that eventually regulate excitability of the motor and other cortical areas involved in motor control.

The nigrostriatal pathway and its intrinsic circuits provide the striatum with modulation from dopamine, acetylcholine, GABA, nitric oxide, and adenosine. Neurochemical analyses of human brain tissues and hemidystonic primates show that the dopaminergic system may be involved in dystonia. Primates treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injections, that eventually developed Parkinson’s disease, first displayed symptoms of dystonia. Furthermore, positron emission tomography studies showed that during dystonic symptoms there was also a decrease in dopamine D2 receptor binding in the putamen (Perlmutter et al., 1998). These studies suggest an intact, but abnormally functioning dopaminergic system may be involved in dystonia, since dystonia symptoms appear only before the neurotoxic effects of MPTP.

Animal Models of DYT1 dystonia

Animal models of neurological diseases are extremely useful for understanding the pathophysiology of the disorder and for developing effective therapeutic treatments. Past attempts using experimentally induced dystonia in animals (Berardelli et al., 1998) and genetic animal models (Richter and Loscher, 2000) with natural spontaneous mutations have provided clues about the nature of the disorder. These models have contrib-
uted substantially to our understanding of the pathophysiology of dystonia. However, these animal models have limitations in identifying the primary insults of dystonia since the lesions produced or genes mutated in these animal models are different from those observed in human patients with the disease. As mentioned previously, cloning of the DYT1 gene in humans and subsequent identification of the mouse homolog (Ozelius et al., 1999) made it possible to use reverse genetics to create animal models of DYT1 primary dystonia. Sharma, Shashidharan, and their respective colleagues, as well as Shashidharan’s laboratory reported the creation of transgenic lines that overexpress human mutant torsinA that show motor deficits (Sharma et al., 2005; Shashidharan et al., 2005). The Li laboratory successfully generated a knock-in (KI) of Dyt1ΔGAG (Dang et al., 2005).

**The Dyt1ΔGAG knock-in mouse model of DYT1 dystonia**

The KI of Dyt1ΔGAG mice harbor a single mutation in the mouse Dyt1/Tor1A gene, which mimics the clinically identified mutation, by removing a GAG codon that codes for one of the pair of glutamic acid residues in the C-terminus of the torsinA protein (Dang et al., 2005). These mice are heterozygous for the KI mutation, which make them analogous to human patients with DYT1 dystonia.

While KI mice do not have overt dystonic postures and abnormal movements, they do have motor behavioral deficits (Dang et al., 2005). KI mice were evaluated in a series of motor behaviors to determine if KI mice have any motor impairment. Body form and postures were observed followed by rotarod, beam-walking, pawprint analysis, and open field tests. At 6 months of age, KI mice were similar to wild-type mice and
showed no observable hindlimb extension or truncal arching. Normal splaying of the hindlimbs and righting reflexes were also observed. On the rotarod performance, both KI and control wild-type (WT) mice learned to walk on the rotating rod and improved by trial with no statistically significant difference observed between the two genotypes. On the beam-walking test, male KI mice displayed significantly more slips than male WT mice. There was no significant difference in slip number between female KI and female WT mice. There was also no significant difference between the two genotypes in latency to cross the beam in both male and female mice. In pawprint analysis, both stride length and base length were not significantly different between male KI and male WT mice, but there was a significant increase in overlap length. There was no significant difference in pawprint analysis between female KI and female WT mice. In the open field apparatus, male KI mice had a significant increase in overall activity as measured by an increase in horizontal activity, total distance traveled and marginal distance traveled compared to male WT mice. There was no significant difference between the female KI and female WT mice. Regardless of gender, KI mice also had an increase in clockwise circling behavior compared to WT mice.

Measurements of striatal dopamine and its metabolites DOPAC and HVA showed only a significant decrease in HVA in male KI mice compared to male WT mice. Pathologically there were no observable anatomical changes in the basal ganglia, cerebellum, or cerebral cortex, but immunohistochemistry revealed an increase in torsinA and ubiquitin inclusions in the brain stem of male KI mice similar to human DYT1 patients. Female KI mice had no observable anatomical changes and no torsinA and ubiquitin inclusions in their brain stems.
Dyt1AGAG knock-in mice show impaired skill transfer

In addition to the above characterization, KI mice were examined for impairments in motor learning. Non-symptomatic mutant DYT1 carriers have been found to have impaired sequential motor learning (Ghilardi et al., 2003). A transgenic mouse model over-expressing mutant human torsinA has also been shown to have deficient performance on the rotarod task (Sharma et al., 2005). Unlike these transgenic mice, the Li lab’s KI mice did not display a rotarod deficit (Dang et al., 2005). However, to determine if other types of motor learning are affected, in our preliminary studies we tested the KI mice on a novel skill transfer task. The motor task involved forcing mice to run on a treadmill with increasing speeds over a two-week training period. They were then tested on an accelerating rotarod test, which additionally forced them to maintain effective balance. Our preliminary studies showed that WT mice that were trained on the treadmill significantly improved their performance on the rotarod from trial to trial (trial 1: P = 0.16, trial 2: P = 0.02, trial 3: P = 0.004) in comparison to untrained WT mice who were placed on the treadmill with the belt turned off. This improved performance was detectable even on the first day of training. Similar to the wild-type mice, KI mice that were trained on the treadmill showed an initial increase in performance on the rotarod during trial 1 (P = 0.05), in comparison to KI mice that were not pre-trained on the treadmill. However, unlike the wild-type mice, this improvement was not sustained in subsequent trials (trial 2: P = 0.68, trial 3: P = 0.70). These results suggest that trained WT mice had enhanced motor learning abilities due to their previous training on the treadmill, while KI mice were unable to transfer their motor skills from the treadmill training to the rotarod task.
Overall, this preliminary investigation shows that since KI mice show a motor skill transfer deficit, they may provide a good mouse model of DYT1 dystonia and can potentially be a good model to test experimental therapeutics for DYT1 dystonia.
THP RESCUES THE MOTOR SKILL TRANSFER DEFICIT IN DYT1ΔGAG KI MICE

Background and Rationale

Anticholinergics are a major category of medications long used to treat generalized dystonia (Fahn, 1983). The use of anticholinergics evolved with little understanding of the pathophysiology of dystonia and appeared to have been spurred by its successful use in treating other movement disorders. One of the most widely used anticholinergics is trihexyphenidyl (THP). THP has been shown to be slightly more effective at blocking M1 muscarine acetylcholine receptors than other muscarinic acetylcholine receptors (mAchRs) (Giachetti et al., 1986). We tested the efficacy of THP in reversing the motor skill transfer deficit in KI mice.

Methods

The task performed to determine reversibility of the motor learning deficit with THP was done with a total of 64 males, half injected with THP intraperitoneally (+THP) every other day from beginning of treadmill training to the end of the rotarod testing and half injected intraperitoneally with normal saline (-THP) every other day from beginning of treadmill training to the end of the rotarod testing. THP solutions with dose
of 0.8 mg/kg in normal saline were injected two hours before the behavioral test on the
days of treadmill experiments. On the non-testing days, THP was injected in the treated
group in the morning. These mice were on average 8 months old. The rotarod data was
analyzed using SAS 9.1 software. Significance was assigned at the $P \leq 0.05$ level. Two-
way ANOVA with repeated measurement was used to analyze latency to fall. Trial num-
ber was treated as a repeated variable together with genotype as the main effect. For this
treatment comparison test, repeated measures statistics on latency to fall data for three
days of testing, three trials each day were compared in the same genotype groups with
and without treadmill training. When significance was reached, pair-wise differences
were then computed to drive least square means and standard errors.

Results

![Rotarod](image)

Figure 1: THP reversed motor learning deficits in *Dyt1* ΔGAG knock-in mice. THP-treated *Dyt1* ΔGAG mice, like WT mice, had accelerated improvement in performance on the rotarod after treadmill training.
As observed in our preliminary study WT mice with previous treadmill running experience performed significantly better on the accelerating rotarod test, than untrained WT mice without THP, (P = 0.05, Fig. 1). WT mice treated with THP also showed heightened performance on the rotarod with prior treadmill training (P = 0.02). There was no statistical difference of the drug in WT mice on the effects of training. As determined previously, KI mice did not show enhanced performance with treadmill training (P = 0.55). However, with administration of THP, KI mice were also able to achieve higher latency in rotarod performance after treadmill training (P = 0.05), similar to the enhancement in WT mice. These data showed that KI mice show a statistically significant motor learning transfer deficit, as observed in the preliminary studies. Importantly, administration of THP could reverse the motor learning transfer deficit in KI mice, such that the motor learning transfer in KI mice was similar to that seen in wild-type mice.

**Discussion**

We previously reported that KI mice do not display deficits in the rotarod performance (Dang et al., 2005). However, KI mice lacked the accelerated performance improvement on the rotarod after treadmill training that was observed with WT mice. Interestingly, this deficit in KI mice could be ameliorated with THP. From this behavioral task perspective, we hypothesize that WT mice, and not KI mice, are able to acclimate to the accelerated running of the treadmill while they also learn to balance on the rotarod. From the perspective of neuronal activity, this skill transfer could represent a prior priming of the neuronal apparatus involved in motor learning, so that further learning is more easily achieved in WT mice. The rescue of HFS-LTD in corticostriatal tissue slices (data
not shown) and the behavioral abnormalities with THP in KI mice point to aberrant synaptic plasticity as a possible mechanism for the development of dystonia-associated symptoms. Another important conclusion from our studies is that KI mice may be a good model for testing pharmaceutical therapeutics and determining mechanism of disease.
DYT1ΔGAG KI MICE ARE PARTIALLY RESISTANT TO FPL64176-
INDUCED DYSTONIC-LIKE SYMPTOMS

Background and Rationale

KI mice show a deficit in high frequency stimulation induced long-term depression (HFS-LTD) (Dang et al., 2011). Corticostriatal HFS-LTD has been shown to require D2 dopamine receptor (D2R)-induced inhibition of acetylcholine release from interneurons, which normally act on mAchRs of medium spiny neurons (Wang et al., 2006). This inhibition of mAchR activation removes the inhibition of calcium influx through the Cav1.3 calcium channels in response to synaptic depolarization. We hypothesized that because of a decrease in D2R in KI mice (Dang et al., 2011); acetylcholine release may not be properly suppressed during HFS, causing an over-activation of mAchRs. This overactivation would cause KI mice to have a greater inhibition of Cav1.3 calcium channels, which will alter their behavioral response to calcium channel activation.
Methods

To explore this possibility, we examined the ability of the Cav1.3 calcium channel agonist, FPL64176, to produce dystonic-like postures and behaviors in wild-type and KI mice. This calcium channel activating drug is known to produce dystonic-like behavior in wild-type mice (Jinnah et al., 2000). Ten WT and six KI mice were injected subcutaneously on four consecutive days with escalating doses of FPL64176 (2, 4, 8, 12 mg/kg). Mice were placed in a clear plastic box 18 x 30 cm similar to their home cage. The dystonic-like movements and postures were assessed on a four point scale for one minute at 10 minute intervals for 1 hour by observers blinded to genotype. Data was analyzed by using logistic regression (GENMOD) with GEE model and normalized to WT mice.
Both KI and WT mice developed FPL64176-induced dystonic-like postures and behaviors. However, on the four-point scale which semi-quantitatively represents the severity of dystonic-like postures (1 showing normal behaviors and postures, but slight slowness of movements to 4 showing severe impairments with no ambulation, sustained muscle contractions, and not upright a majority of the time) KI mice displayed significantly less overall severe dystonic-like postures and behaviors across all FPL64176 doses.

Figure 2: Calcium channel agonist FPL64176 causes dystonia-like movements in WT mice, an effect that is attenuated in Dyt1 ΔGAG mice. Inset: ten minutes after the injection of FPL64176, the representative Dyt1 ΔGAG mouse (top) demonstrated slowness of movement, but remained upright and able to respond to tactile stimulation. The WT mouse (bottom) had sustained abnormal postures and was not able to remain upright during the majority of the 10 minutes. (Dang et al., 2011).
On average, the KI mice responded to the drug treatment with behavior that was semi-quantitively measured as a half point less severe phenotypically than WT littermates. Scores were fit to a normal distribution and normalized to WT mice. Overall these data provide additional evidence for the pathway involved in DYT1 dystonia in our KI mice.

**Discussion**

Our data show that KI mice are partially resistant to the behavioral changes induced by FPL64176, a Cav1.3 channel agonist. When applied to brain tissue, FPL64176 has been demonstrated to induce LTD, which is sufficiently strong that no further HFS-LTD induction could be achieved (Adermark and Lovinger, 2007). Given the partial resistance to FPL64176 demonstrated by KI mice, we speculate that the reverse of this occlusion phenomenon may be occurring in our mouse model, whereby the suppression of calcium channel opening, achieved by modulation from the interneurons, may be strong enough to attenuate the opening of channels by a direct agonist.

We speculate that KI mice have aberrant D2R activity that causes cholinergic interneurons to be disinhibited from secreting acetylcholine. This acetylcholine acts on medium spiny neuron mAchRs to inhibit the activation of Cav1.3 channels. The inhibition of Cav1.3 channels reduces the release of endocannabinoids, thought to be responsible for the downregulation of glutamate release that in WT mice leads to HFS-LTD (Calabresi et al., 1992, Wang et al., 2006). Consistent with this model is the restoration of skill transfer in KI mice by an anticholinergic such as THP, which bypasses the D2R
deficiency and acts directly on the post-synaptic mAChRs to rescue the HFS-LTD and motor learning skills.
ELECTROMYOGRAPHIC ANALYSIS REVEAL DYT1ΔGAG KI MICE HAVE SUSTAINED MUSCLE CONTRACTIONS

Background and Rationale

KI mice have been shown to have behavioral deficits similar to deficits reported in DYT1 dystonia patients. However, as reported above, KI mice are not known to have overt dystonia or sustained co-contractions of agonist and antagonist muscles causing twisting and repetitive movements. Recently, a mouse model overexpressing human mutant torsinA revealed sustained co-contractions by electromyographic recordings, however, these mice subsequently lost the transgene expression casting some doubt on the validity of this mouse model (Chiken et al., 2008).

Methods

To determine if our KI mice have sustained muscle contractions, we performed electromyography (EMG) analysis on KI mice. An F20-EET transmitter (Data Science International) is light (only 3.9 g.) and small (1.9cc) making it ideal to use in mice to record two muscle EMGs simultaneously in our case a major hindlimb flexor and extensor muscle. After animals were put under anesthesia with a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine, the two biopotential electrodes were placed in the rectus femoris and bicep femoris, with the excess wire and transmitter implanted subcutaneously. Ani-
mals were allowed to recovery from surgery in their home cage for 24-48 hrs. before the transmitter was turned on remotely and EMG recorded overnight with video monitoring. EMG recordings were digitized and band pass filtered at 1-100 hertz. Number of pairs of animals (n=3).

Results

![Figure 3: Example EMG traces from the bicep femoris of Dyt1 ΔGAG knock-in mice and WT control mice. (A) Regardless of protocol applied sustained contraction was detected in the EMG traces from Dyt1 ΔGAG knock-in mice. (B) WT control mice never showed sustained contraction.]

WT and KI mice showed very low baseline recordings of approximately 3 to 4 micro-volts, and alternating, repetitive contractions of agonist and antagonist muscles when the mice walked (Figure 3B). However, sometimes KI mice showed large, sustained contractions that were never seen in WT mice (Figure 3A).

We *a priori* defined sustained contraction as those contractions that were three, four, or five times above baseline and that lasted at least 10 seconds. Depending upon the
protocol applied, KI mice showed varying levels of sustained contractions (Table 1) over a 12 hour dark period. These data show that KI mice have sustained muscle contractions and validate the effectiveness of our mouse model to DYT1 dystonia.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>No. of Sustained Contractions in ∆GAG1</th>
<th>No. of Sustained Contractions in ∆GAG2</th>
<th>No. of Sustained Contractions in ∆GAG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: ≥20 µV for 10 sec.</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2: ≥16 µV for 10 sec.</td>
<td>4</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>3: ≥13 µV for 10 sec.</td>
<td>41</td>
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</tbody>
</table>

Table 1: Number of sustained contractions in three Dyt1 ∆GAG KI mice over 12 hours recorded during the “lights off” period. Although, the number of sustained contractions in Dyt1 ∆GAG knock-in mice varies, WT control mice never showed sustained contraction.

**Discussion**

We show evidence of abnormal electromyographic recordings in hindlimb muscles in our KI mice. Electromyography is a powerful tool for investigating the bioelectrical properties in electrically excitable tissue such as muscles. With recent advances in
technology, transmitters and wires have become sufficiently small to examine these properties in mice. The finding that KI mice have sustained muscle contractions provides evidence that KI mice do have clinically defined dystonia, providing additional evidence that our KI mice provide a useful model of DYT1 dystonia.
TORSINA$^{AE}$ ACTS THROUGH A GAIN-OF-FUNCTION MECHANISM TO ALTER PRE-SYNAPTIC FUNCTION IN THE HIPPOCAMPUS

**Background and Rationale**

In an attempt to determine whether the consequence of torsinA$^{AE}$ is a gain-of-function, a loss-of-function, or both and whether torsinA$^{AE}$ affects synaptic function ex vivo, we performed electrophysiological recordings in a model glutamatergic synapse in acute hippocampal slices from three lines of mutant mice. The first mouse line was a heterozygous knock-out mouse (+/Δ), as a knockdown model of torsinA. The second mouse line, a conditional knock-out mouse (cKO), was investigated as a model of complete knock-out of torsinA. The third mouse line, a Dyt1 ΔGAG knock-in mouse (KI), was analyzed as a model of the human mutation (Dang et al., 2005).

All experiments were carried out in compliance with the USPHS Guide for Care and Use of Laboratory Animals and approved by the IACUC at the University of Alabama at Birmingham (UAB). Dyt1 ΔGAG heterozygous knock-in (KI), heterozygous knock-out (+/Δ), and cerebral cortex and hippocampus-specific conditional knock-out (cKO) mice were prepared and genotyped by PCR as previously described (Dang et al., 2005, Yokoi et al., 2008, Yokoi et al., 2009). The KI mice harbor a single mutation in the Dyt1/Tor1a gene mimicking the clinically identified mutation by removing a GAG sequence that codes for one of the two glutamic acid residues in the C-terminus of the
torsinA protein (Dang et al., 2005). The cKO mice were generated by breeding mice harboring two *loxP* sites flanking two exons of the *Dyt1/Tor1a* gene with mice that express cre-recombinase driven by the cerebral cortex and hippocampus glutamatergic neuron-specific promoter Emx-1 (Emx1-cre) (Guo et al., 2000, Chan et al., 2001). This selectively leads offspring with a loss of the *Dyt1/Tor1a* gene in these neurons (Yokoi et al., 2008). Finally, we have generated +/-Δ mice. These mice were generated by breeding mice that harbor two *loxP* sites flanking the two exons of the *Dyt1/Tor1a* gene with mice that express cre-recombinase driven by the ubiquitously expressed promoter cytomegalovirus (CMV-cre) (Schwenk et al., 1995). The male +/-Δ mice were then bred with wild type female mice to generate more +/-Δ mice for experiments. These mice demonstrate a knockdown of torsinA in all cells where the *Dyt1/Tor1a* gene is naturally expressed. For the following experiments, only male mice were used. All mice were housed under a 12-hour light 12-hour dark cycle with *ad libitum* access to food and water.

**Methods**

*Preparation of Hippocampal Slices*

Field recordings were performed in 6 KI and 5 matched wild-type littermate control mice, 4 +/-Δ and 6 matched wild-type littermate control mice, and 4 cKO and 3 matched control littermate mice. Hippocampi of adult mutant or control mice were rapidly removed and briefly chilled in ice-cold cutting saline (110 mM sucrose, 60 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 28 mM NaHCO3, 5 mM D-glucose, 500 µM CaCl2, 7 mM MgCl2, and 600 µM ascorbate). Transverse slices 400-µm thick were prepared with a Vibratome and maintained at least 45 min. in a holding chamber containing
50% artificial cerebral spinal fluid (aCSF) (125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH$_2$PO$_4$, 25 mM NaHCO$_3$, 25 mM D-glucose, 2 mM CaCl$_2$, and 1 mM MgCl$_2$) and 50% cutting saline. The slices were then transferred to a recording chamber and perfused (1 ml/min.) with 100% aCSF. Slices were allowed to equilibrate for 60–90 min. in a Fine Science Tools interface chamber at 30°C. All solutions were continuously bubbled with 95% O$_2$/5% CO$_2$.

Field Recording

Set-up and Electrode Placement. For extracellular field recordings, glass recording electrodes were pulled from capillary glass tubes using a horizontal electrode puller (Narishige), and filled with aCSF. The input resistance of each electrode was tested by applying a current pulse and then adjusting the tip until a resistance of 1–3 MΩ was obtained. These recording electrodes were placed in stratum radiatum of hippocampal area CA1. Test stimuli were delivered to the Schaffer collateral/commissural pathway with bipolar Teflon coated platinum stimulating electrode positioned in stratum radiatum of area CA3. Responses were recorded using AxoClamp pClamp8 data acquisition software. Excitatory Post-Synaptic Potential (EPSP) slope measurements were taken after the fiber volley to eliminate contamination by population spikes.

Paired pulse ratio. Paired pulse ratios (PPRs) was measured at various inter-stimulus intervals (10, 20, 50, 100, 150, 200, 250, and 300 msec.). All experimental stimuli were set to an intensity that evoked 50% of the maximum field EPSP (fEPSP)
slope. Averages were compared by Student’s t-test using n (number of slices) as the level of analysis. Significance was assigned at P < 0.05.

*Long term potentiation.* Followed by at least 20 min. of stable baseline recording, long-term potentiation (LTP) was induced with two, 100 Hz tetani (1 sec.), with an interval of 20 sec. between tetani. Synaptic efficacy was monitored by recording fEPSPs every 20 sec. beginning 0.5 hr prior to and ending 3 hrs. after the induction of LTP (traces were averaged for every 2 min. interval). For statistical comparison, all traces were averaged over 2 min. intervals from 36 min. to 176 min., when a majority of recordings ceased. The first 35 min. were excluded to prevent averaging the post-tetanic potentiation. Statistics were performed using SAS/STAT Analyst software (version 9.1) for LTP with genotype, slice, and time considered in the model and compared by repeated measure ANOVA. Significance was assigned at P ≤ 0.05.

*Input-Output Curves.* Test stimuli were delivered and responses recorded at 0.05 Hz; every six consecutive responses over a 2 min. period were pooled and averaged. fEPSPs were recorded in response to increasing intensities of stimulation (from 2.5 µA to 45.0 µA). Averages of individual stimulus intensities were compared by Student’s t-test. Significance was assigned at P < 0.05.

*Whole-cell recordings*

A Zeiss Axioskop FS microscope (Zeiss, Thornwood, NY, USA), equipped with Nomarski optics, 40× water immersion lens and infrared illumination were used to view
neurons in the slices. aCSF was heated to 32–35°C by an in-line heater (Warner Instruments, Hamden, CT). The temperature was monitored by a thermometer placed in the recording chamber. Cells were labeled intracellularly with biocytin (0.5%; Sigma, St. Louis, MO, USA) and processed as previously described (Campbell and Hablitz, 2004) to confirm identification. Whole-cell voltage-clamp recordings were obtained as described previously (Campbell and Hablitz, 2004). Patch electrodes had an open tip resistance of 3–4 MΩ. Tight seals (>2 GΩ before breaking into whole-cell mode) were obtained under visual guidance. Series resistances during recording were allowed to vary from 10 to 20 MΩ and were not compensated. Recordings were terminated whenever significant increases (>20%) in series resistance occurred. The intracellular solution for recordings contained 125 mM K gluconate, 10 mM KCl, 10 mM HEPES, 2 mM Mg-ATP, 0.2 mM Na-GTP, and 0.5 mM EGTA. Osmolarity and pH were adjusted to 290 mOsm and 7.3, respectively. All recordings were made in the presence of 10 µM bicuculline (Sigma, St. Louis, MO) in the bath solution to block GABA_A receptors. For recording miniature Excitatory Post-Synaptic Currents (mEPSCs), 1 µM tetrodotoxin (TTX; Sigma, St. Louis, MO) was also added to the bath solution to block voltage-dependent sodium channels. Spontaneous and mEPSCs were analyzed using the Mini Analysis program from Synaptosoft, Inc. (Decatur, GA, USA). Frequency, amplitude, rise and decay time of spontaneous EPSCs (sEPSCs) and mEPSCs were averaged across animals of the same genotype and compared by Student’s t-test by using the number of neurons (n) as the level of analysis. Significance was assigned at P < 0.05.
Results

We first examined the hippocampal expression levels of torsinA in each line of mice by Western blot analysis. Heterozygous knock-out mice showed a significant reduction in hippocampal torsinA level when compared to wild-type control littermates (33.1%; P=0.004; n=4 each; Figure 4A). Conditional knock-out mice had the most dramatic reduction in torsinA level when compared to their control littermates (12.9%; P=0.00001; n=5 control with 3 cKO; Figure 4B). TorsinA level was also significantly reduced in KI mice when compared to wild-type control littermates (66.4%; P = 0.04; n=3 each; Figure 4C). The low level of torsinA protein detected in cKO mice is likely a cause of torsinA expression in inhibitory neurons, since Emx1-cre is only expressed in glutamatergic neurons (Guo et al., 2000, Chan et al., 2001). Additionally, it has previously been shown that wild-type torsinA is degraded through the proteasome pathway, while torsinA \( ^{\gamma E} \) is degraded via both the proteasome and lysosomal-autophagy pathways, thus possibly explaining the reduced levels of torsinA in KI mice (Giles et al., 2008, Gordon and Gonzalez-Alegre, 2008). These data are consistent with a reduction of torsinA in KI and +/-Δ mice, and likely a complete loss of torsinA in glutamatergic neurons in cKO mice in the hippocampus.
In cultured cell lines overexpressing human mutant torsinA, it has been demonstrated that the expression of genes associated with glutamate receptor-mediated synaptic plasticity are altered (Grundmann et al., 2007, Martin et al., 2009). In addition, glutamate

Figure 4: **TorsinA level is reduced in KI and +/-Δ, but nearly absent in cKO mice.** Representative band images of western blot (left) and the quantified hippocampal torsinA levels (right) from WT and +/-Δ (A), control (Ctrl) and cKO (B), and WT and KI mice (C). The torsinA levels were normalized to β-tubulin or GAPDH and the vertical bars represent means ± SEM. *$P< 0.05$, **$P < 0.01$, and ***$P < 0.005$. 

In cultured cell lines overexpressing human mutant torsinA, it has been demonstrated that the expression of genes associated with glutamate receptor-mediated synaptic plasticity are altered (Grundmann et al., 2007, Martin et al., 2009). In addition, glutamate
receptor-mediated LTP, a form of synaptic plasticity in the CA1 region of the hippocampus has been well documented and is highly reproducible. Therefore, we performed field recordings in hippocampal slices to determine whether CA1 LTP was altered. We have previously reported that KI mice showed no change in CA1 LTP compared to wild-type control littermates (Yokoi et al., 2009). Unlike KI mice, +/Δ mice had significantly enhanced CA1 LTP compared to their wild-type control littermates (WT, 132% ± 6%, 29 slices from 5 mice; +/Δ, 153% ± 5.7%, 28 slices from 6 mice, P<0.05; Figure 5A).

Similar to +/Δ mice, cKO mice showed enhanced CA1 LTP compared to control littermates (Ctrl, 142% ± 8.5%, 18 slices from 4 mice; cKO, 173% ± 13%, 8 slices from 3 control mice; P=0.05; Figure 5B). These data suggest that knockdown or knock-out of wild-type torsinA leads to enhanced CA1 LTP. In contrast, KI mice have normal CA1 LTP, as previously reported (Yokoi et al., 2009).
Figure 5: LTP in the CA1 region of hippocampus of +/Δ and cKO is significantly enhanced. Compared to their control mice, there was a significantly enhanced LTP in +/Δ (A), and cKO mice (B), but not in KI mice, as reported previously (Yokoi et al., 2009). Filled circles denote data from WT or control littermates. Open circles were from mutant mice.
With a difference in long-term synaptic plasticity among the three lines of *Dyt1/Tor1a* mutant mice, we next examined if there was also a difference in short-term plasticity. We examined paired pulse ratios (PPRs) to explore an indirect measurement of the probability of synaptic vesicle release in all three lines of *Dyt1/Tor1a* mutant mice. In addition, PPRs are inversely proportional to the probability of synaptic vesicle release. PPRs at four different inter-stimulus intervals were significantly enhanced in KI mice compared to wild-type control littermates (20 msec. WT 18.0 ± 4.7, KI 35.7 ± 5.4; P<0.05; 100 msec. WT 18.0 ± 3.0, KI 35.5 ± 6.5; P<0.05; 250 msec. WT 1.63 ± 2.5, KI 11.5 ± 3.3; P<0.05; 300 msec. WT 0.64 ± 2.7, KI 9.06 ± 3.6; P<0.05; Figure 6E). In contrast, neither the +/-Δ nor the cKO mice showed enhanced PPRs at any inter-stimulus interval compared to their respective wild-type control or control littermates (Figures 6A and 6C). To distinguish between a pre-synaptic or post-synaptic mechanism for the enhanced PPRs in KI mice, we obtained input-output curves to measure the post-synaptic potential slope versus varying stimulus intensities. Here we use the term pre-synaptic loosely to refer to the CA3 Schaffer collaterals. In addition, pre-synaptic also refers to the group of neurons that control CA3 neurons excitability, by either direct or indirect synaptic connections. Finally, a combination of these two alternatives would also be referred to as pre-synaptic. CA1 pyramidal neurons are referred to as post-synaptic. The absolute values of the input-output relationship slopes were plotted in all three lines of mice. KI mice showed no change in their input-output relationship from wild-type control littermates (Figures 6F). Similar to KI mice, +/-Δ and cKO mice did not significantly differ at any stimulus intensity from their wild-type control or control littermates (Figure 6B and 6D). These data suggest that knockdown or knock-out of torsinA have no short-
term plasticity alteration, while KI mice have a short-term plasticity alteration. This alteration in short-term plasticity in KI mice is likely a pre-synaptic functional alteration that may be a property of the CA3 neurons. Alternatively, this pre-synaptic functional alteration may be a property of the group of neurons that control CA3 neurons’ excitability, either directly or indirectly.
Figure 6: Paired pulse ratios are significantly enhanced only in KI mice. PPRs are enhanced in KI mice (E), but not in +/Δ (A) or cKO mice (C). The KI mice showed significantly enhanced PPRs at four inter-stimulus intervals (20, 100, 250, and 300 msec.). The input-output curves in +/Δ (B), cKO (D), and KI mice (F) were unaffected. Filled circles denote data from WT or control littermates. Open circles were from mutant mice. *P<0.05.
Figure 7: KI mice had significantly enhanced sEPSC frequency. (A) Representative traces for sEPSCs. KI mice had a significantly decreased frequency of spontaneous excitatory post-synaptic currents (B), but no change in either the amplitude (C) or kinetics (rise or decay time) of these events (D). The vertical bars represent means ± SEM. *P<0.05.
To further explore the enhanced PPRs in KI mice spontaneous and miniature excitatory post-synaptic currents (sEPSCs and mEPSCs, respectively; Figures 7A and 8A) were recorded from CA1 pyramidal cells. Both pre-synaptic and post-synaptic measurements were analyzed for sEPSCs and mEPSCs. The pre-synaptic measurement of frequency of the occurrence of sEPSCs and mEPSCs was measured. The post-synaptic measurements of amplitude and rise and decay times of these events were also measured. There was a significant decrease in sEPSC frequency compared to wild-type control littermates (WT, 0.85 ± 0.10; KI, 0.59 ± 0.08, P<0.05; Figure 7B), but there was no significant change in sEPSC amplitude in KI mice compared to wild-type control littermates (WT, 13.1 ± 0.8, KI, 12.6 ± 0.5, P>0.05; Figure 7C).
Figure 8: KI mice had no change in mEPSCs. (A) Representative traces for mEPSCs. KI mice had no change in frequency (B), amplitude (C), or kinetics (rise or decay time) (D) of miniature excitatory postsynaptic currents. The vertical bars represent means ± SEM.
In addition, no significant change in sEPSC rise and decay times were detected in KI mice compared to wild-type control littermates (WT rise, 2.25 ± 0.03, KI rise, 2.1 ± 0.06, P>0.05 and WT decay, 5.4 ± 0.1, KI decay, 4.9 ± 0.2, P>0.05; Figure 7D). In contrast to sEPSCs, there was no significant change in the mEPSC frequency in KI mice compared to wild-type control littermates (WT, 0.40 ± 0.09, KI, 0.42 ± 0.08, P>0.05; Figure 8B). Similar to sEPSCs, mEPSC amplitude (WT, 12.0 ± 0.4, KI, 12.5 ± 0.4, P>0.05; Figure 8C) and rise and decay times were unchanged in KI mice compared to wild-type control littermates (WT rise, 2.2 ± 0.04; KI rise, 2.2 ± 0.05, P>0.05; WT decay, 4.75 ± 0.25; KI decay, 5.1 ± 0.3, P>0.05; Figure 8D). These data provide additional support for a pre-synaptic alteration in KI mice. In addition, along with the enhanced PPRs, the decrease in sEPSC frequency may suggest a decrease in the excitation of the group of neurons that contribute, either directly or indirectly, to CA3 neurons excitability.

**Discussion**

We provide evidence for a gain-of-function of the Dyt1 ΔGAG mutation in the hippocampus. We showed enhanced PPRs were present in Dyt1 ΔGAG KI mice, but not in Dyt1 heterozygous knock-out, +/-, or Dyt1 conditional knock-out, cKO, mice. We also showed that sEPSCs, but not mEPSCs were affected in KI mice using whole-cell recordings. We found a decrease in frequency of sEPSCs, but no change in the frequency of mEPSCs. There was no change in amplitude or kinetics for either sEPSCs or mEPSCs in KI mice. Our data implies that the Dyt1 ΔGAG mutation leads to a pre-synaptic functional alteration *ex vivo*, consistent with recent findings that have implicated torsinA^{ΔE} in affecting vesicular recycling dynamics *in vitro* (Granata et al., 2008). Finally, we showed
+/Δ and cKO mice had enhanced CA1 LTP, while KI mice do not have enhanced CA1 LTP (Yokoi et al., 2009). This implies that knockdown or knock-out of wild-type torsinA alone leads to enhanced CA1 LTP.

In DYT1 dystonia, there is considerable evidence that the Dyt1 ΔGAG mutation is a gain-of-function. For example, in Drosophila melanogaster and various mammalian cell lines overexpressing torsinAΔE resulted in a redistribution of torsinAΔE to the nuclear envelope (Hewett et al., 2000, Kustedjo et al., 2000, Kustedjo et al., 2003, Gonzalez-Alegre and Paulson, 2004, Goodchild and Dauer, 2004, Koh et al., 2004, Naismith et al., 2004). Redistributed torsinAΔE has been reported to recruit wild-type torsinA from the endoplasmic reticulum (Torres et al., 2004, Gonzalez-Alegre et al., 2005) and may lead to a loss-of-function of wild-type torsinA. Studies have also found that torsinAΔE, but not wild-type torsinA, interact with proteins involved in dopamine synthesis and storage in cultures of mammalian cells (Misbahuddin et al., 2005, O'Farrell et al., 2009). These latter studies are similar to patients, DYT1 ΔGAG mutation carriers, who have dopamine and dopamine metabolite alterations (Furukawa et al., 2000, Augood et al., 2002). So far, however, the evidence for torsinAΔE being a gain-of-function has come mainly from overexpression of torsinAΔE in cell culture studies without proper control for the level of wild-type torsinA. Here, we further provide evidence for a gain-of-function of torsinAΔE through demonstration of enhanced PPRs in KI mice. No enhanced PPRs were found in +/Δ or cKO mice, therefore, the enhanced PPRs cannot be explained simply as a reduced level of torsinA protein in KI mice. The enhanced PPRs, which implies a decrease in the probability of neurotransmitter release, to our knowledge, is the first demonstration of a gain-of-function of mutant torsinA ex vivo.
The cellular mechanism of this gain-of-function of torsinA$^{\Delta E}$ is an exciting new direction of future research in DYT1 dystonia. There are two possible outcomes of the gain-of-function of torsinA$^{\Delta E}$. TorsinA$^{\Delta E}$ may have a new function that wild-type torsinA does not, which may cause abnormal cellular function in cells that express torsinA. Another possibility is that torsinA$^{\Delta E}$ may prevent a normal function, similar to a dominant negative effect, which may prevent a normal cellular function in cells that express torsinA. Future research will determine which of these possibilities are correct and with this discovery we may be closer to discovery more effective therapies to treat DYT1 dystonia.

There are many additional studies which provide evidence that wild-type torsinA may have additional cellular functions. One recent study showed that wild-type torsinA and torsinA$^{\Delta E}$ interact with snapin and promoted vesicular recycling dynamics in vitro (Granata et al., 2008). Snapin was originally identified as a synaptosomal-associated protein of 25 kDa (SNAP-25) binding protein (Ilardi et al., 1999). Snapin has since been shown to facilitate regulated exocytosis. Specifically, there is an increase in the release probability and rate of depression during high-frequency stimulation in cultured cortical neurons from snapin knock-out mice (Pan et al., 2009). A decrease in calcium-dependent neurosecretion in cultured chromaffin cells from snapin knock-out mice has also been reported ((Tian et al., 2005, Pan et al., 2009). We provide additional electrophysiological evidence that the gain-of-function of mutant torsinA results in a decrease in pre-synaptic function. Specifically, the enhanced PPRs and decreased sEPSC frequency could be consistent with torsinA$^{\Delta E}$ negatively affecting neurotransmitter exocytosis or endocytosis. Determining the exact mechanism or mechanisms of how torsinA$^{\Delta E}$ alters pre-synaptic function, are exciting future studies.
Finally, our data suggest knockdown or knock-out of wild-type torsinA, as modeled by +/Δ and cKO mice, enhanced CA1 LTP. In contrast, our KI mice did not exhibit enhanced CA1 LTP. Other groups have demonstrated enhanced synaptic plasticity alterations similar to our +/Δ and cKO mice. For example, Pisani and colleagues have demonstrated enhanced corticostriatal LTP, absence of corticostriatal long-term depression (LTD), and absence of depotentiation (Martella et al., 2009). Pisani and colleagues did not find electrophysiological deficits in mice overexpressing human wild-type torsinA or non-transgenic mice. These data collectively imply perturbing the level of torsinA may interfere with LTP. Interestingly, KI mice have no enhanced CA1 LTP despite have a reduced protein level of torsinA. TorsinA\textsuperscript{ΔE} may have no effect on CA1 LTP or alternatively the reduction in torsinA protein level to 66% of wild-type may not be sufficient to lead to enhanced CA1 LTP. As for the mechanism underlying the enhanced CA1 LTP in +/Δ and cKO mice, we propose it to be linked to a change in the expression of several genes involved in energy metabolism or redox state, similar to a cell culture study (Martin et al., 2009).

In conclusion, our data suggests a gain-of-function of torsinA\textsuperscript{ΔE} and that this gain-of-function of torsinA\textsuperscript{ΔE} leads to enhanced PPRs, a decrease in sEPSC frequency, but no change in CA1 LTP in the hippocampus. How torsinA\textsuperscript{ΔE} leads to these electrophysiological changes and whether similar electrophysiological changes, if present throughout the basal ganglia circuits, relate to DYT1 dystonia are not completely understood and remain to be investigated. With a better understanding of how torsinA\textsuperscript{ΔE} leads to the pathophysiology of DYT1 dystonia, it is hoped that novel therapeutics will be developed to provide relief from this debilitating disorder.
REFERENCES


APPENDIX I

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: January 27, 2010
TO: Li, Yaqing
    CIRC-546
    996-6299

FROM: Judith A. Kapp, Ph.D., Chair
       Institutional Animal Care and Use Committee

SUBJECT: NOTICE OF APPROVAL - Please forward this notice to the appropriate granting agency.

The following application was reviewed and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on January 27, 2010.

Title: Pathophysiology of DYT1 Dystonia: Targeted Mouse Models
Sponsor: NIH

This institution has an Animal Welfare Assurance on file with the Office for Protection from Research Risks (Assurance Number A3255-01) and is registered as a Research Facility with the United States Department of Agriculture. The animal care and use program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).
NOTICE OF APPROVAL

DATE: January 27, 2010

TO: Li, Yuging
   CIRC-545
   996-6299

FROM: 
Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee

SUBJECT: Title: Pathophysiology of DYT1 Dystonia: Targeted Mouse Models
         Sponsor: NIH
         Animal Project Number: 100108196

On January 27, 2010, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

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Animal use is scheduled for review one year from January 2010. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 100108198 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.