

# Expert Opinion

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## Animal models for drug discovery in dystonia

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Dystonia is a neurological disorder characterized by involuntary twisting movements and unnatural postures. There are many different forms of dystonia, which affect over three million people worldwide. Effective treatments are available only for a minority of patients, so new treatments are sorely needed. Several animal species have been used to develop models for different forms of dystonia, each with differing strengths and weaknesses. This review outlines the strategies that have been used to exploit these models for drug discovery. Some have been used to dissect the pathogenesis of dystonia for the identification of molecular targets for intervention. Others have been used for the empirical identification of candidate drugs. Therefore, the animal models provide promising new tools for developing better treatments for dystonia.

**Keywords:** animal model, *Caenorhabditis elegans*, dystonia, experimental therapeutics, hamster, mouse, primate, rat

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### 1. What is dystonia?

Dystonia is a neurological disorder defined by characteristic abnormal movements [1,2]. The chief underlying problem is excessive contraction of muscles for an intended movement. The primary muscles needed for the movement contract too strongly and nearby muscles, that are not normally needed, also contract. This overflow contraction sometimes spreads to muscles that oppose the actions of the primary muscles. The final movement depends on the patterns and strengths of the muscles involved.

In the mildest cases, dystonic movements may appear as exaggerations of normal actions. In moderately affected cases, abnormalities are more obvious with movements that are stiff, slow, twisting or combined with a coarse tremor-like action. In severe cases, dystonic movements may lead to discomforting posturing or fixed deformities. Dystonia is a chronic disorder. Once it begins, it rarely remits and often progresses.

Dystonia is not one disorder, but many [3-5]. The many different forms are classified by three overlapping systems, each with different implications for therapy. The simplest method of classification is based on the body part involved. The focal dystonias are the most common. They affect an isolated body region, such as the neck (cervical dystonia), the eyes (blepharospasm), the hand (writer's cramp) or the larynx (spasmodic dysphonia). The segmental dystonias involve two or more contiguous regions, such as the eyes and mouth (Meige syndrome) or the neck and one arm. Generalized dystonias exhibit broader involvement. The extent of the involvement influences the choices for therapies.

Another important element for classification is age at onset. Adult-onset dystonias are most often focal or segmental, non-associated with other neurological

defects or degeneration, and exhibit limited progression. Most are sporadic, although there are clues that suggest an inherited predisposition with reduced penetrance. In contrast, childhood-onset cases are more likely to be familial and more often progress to generalized involvement. In children, there are also more forms with a higher likelihood of being associated with other neurological defects or evidence of neurodegeneration.

The third scheme for classification involves the etiology (Box 1). When dystonia occurs without other neurological problems, it is classified as primary dystonia. Dystonia can also be secondary to a wide variety of nervous-system insults, including cerebrovascular disease, tumors, toxins or medications, metabolic abnormalities and infectious or inflammatory processes. Dystonia is a frequent feature of numerous developmental or degenerative diseases, many of which are inherited.

### 2. How many people are affected?

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Dystonia is sometimes described as a rare or orphan disease. Although individual dystonias may be uncommon, as a group they are not rare. Estimates of the number of people affected are limited by the lack of comprehensive epidemiological studies. For the primary dystonia, several studies have provided prevalence estimates of 370 per million [6]. These figures translate to more than three million people worldwide. However, these figures reflect significant underestimates because the many different manifestations of dystonia are not well recognized and many patients go undiagnosed. Others do not seek medical attention because of the view that effective treatments are lacking.

More importantly, the estimates of people affected include only those with primary dystonia. Estimates increase dramatically if secondary dystonias are included. For example, Parkinson's disease (PD) affects ~ 1% of people > 65 years of age. Approximately a third of them suffers from dystonic movements at some point in their illness [7,8]. Dystonia is even more common in the related Parkinson-like degenerative disorders. Another example is cerebral palsy, which occurs with an incidence of 1.5 per 1000 live births per year. These patients are often diagnosed with spasticity, yet a third exhibits dystonic movements and, in some cases, dystonia predominates [9-11]. If these additional populations of secondary dystonia are included, disorders associated with dystonia are not rare, they are common.

### 3. What treatments are already available?

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Effective medications exist for only a tiny fraction of the patients with specific forms of dystonia [12,13]. Levodopa provides an excellent response for patients with dopa-responsive dystonia, but little or no response in others. Dystonia is frequent in Wilson's disease, for which copper-chelating agents can stop the progression and

sometimes reverse it. Aside from a few rare conditions, broadly effective medications are lacking.

Anticholinergics, such as trihexyphenidyl, are often prescribed for many dystonias. They have a modest efficacy in most adults and the doses required cause multiple undesirable side effects that limit their use. They are better tolerated in children, although satisfactory responses are achieved in the minority. Benzodiazepines, baclofen and other muscle relaxants are frequently prescribed. Some patients note partial benefits from these medications, but side effects are common.

Botulinum toxins provide an effective alternative to oral medications in some cases. Because they must be injected into involved muscles, they are most suited for patients with limited involvement. They provide the best option for focal dystonias, such as cervical dystonia, blepharospasm and spasmodic dysphonia. The botulinum toxins can also be used to target the most discomforting features in patients with broader involvement in segmental and generalized dystonia, but delivery to all affected muscles is not practical. Along with their benefits, they have some drawbacks. The injections must be repeated every 3 – 4 months when benefits begin to wane, and efficacy is lost in some patients who develop a resistance.

The limited availability of effective medical treatments has led to an increasing interest in surgical options. Intrathecal delivery of baclofen via a subcutaneous pump can help in those with a prominent involvement of the lower body, especially children [11,14,15]. Selective peripheral denervation can provide relief in cervical dystonia [16]. Deep brain stimulation is helpful in patients with generalized dystonia and is increasingly used in focal and segmental dystonias [17]. Because of the surgical risks, these procedures are usually reserved for medically refractory cases. In addition, they are offered by relatively few centers with specialized experience in dystonia and are not widely available.

### 4. What animal models are available for dystonia?

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#### 4.1 Rodent models: etiologic

Multiple rodent models are available presently. They can be grouped into two main categories (Figure 1). One category includes the models in which a trigger known to cause human dystonia is reproduced in an animal. Most frequently, this involves the generation of mice with a gene defect linked with human dystonia. They were reviewed recently [18] and only those relevant to drug discovery are summarized here.

Etiologic models for drug discovery have been most actively pursued for DYT1 dystonia, a form of generalized primary dystonia caused by an in-frame three-base-pair deletion of GAG in the *TOR1A* gene. This mutation results in the omission of the amino acid glutamate in the protein, torsinA [19]. Recent research suggests that torsinA is a

**Box 1. Dystonia etiologies.****Primary (isolated dystonia)**

Inherited: early onset and adult onset, generalized or focal

Idiopathic: torticollis, blepharospasm, spasmodic dysphonia etc.

**Dystonia plus syndromes (dystonia plus other telltale features)**

Dystonia/parkinsonism: DOPA-responsive, dopamine agonist responsive, rapid-onset dystonia/myoclonus

**Secondary (known environmental cause)**

Perinatal injury: hypoxia/ischemia, kernicterus

Infectious/inflammatory: viral, bacterial, fungal, tuberculous, prion-related

Autoimmune/paraimmune: demyelination, lupus, anticardiolipin, Reye's syndrome, subacute sclerosing pan-encephalitis

Trauma: brain, spinal cord, peripheral nerves

Neoplasm: direct effect or paraneoplastic

Vascular: ischemic stroke, hemorrhagic stroke, vessel malformations

Drugs: dopamine-related, SSRI, anticonvulsants, cocaine, MPTP, ergots

Toxins: cyanide, manganese, carbon monoxide, carbon disulfide, disulfiram, methanol, 3-nitropropionic acid

Other: hypoparathyroidism, central pontine myelinolysis, cervical stenosis, congenital

**Hereditary/degenerative (recognized syndrome with or without known cause)**

Parkinsonian: idiopathic Parkinson's disease, corticobasal degeneration, progressive supranuclear palsy, multiple system atrophy

Trinucleotide repeat diseases: Huntington's disease, Machado-Joseph disease and other spinocerebellar ataxias, dentate-rubropallidolusian atrophy

Lysosomal: metachromatic leukodystrophy, GM1 and GM2 gangliosidosis, Niemann-Pick C, Krabbe disease, ceroid lipofuscinosis

Amino acidurias: homocystinuria, Hartnup disease

Organic acidurias: glutaric aciduria I, methylmalonic aciduria

Mitochondrial: Leigh and Leber disease, dystonia/deafness syndrome

Metal/mineral metabolism: Wilson disease, Hallervorden-Spatz, Fahr's disease

DNA handling: ataxia-telangiectasia, Cockayne syndrome, xeroderma pigmentosa, Rett syndrome

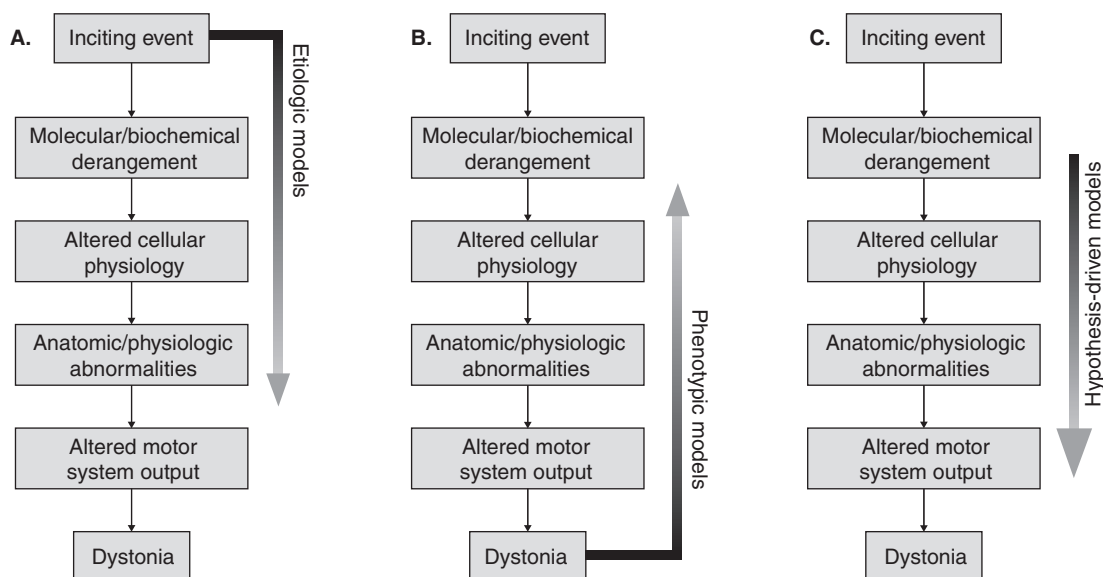
Miscellaneous: Lesch-Nyhan disease, Pelizaeus-Merzbacher disease, neuroacanthocytosis, adult and infantile striatal necrosis

molecular chaperone required for protein folding and the membrane structure of the nuclear envelope or endoplasmic reticulum. The mutant protein exerts a dominant effect through mechanisms that are not yet fully understood. At the cellular level, the consequences of expressing the mutant protein include the disruption of the nuclear envelope with the formation of perinuclear inclusions [20,21]. Neurodegenerative changes are not seen; dystonia is thought to arise from changes in the physiology of motor circuits in the brain.

Several aspects of DYT1 dystonia make it a good candidate for exploring treatments. First, there is evidence that symptoms are reversible. Marked improvements in symptoms are seen in response to deep brain stimulation and, to a lesser extent, with anticholinergic drugs. Second, it is dominantly inherited, but has a penetrance of only 30%, with the disease emerging in almost all cases by 30 years of age. The low penetrance suggests a near-normal

functional state in the brain that may be tipped off balance by other genetic or non-genetic factors within an isolated window of vulnerability. A relatively modest intervention to restore the balance during a critical developmental window may be sufficient to prevent the disease permanently. Third, essentially all cases carry the same mutation. This uniformity permits the design of a single therapeutic reagent targeting the same mutation or common downstream pathophysiological consequences.

Two categories of genetically engineered mouse models have been generated to study the consequences of the mutation *in vivo*. The first category includes transgenic mice expressing a copy of the mutant gene along with normal endogenous genes (Table 1). Several lines were produced in which the mutant gene is expressed from the mammalian neuron-specific enolase promoter [22], the human cytomegalovirus promoter [23] or the mammalian prion protein promoter [24]. The lines were evaluated with



**Figure 1. Different types of animal models for dystonia.** **A.** Etiological models refer to those in which a cause for human disease is replicated in an animal. Causes may include gene defects or toxins known to cause the human disease. Examples include mutant DYT1 mouse models for dystonia or MPTP-induced parkinsonism. **B.** Phenotypic models are those that exhibit a motor disorder that fulfills clinical and electrophysiological criteria for human disease. Examples for dystonia include the *dt* rat, *dt<sup>sz</sup>* hamster and the tottering mouse. **C.** Hypothesis testing models are those in which some intermediate event thought to participate in the pathophysiological pathway for dystonia is reproduced in an animal. Examples include surgical or 6OHDA lesions of nigrostriatal dopamine neurons for Parkinson's disease.

MPTP: 1-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine.

regard to predicted abnormalities at the behavioral, neurochemical and histopathological levels. None exhibits a motor disorder resembling human dystonia, but some detectable motor anomaly was uncovered in most. Histologically, most exhibit abnormal perinuclear aggregates in different brain regions (Table 1). Biochemically, several exhibit changes in brain monoamines. However, most changes are small and inconsistent across the lines.

It is noteworthy that transgenics expressing normal rather than mutant human torsinA from the prion protein promoter, also exhibit several abnormalities, including changes in motor behavior and perinuclear inclusions [24]. This finding indicates that some abnormalities in the transgenics may reflect the consequence of overexpression, rather than a pathological consequence of the mutant torsinA. In all of the transgenic animals, large amounts of mutant protein are expressed in many brain regions, in comparison with the lower and more regionally selective expression in the normal brain. Therefore, the phenotype of the transgenics might be broader than when mutant torsinA is expressed under its natural promoter.

In principle, knock-in mice in which one copy of the endogenous gene is changed to the human mutant form may provide more faithful models. In this case, the mutant gene is expressed by its natural promoter, so the levels and regional patterns of expression more closely resemble the disease state. The more natural pattern of expression avoids

potential problems associated with phenotypic consequences of overexpression or expression of mutant protein in the wrong brain regions. One line of knock-in mice was reported to exhibit mild hyperactivity and impaired performance on the beam walking test without overt dystonia in the heterozygous state [25]. Homozygous knock-ins die at birth [21]. Histologically, perinuclear aggregates were seen in the brainstem.

In summary, multiple lines of mutant mouse models have been generated for DYT1 dystonia. Each has a slightly different phenotype. None has a motor disorder resembling human dystonia, although each has some measurable motor or histopathological abnormality.

#### 4.2 Rodent models: phenotypic

The second category of animal models includes those that exhibit abnormal movements resembling human dystonia [18]. Included are mice, rats and hamsters. Several were discovered as spontaneous occurrences in breeding colonies. Others were discovered via pharmacological studies or after targeted gene alterations. The best characterized ones all have clinical and electrophysiological features consistent with human dystonia.

The dystonia musculorum mouse [26] and the dystonic *dt* rat [27] were among the first of the spontaneously occurring inherited phenotypic dystonia models to be described. In both models, severe generalized dystonia emerges during

**Table 1. Genetically engineered mouse models for DYT1 dystonia.**

Type	Promoter	Protein expression	Neurochemical phenotype	Anatomical phenotype	Motor phenotype
Transgenic [22]	NSE	3 – 8 ×	Striatal DA increased 39%*	Perinuclear aggregates stained for torsinA and ubiquitin <sup>‡</sup>	Hindlimb clasping, marked hyperactivity, circling <sup>§</sup>
Transgenic [23,79]	CMV	2 – 4 ×	Striatal DA release impaired	Not reported	Limited improvement on repeated Rotarod testing in old animals
Transgenic [24]	Prion protein	2 – 6 ×	Brainstem 5-HT and 5-HIAA increased	Perinuclear aggregates stained for torsinA and ubiquitin <sup>¶</sup>	Limited improvement on repeated Rotarod testing in old animals
Knock-in [25]	Natural	Normal	Striatal HVA reduced 27%	Perinuclear aggregates stained for torsinA and ubiquitin <sup>#</sup>	Mild hyperactivity, poor performance on beam walking test

\*DA release observed only in the 40% of animals affected with abnormal motor phenotype; behaviorally normal animals showed 18% increase.

<sup>‡</sup>Abnormal histology limited to pons, pedunculopontine nuclei, periaqueductal gray.

<sup>§</sup>Only 40% of animals showed the abnormal motor phenotype.

<sup>¶</sup>Abnormal histology noted for pedunculopontine nuclei, periaqueductal gray, brainstem raphe, hypothalamus, cerebellum.

<sup>#</sup>Abnormal histology limited to pons.

5-HIAA: 5-Hydroxyindole acetic acid; 5-HT: 5-Hydroxytryptamine; CMV: Cytomegalovirus; DA: Dopamine; HVA: Homovanillic acid; NSE: Neuron-specific enolase.

early development. Although they have been used extensively for neuropathological and physiological studies, few studies have focused on drug discovery. A major reason is that the motor disorder is sufficiently severe to compromise the viability. Special procedures are required to keep the animals alive, especially during the early development. Another reason is that the severity of the motor disorder leads to ongoing concerns regarding the health of animals that challenge efforts to develop and interpret measures of improvement or worsening in response to drug challenges. Therefore, these models highlight some of the technical challenges associated with drug discovery for dystonia.

Pharmacological studies relevant to drug discovery have been pursued most extensively in the *dt<sup>sz</sup>* hamster, an inherited model for paroxysmal generalized dystonia [28,29]. Although paroxysmal dystonia is an uncommon form of human dystonia, the model has a number of attractive features for drug discovery. Because dystonia emerges in discrete attacks lasting 3 – 5 h, the animals are able to maintain nutrition and hygiene during the interictal periods, so they suffer minimal morbidity or mortality. As the attacks are triggered reliably by a number of influences, it has been possible to develop quantitative rating scales for a rigorous measurement of changes in the frequency and severity of dystonia in response to experimental manipulations. Although the genetic basis for dystonia in *dt<sup>sz</sup>* hamster is unknown, a number of physiological studies have revealed abnormalities among motor control pathways relevant to dystonia. Most notably, dystonia appears to correlate with overactivity of the striatal projection neurons and reduced basal ganglia output [30].

The tottering mutant mouse also has been the subject of several pharmacological studies relevant to drug discovery for dystonia. Tottering mice also exhibit paroxysmal dystonia, which is easily experimentally induced by stress, caffeine or ethanol. Their attacks of generalized dystonia last for 30 – 40 min and are readily quantified, and the relatively normal interictal periods allow the mice to remain healthy and viable. Therefore, these mice offer the same advantages as the *dt<sup>sz</sup>* hamster as a tool for drug discovery. Tottering mice carry a mutation in the *Cacna1a* gene, which encodes the  $\alpha_1$  subunit of the Cav2.1 (P/Q-type) voltage-dependent calcium channel [31]. These channels are abundantly expressed in the cerebellum, particularly in Purkinje cells, where a 40% reduction in the Cav2.1 calcium-current density is observed [32]. During a dystonic attack, neuronal activation is observed throughout the olivocerebellar circuit, but is absent from the basal ganglia [33]. Lesions that eliminate cerebellar output alleviate dystonic attacks in tottering mice, thus suggesting that the cerebellum is necessary for the expression of dystonia [34].

### 4.3 Primate models

Although the majority of research has focused on rodent models for dystonia, other species also provide valuable tools for drug discovery. Non-human primate models are attractive because they most closely resemble humans in their neuroanatomy, neurophysiology and motor behavior. The cognitive abilities of primates also allow for more sophisticated behavioral, learning and movement paradigms than those possible for other common laboratory animals. The success of primate models for advancing the understanding of treatments for PD further reinforces



the value of primate models for dystonia. Finally, primates may be particularly well-suited to study certain treatment modalities, such as deep brain stimulation.

Presently, there is no ideal non-human primate model for dystonia. Dystonia is observed in several experimental paradigms, but few were developed specifically as models for dystonia. A model of focal hand dystonia related to overuse was developed in owl monkeys trained to maintain all fingers and the thumb in contact with a spring-loaded grip during multiple rapid opening and closing cycles [35]. The monkeys made as many as 3000 stereotyped cycles in daily training periods lasting up to 2 h. After 5 weeks of training, the performance declined in 3 of 4 monkeys due to the emergence of abnormal movements resembling hand dystonia. This paradigm is suggestive of task-specific dystonias that develop in humans following overuse.

Dystonic movements are also observed in association with destructive lesions or transient pharmacological inactivation of specific brain regions. Rhesus monkeys with lesions of the internal segment of the globus pallidus developed abnormal postures of the contralateral limbs similar to what is seen in human dystonia [36]. Similar phenomena were reported for rhesus monkeys following lesions of the posterior putamen [37]. Midbrain lesions were reported to cause abnormal movements of the head and neck resembling cervical dystonia in several primates [38-40]. These findings in non-human primates are suggestive of dystonia that develops following focal lesions of similar regions in humans [41].

Dystonia is also observed as an accompanying feature of some primate models of PD. Multiple primate species developed hemidystonia a few days after a single intra-carotid infusion of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) [42,43]. The dystonia diminishes after 4 – 5 weeks and is followed by the development of hemi-Parkinsonism. In another experimental paradigm, Cynomolgus monkeys with Parkinsonism due to chronic MPTP lesions were treated with dopamine replacement therapy [44]. After 5 months of treatment, they developed dystonic limb movements at peak dose. The dystonia in these models is analogous to that in some patients with early PD, or after chronic treatment [7,8].

Finally, dystonia is observed following an exposure to other toxins. Two of three rhesus monkeys given intravenous manganese chloride developed slowed movements and facial grimacing resembling dystonia [45]. Cynomolgous monkeys also developed dystonia after exposure to 3-nitropropionic acid, with or without MPTP [46,47]. These models are directly relevant to the dystonia that develops in humans suffering from manganese or 3-nitropropionic acid toxicity.

In summary, there are multiple non-human primate models for dystonia. Several are based on biological processes related to human dystonia, but they are technically challenging to generate and maintain. The existing models have been used most for studying the pathophysiology of

dystonic movements. They have not yet been used extensively for developing and testing new treatments.

### 4.4 Simpler organisms

Although primate models are attractive because of their similarity to humans, simpler organisms are attractive for different reasons. The goal in using simpler organisms is not to reproduce all aspects of the disease, but to reproduce a key aspect as a target for drug therapy [48].

Among the simpler organisms for dystonia, the nematode *C. elegans* has received the most attention [49]. It was the first animal to have its genomic DNA sequence fully determined and, thereby, has taken a lead-role in the post-genomic era in terms of its rich bioinformatics databases on gene expression, function and interactions. It is genetically invariant and shares ~ 70% of its genes with humans. It is anatomically defined and its entire cell lineage and complete neuronal connectivity are determined. Compared with the ~ 100 billion neurons of the human brain, *C. elegans* has exactly 302 neurons. Despite its evolutionary distance from humans, its neurons retain many hallmarks of the mammalian neuronal function, including ion channels, neurotransmitters, transporters, guidance cues, receptors and synaptic components. Defects in many of these are associated with simple, but defined behavioral readouts for neuronal function, such as egg-laying or motor coordination. These worms are very easy to grow and maintain in large numbers and at minimal cost.

All of these features make nematodes an attractive experimental tool for drug development and discovery [48,49]. The recent application of *C. elegans* toward human disease research has already provided insights into the function of specific gene products linked to a variety of neurological disorders, including Alzheimer's disease, PD and epilepsy. In the case of a non-degenerative disease such as dystonia, even a slight positive change in torsinA activity might be enough to attenuate the threshold of dysfunction represented by the reduced penetrance of this disease gene. In this context, the identification of a small molecule that could even minimally restore normal activity to the torsinA protein might be of potential therapeutic value.

## 5. What can we learn from other disorders?

### 5.1 Therapeutic goals

Before considering animal models for drug discovery in dystonia, it is useful to consider what has been learned from other disorders. They provide valuable guidance for moving forward and avoiding some common pitfalls in drug discovery.

Animal models for PD have been very helpful in drug discovery. These models can be divided into two main categories. The first includes toxin-based models focused on destroying nigrostriatal dopamine neurons, a key pathological feature in PD [50,51]. The most thoroughly

studied toxins include 6-hydroxydopamine and MPTP, both of which have been used in many species, including rodents and non-human primates. The symptomatic consequences of the loss of dopamine neurons provide objective targets for therapy. These models have been validated for drug discovery as drugs known to be effective in PD are effective in reversing the symptoms in these models.

A second group of PD models is based on genes known to cause a PD-like disorder in humans [52,53]. These include genetically modified mouse models for familial disorders associated with mutations in  $\alpha$ -synuclein, Parkin, DJ-1, LRRK2 and PINK1. The majority of these models lack overt symptoms similar to those occurring in PD, so they have not been used extensively to identify drugs with symptomatic benefit. However, they have been very useful for dissecting pathogenesis and identifying potential biological targets for interventions that might slow progression of disease.

The PD models have made it clear that different therapeutic goals are best approached with different models. The most useful models for identifying drugs to reduce symptoms have limited utility for identifying drugs that slow the progression of the disease. On the other hand, models potentially useful for developing drugs to slow progression may not be appropriate for identifying symptomatic therapies. This issue is important for dystonia. Different treatment strategies and models may be required for preventing the emergence of symptoms in at-risk individuals versus suppressing dystonic symptoms in those already affected.

## 5.2 Eggs in one basket

Several drug discovery programs highlight a common problem in focusing on a single animal model for a human disease with complex etiologies. Motor neuron degeneration in amyotrophic lateral sclerosis (ALS) has been the target of extensive studies of drug discovery with mouse models [54]. These mice have been valuable for elucidating pathogenesis and screening potential new therapies. More than 20 of the most promising drug candidates identified with these mice moved on to clinical trials in humans, but none proved useful. The chief reason for this discrepancy is that the pathological process in the most extensively studied ALS models is not representative of the broader human population. The models are based on defects in superoxide dismutase, which account for only a tiny fraction of human ALS.

Another example is the experimental allergic encephalomyelitis mouse model, based on the immunization of mice against purified myelin-associated proteins, as a model for multiple sclerosis (MS) [55]. This model also has been valuable for elucidating pathogenesis, but its track record for human drug discovery is poor. Again the main reason is that the pathogenesis in this model differs from more heterogeneous causes in the human population.

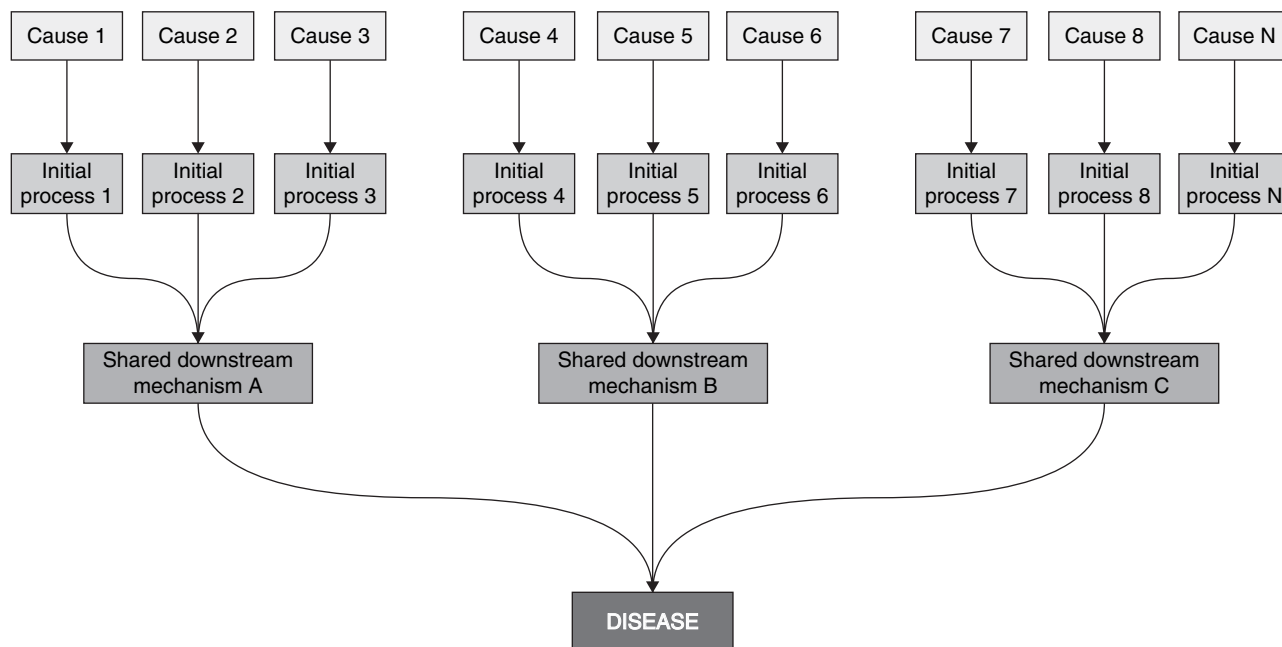
The experiences with ALS and MS models highlight the value of understanding basic pathogenesis for drug discovery. They also emphasize that over-reliance on any single animal model for drug discovery carries a risk. The results obtained may be highly relevant for a small subpopulation of patients affected by a specific pathomechanism and may not translate to populations where the mechanisms are more heterogeneous (Figure 2). This issue is particularly important for dystonia, which has numerous different causes (Box 1).

## 5.3 Rational design versus empirical discovery

The design of rational therapies based on the elucidation of pathogenesis and the identification of valid molecular targets is both logically and intuitively attractive. In reality, it does not always work. The most glaring example involves animal models for stroke. These models have been remarkably valuable for dissecting a complex web of stroke-related events involving thrombolysis, excitotoxicity, oxidative stress, inflammation, calcium influx and apoptosis. Each of these events has served as a molecular target for therapy, with the identification of > 1000 promising drugs. So few of the drugs have proven successful in clinical trials that the animal models have been questioned [56-58]. The poor track record appears to have many causes. They include practical aspects of drug delivery to experimental animals versus real-world stroke patients, poorly validated end points for determining efficacy and weak experimental designs. Furthermore, discrepancies between the most effective drugs found in animals and those actually chosen for clinical trials suggest more complex factors at play, including issues relating to drug cost and safety, regulatory approval and intellectual property [59].

In comparison with the rational design approach, the empirical approach to drug discovery lacks the logic of the pathogenesis and seems intuitively less attractive. However, empirical approaches unencumbered by preconceived notions about pathogenesis can be surprisingly productive. Animal models have played an essential role in the discovery and characterization of all marketed drugs for epilepsy [60]. These drugs were discovered through empirical testing in batteries of animal models of epilepsy. Primate models are rarely used. Virtually all are mouse models and the most widely used are not mechanistically based on any human epilepsy. The two gold standards involve subcutaneous administration of the convulsant pentylenetetrazole and exogenous electroshock in rodents.

It has been argued that the lack of bias with respect to mechanism is an advantage for drug discovery [60]. Unlike models based on rational design, the empirically based models are more likely to uncover drugs that act in new ways and through new targets. One example is levetiracetam, widely recognized as one of the most broadly useful anticonvulsants for human epilepsy. It was first identified via a mouse model with audiogenic seizures, a very uncommon form of human epilepsy. The benefits of



**Figure 2. A conceptual model for the treatment of a disease with heterogeneous causes.** Multiple different etiologies for a disease may provoke different initial pathological processes. The initial processes lead to a cascade of downstream events, ultimately leading to the clinical manifestations of the disease. Because the clinical manifestations and physiological features of many dystonias are similar, it is likely that some of the downstream events are shared at some levels, either at the biochemical or physiological levels. Treating an original cause or a biological process proximal to the cause, such as RNAi therapy for DYT1 dystonia, may be effective in terminating downstream pathological processes leading to manifestations of disease. Treating distal or downstream processes more proximal to the manifestations of the disease, such as the use of botulinum toxin, interrupts expression of symptoms regardless of cause. Proximal interventions are likely to be most useful for specific diseases, whereas distal interventions may have broader applicability.

levetiracetam were later confirmed in several other seizure models. Its mechanism was initially thought to involve sodium channels or GABA-related neural transmission, two of the most favored mechanistic targets in epilepsy. However, further studies revealed it to have an entirely novel molecular target, the synaptic vesicle protein SV2A. This protein now serves as a new rational target for additional drugs [60].

The stroke and epilepsy models provide important lessons for moving forward with dystonia models. Rational design of therapies based on known or presumed pathomechanisms is intuitively attractive, but does not guarantee success. Useful drugs can be discovered using models with little or no relation to pathomechanisms defined in humans.

## 6. How can available models facilitate drug discovery in dystonia?

For research into new treatments, an important concern for any model is that it be validated for drug discovery. This validation requires the demonstration that drugs known to be effective in the human disease can be identified by the model. Conversely, drugs that do not work in the human disease should not provide a high false positive rate by the model. For dystonia, the paucity of effective medications

challenges efforts to validate any model. In this setting, two strategies are being pursued. One involves exploring pathogenesis to identify putative biological targets for drug intervention. This strategy has been most actively explored through genetic models of mutant torsinA for DYT1 dystonia. Another strategy involves empirically testing drugs for their ability to attenuate dystonic movements in animals. The phenotypic models are well suited for this strategy.

### 6.1 Exploring treatments via etiologic models

Each of the many mutant mouse lines available for DYT1 dystonia exhibits some abnormal phenotype that could serve as an end point for drug intervention. Ideally, this end point should meet certain criteria. From a practical perspective it should be easy to evaluate and readily quantifiable, to allow for a rapid throughput of multiple potential drugs. The end point should also exhibit a high signal-to-noise ratio (difference between disease and normal state), so that it is possible to detect drugs with partial efficacy for further development. The end point also should be reliable with little experimental variation, to permit screening candidate drugs in small numbers of animals. Most importantly, the end point should be unequivocally linked to the pathogenesis of dystonic movement, which is the ultimate target of drug intervention.



None of the consequences of expressing mutant torsinA in mice meet all of the ideal criteria, although some come close. For example, the neurochemical abnormalities involving monoamines provide a potential read-out for drug intervention. They are readily quantifiable by automated methods and there are good reasons to suspect that the changes are relevant to dystonic movement. However, differences between normal and disease states are small and experimental variation too high to serve as a useful end point. Many potential drugs could be overlooked unless large numbers of animals are tested.

Another potential end point is the pathological inclusions that are observed in transgenic and knock-in mice expressing the mutant allele, or in cultured mammalian cells. They are readily quantified via systematic sampling and image analysis. The signal-to-noise ratio is excellent, as they are absent in normal samples. Measurement variability is likely within acceptable limits. The main limitation is that using the inclusions as an end point requires the assumption that they are pathologically linked to the expression of dystonia or that they serve as a reliable surrogate marker of dystonia. Data addressing this assumption are not available.

Several of the behavioral abnormalities could serve as alternative end points. Hyperactivity is readily quantified with automated devices, but the relatively modest increases in activity in most of the mutant mouse lines means a small signal-to-noise ratio. Abnormalities on Rotarod are also reliably quantified via automated devices. Limitations of this end point include its labor intensiveness, small signal-to-noise ratio, relatively large experimental variation and usefulness only in animals aged  $\geq 6$  months. The beam walking deficits could serve also as end points for drug testing. Its limitations are similar to those of the Rotarod. It is labor-intensive as it is not readily automated and requires manual observation of each animal over multiple trials. It also suffers a small signal-to-noise ratio and relatively large experimental variation. A weakness shared by all of the behavioral readouts is an uncertain relationship between the pathogenesis of the end point and the pathogenesis of human dystonic movements. Although any of the behavioral end points could be used for drug screening, more robust end points and additional information on the relationship between the end point and the ultimate target of therapy would be valuable.

Nevertheless, several strategies for drug discovery can proceed while pathogenesis is being worked out. For example, even without further understanding of the underlying biology, the dominant nature of DYT1 dystonia provides a clear target for therapy. Suppressing expression from the mutant allele should be therapeutic. One way to do this involves RNA interference (RNAi), a naturally occurring mechanism of post-transcriptional gene silencing [61]. It is used as a tool for silencing specific gene products in a spatially and temporally controlled manner. It holds promise

for the development of novel therapies for human disease, by preventing the synthesis of disease-causing proteins.

Several questions have to be answered before human RNAi therapeutic trials can begin. These include what, how, where and when the therapeutic agent should be applied. Cell-culture models have provided answers to the first two questions. Neuronal delivery of RNAi reagents can be accomplished via neurotrophic recombinant viruses as a vehicle. Animal models are required to provide answers to the remaining questions and demonstrate feasibility *in vivo*. The ideal model would be a mammal that replicates all genetic, molecular, pathological and behavioral aspects of the disease. However, as is true for most genetic models, no single model recapitulates all the features of the human disease. The minimum requirement for an animal model is that normal and mutant torsinA be expressed in neurons and reliably detected at the message and protein levels to determine the degree of silencing. To demonstrate the allele-specific silencing of mutant rather than normal protein, the ideal model is a knock-in mouse, where one copy of the normal allele has been replaced with a mutant allele to yield a protein expression that is regionally and quantitatively similar to that occurring normally. Even if the heterozygous knock-in model displays only a subtle disease phenotype, molecular studies would allow the assessment of allele-specificity of the RNAi reagent. To identify a physiological effect of the RNAi reagent, a model in which mutant torsinA causes a measurable disease phenotype is needed. Although desirable, the presence of a dystonic phenotype is not required.

*C. elegans* has also been used to establish assays that enable the detection of functional changes in torsinA activity [62]. This assay has been exploited to screen a collection of 240 off-patent, FDA-approved drugs that are chemically and therapeutically diverse. These drugs were prescreened from a larger library of nearly 1000 such molecules for toxicity to worms, thereby enabling the maximum dosing of the molecules. The screen was performed in a matter of months at a cost of  $< \$50,000$ , excluding personnel. Five drugs were found to either specifically enhance the normal torsinA or inhibit the mutant torsinA activity. These drugs are proceeding through secondary testing in other model systems and medicinal chemistry and continued target validation are being used to optimize ideal molecules for human clinical trials. Taken together, these data highlight the utility of the nematode system for the rapid identification of lead molecules for therapeutic development in dystonia.

## 6.2 Exploring treatments via phenotypic models

A clear advantage of the etiologic models of DYT1 dystonia is that they begin with a pathological process known to cause one form of human dystonia. Therefore, they are likely to identify valid molecular targets for therapy. From a technical perspective, one disadvantage is that none exhibits

a motor disorder resembling human dystonia. As a result, alternative end points must be chosen to evaluate drug treatments, with the assumption that the chosen end points correlate with dystonia. From a conceptual perspective, another limitation is that drugs designed to address specific pathological consequences of mutant torsinA may not be applicable to other forms of dystonia, analogous to drug discovery models for ALS and MS (Figure 2).

An advantage of the phenotypic models for drug discovery is that they display the motor disorder that is the target of therapy. Analogous to models used for drug discovery in epilepsy, the phenotypic dystonia models can facilitate drug discovery in two different ways. They can be used empirically to screen potential new therapies and they can point to new molecular targets. Empirical testing usually is not entirely random, but driven instead by hypotheses or knowledge of the pathogenesis of dystonia from humans or other models. A frequently cited potential disadvantage of empirical testing in phenotypic rodent models is that drugs found to be effective for reducing dystonia in the model may have no efficacy in humans. This may occur, for example, if the underlying pathogenesis of dystonia in the model differs from that of humans. One way to avoid this pitfall is to test drugs using multiple unrelated models and proceed only with those that are effective in more than one model, similarly to the strategy used for epilepsy. Presumably, drugs effective in multiple models with different (and sometimes unknown) pathomechanisms operate on biological processes that are shared among different dystonias (Figure 2).

Pharmacological investigations have been pursued most extensively in the *dt<sup>sz</sup>* mutant hamster with paroxysmal dystonia [28,29]. Multiple pharmacological studies have shown that drugs sometimes helpful in human dystonia also suppress dystonia in the hamsters (Table 2). For example, benzodiazepines and baclofen improve dystonia in the hamsters, in keeping with the effects of these drugs in humans. These results together with physiological studies suggest GABA-related mechanisms to be useful targets for drug development. In recent studies, it was hypothesized that the hyperpolarization of overactive basal ganglia neurons might attenuate dystonia by reducing their activity. Indeed, the Kv7.2/3 potassium channel openers, retigabine and flupirtine, were found to improve dystonia in the hamster [63]. These channels now provide a novel molecular target for dystonia.

The tottering mouse model provides another example where a phenotypic model has provided insights for potential new molecular targets for dystonia. This mouse carries a mutation in the gene encoding the  $\alpha_1$  subunit of the Cav2.1 calcium channel [31]. Several additional genetic mouse models that carry mutations in this same gene also express dystonia [64]. These include paroxysmal dystonia in rocker mice and chronic generalized dystonia in leaner and targeted knockouts. When the tottering gene defect was

initially identified, this channel was not associated with human dystonia. Instead, defects in the gene encoding Cav2.1 channels in humans were associated with episodic ataxia type 2, familial hemiplegic migraine or spinocerebellar ataxia type 6, depending on the specific mutation. It is now recognized that inherited defects in the Cav2.1 gene in humans can also result in focal or segmental dystonia [65,66]. These findings make the tottering mouse mutant both a genotypic and phenotypic model of dystonia.

Abnormal calcium handling caused by mutations in many different proteins, not just the  $\alpha_1$  subunit of the Cav2.1 calcium channel, can produce dystonia. The Cav2.1 calcium channel functions as a multimeric complex that includes  $\beta$ ,  $\alpha_2\delta$  and sometimes  $\gamma$  auxiliary subunits in addition to the  $\alpha_1$  subunit. Lethargic mice express paroxysmal generalized dystonia caused by a mutation in the  $\beta$  subunit whereas stargazer mice exhibit cervical dystonia as a result of a mutation in the  $\gamma$  subunit. The Cav2.1 channels are not the only calcium channels implicated in dystonia. Activation of Cav1.2/1.3 (L-type) calcium channels in normal mice evokes generalized dystonia [67]. Furthermore, the disruption of intracellular calcium handling via defective inositol triphosphate receptor signaling causes dystonia, as illustrated by the *opisthotonus* mouse mutant [68,69]. Thus, a number of mouse models have defined defects in calcium signaling as a common pathogenic mechanism underlying dystonia.

The gene defect in tottering mice has provided a unique lead for drug discovery in dystonia. Although the primary defect of tottering mice is in Cav2.1 channels, the dystonia results from the compensatory upregulation of Cav1.2/1.3 (L-type) calcium channels. Blockade of the defective Cav2.1 channels in tottering mice is ineffective against the dystonia, but dystonia is suppressed by dihydropyridine calcium channel antagonists, such as nifedipine, which are L-type calcium channel blockers [70]. This same class of drugs can suppress dystonic movements in other models, including lethargic and stargazer mutant mice, normal mice treated with  $\pm$ BayK 8644 and the *dt<sup>sz</sup>* mutant hamster [67,71-73]. The converging evidence from multiple unrelated animal models, together with indirect evidence from the clinical literature suggesting Cav1.2/3 calcium channel antagonists can suppress dystonia associated with tardive dyskinesia [74], recently led to a pilot clinical trial of nifedipine for generalized dystonia (unpublished).

## 7. Pathogenesis of dystonia: divergent or convergent mechanisms?

### 7.1 Divergent mechanisms

The etiologies for dystonia are clearly heterogeneous and complex (Box 1). At the biochemical level, dystonia may arise from a very diverse array of processes. These include disorders of neurotransmission, basic metabolic processes, mitochondrial function, metal and ion homeostasis, DNA handling and others. At the anatomical level, dystonia may be

**Table 2. Drug trials in *dt<sup>sz</sup>* hamster model.**

Target system	Improved	Worsened
<b>Acetylcholine</b>		
Biperidine (antagonist)	0	0
Trihexyphenidyl (antagonist)	+	
Pilocarpine (agonist)		++
<b>Dopamine</b>		
Apomorphine (D <sub>1</sub> /D <sub>2</sub> agonist)		++
Levodopa (precursor)		+++
GBR-12909 (uptake inhibitor)		+++
Haloperidol (antagonist)	++	
Clozapine (atypical antagonist)	++	
<b>Serotonin</b>		
8-OH-DPAT (5-HT <sub>1A</sub> agonist)		++
(+)WAY-100135 (silent 5-HT <sub>1A</sub> antagonist)		++
SDZ 216-525 (5-HT <sub>1A</sub> antagonist)		+
DOI (5-HT <sub>2</sub> agonist)		+
Ritanserin (5-HT <sub>2</sub> antagonist)		+
<b>Noradrenaline</b>		
Pindolol (5-HT/β-adrenoceptor antagonist)	+	
<b>GABA</b>		
Muscimol (GABA <sub>A</sub> agonist)	+++	
Diazepam (benzodiazepine)	+++	
Phenobarbital	++ (acute)	+++ (chronic)
Baclofen (GABA <sub>B</sub> agonist)	+++	
Aminoxyacetic acid (GABA-T-inhibitor)	+	
Tiagabine (GAT1 inhibitor)	+++	
Bicuculline (GABA <sub>A</sub> antagonist)		+
Pentylentetrazol (GABA <sub>A</sub> antagonist)		+
Flumazenil (benzodiazepine antagonist)		++
<b>Glutamate</b>		
Dicozilpine (NMDA receptor antagonist)	+	
CGP-37849 (NMDA receptor antagonist)	+	
Memantine (NMDA receptor antagonist)	+	
NBQX (AMPA receptor antagonist)	++	
(+)HA-966 (NMDA, antagonist glycine binding site)	++	

**Table 2. Drug trials in *dt<sup>sz</sup>* hamster model (continued).**

Target system	Improved	Worsened
Ro 61-8048 (kynurenine 3-hydroxylase inhibitor)	++	
Nitric oxide synthase inhibitors		++
RO 25-6981 (NR <sub>2B</sub> selective antagonist)		+
Ifenprodil (polyamine binding site)		+++
<b>Opiates</b>		
U50,488H (κ opioid receptor agonist)	+++	
Naloxone (opioid receptor antagonist)	0	0
<b>Cannabinoids</b>		
(+)WIN 55,212-2 (CB receptor agonist)	+	
SR-141716A (CB1 receptor antagonist)	0	0
<b>Adenosine</b>		
Cyclopentyladenosine (A <sub>1</sub> receptor agonist)		++
CGS-21680 (A <sub>2A</sub> agonist)	+++	
Caffeine, theophylline (A <sub>1/2A</sub> antagonists)		+++
DPCPX (A <sub>1</sub> antagonist)		++
DMPX, ZM 241385 (A <sub>2</sub> antagonists)	0	0
<b>Calcium channels (L-type)</b>		
Nimodipine (channel blocker)	++	
Diltiazem (channel blocker)	++	
(±)-BAY K-8644 (channel agonist)	0	0
<b>Sodium channel blockers</b>		
Diphenylhydantoin		+
Lamotrigine		+++
Riluzole		+++
<b>Potassium channel (K<sub>v</sub>7.2/3)</b>		
Retigabine (channel opener)	+++	
Flupirtine (channel opener)	+++	
XE-991 (channel blocker)		++
<b>Others</b>		
Acetazolamide (carbonic anhydrase inhibitor)	++	
Carbamazepine (antiepileptic drug)	0	0
Gabapentin (antiepileptic drug)	+	
Levetiracetam (antiepileptic drug)	++	
Deep brain stimulation	++	

associated with no apparent structural defect, subtle microscopic anomalies or gross structural defects in different areas of the nervous system. In view of the remarkably diverse causes for dystonia, defining the most appropriate molecular targets to find drugs with broad efficacy seems to present an overwhelming challenge.

### 7.2 Convergent mechanisms

Despite etiological heterogeneity, the similarities that define the clinical manifestations of dystonia argue that the many different causes share common mechanisms (Figure 2). These shared mechanisms may exist at different levels including biochemical, anatomical or physiological. At the biochemical level, for example, dysfunction of dopaminergic neurotransmission is a common feature for several, but not all, dystonias. Dystonia occurs in developmental disorders associated with abnormal dopamine metabolism, in degenerative disorders affecting dopamine neurons and as a side effect of acute or chronic dopamine receptor antagonists [75]. Therefore, manipulating dopamine neurotransmission becomes an attractive therapeutic strategy for a group of disorders. At the anatomical level, many dystonias are associated with a dysfunction of the basal ganglia motor pathways [76]. The shared neuroanatomical substrates provide another focus for therapeutic intervention and may explain the broad efficacy of deep brain stimulation in many different dystonias. At the physiological level, many dystonias also have been associated with abnormally enhanced cortical excitability or abnormal motor learning due to aberrant neural plasticity [76,77]. Therefore, drugs aimed at reducing cortical excitability or reversing aberrant neuroplasticity may prove useful. Finally, excessive muscle contraction as the defining characteristic for all dystonias provides an obvious final common target for which botulinum toxins or other muscle relaxants can be targeted.

Although the etiologies of dystonia are diverse, the identification of shared features in pathogenesis as targets for therapy provides a less daunting task than independently identifying molecular targets for each (Figure 2). The problem of markedly diverse etiologies converging on a few shared final common mechanisms is also seen in the epilepsies, where drugs aimed a few targets, such as GABA neurotransmission or cortical excitability, are effective in broad groups of patients, regardless of diverse molecular etiologies.

## 8. Conclusions

There are multiple animal models for drug discovery in dystonia. Etiologic mouse models for specific inherited dystonias have been useful for exploring their pathogenesis and identifying potential molecular sites for intervention. The design of rational therapies for these targets holds promise for interrupting the pathological processes leading to dystonia in these conditions. There are also multiple phenotypic rodent models in which to test empirically the effect of

drugs on dystonic movements. These models have pointed to novel molecular targets for symptomatic control of dystonic movements. Other promising species such as primates and *C. elegans* are under development for dystonia.

## 9. Expert opinion

Dystonia is sometimes labeled a rare disease. Although this may be true for individual dystonic disorders, as a collective group they are not rare. At the same time, there is a paucity of broadly effective medications for treatment. The prevalence combined with the limited treatments leaves a great opportunity for the development of new therapeutics. Relative to the other neurological disorders described in this review, our understanding of the biology and potential treatments for dystonia is in its infancy. This position can be exploited by learning from similar efforts in other neurological diseases. The most efficient path for moving forward with drug discovery is best charted by copying the most successful strategies and avoiding known pitfalls.

The remarkable heterogeneity of manifestations and causes for dystonia often leads to the question: where do we begin? A frequent proposal is to begin with one or more model diseases, with the hope that the results will be applicable to the broader group. This proposal is based on successes in other diseases such as PD, where detailed studies of rare familial forms of Parkinsonism have provided novel insights into molecular mechanisms of pathogenesis relevant to the more common sporadic disease [52,53,78]. It is not likely that this approach can be used in dystonia research. The majority of PD and related syndromes share a relatively circumscribed pathomechanism that involves death or dysfunction of nigrostriatal neurons or targets. In comparison, dystonia arises from an unusually diverse array of biological processes affecting multiple different areas of the nervous system in different ways [3-5]. Focusing on shared mechanisms is likely to be of broader benefit than focusing on a few model diseases.

Another question that often arises is: what is the best animal model for drug discovery? Experience with models for ALS and MS shows that focusing on one best model carries a risk that results will be relevant only for the chosen model. Batteries of models, analogous to those used in epilepsy, avoid the idiosyncracies of individual models. A related question is whether etiological models engineered to match a specific human dystonia are better for drug discovery than phenotypic models where the pathomechanism is unrelated or even unknown. The experience with models for PD indicates that the answer depends on the goal of therapy. If the goal is to interrupt the pathogenesis of disease at an early stage to prevent the symptoms, models that closely replicate the pathogenesis in humans are essential. Because these models do not exhibit dystonic movements, it is uncertain if they can be used for testing symptomatic therapies. In this case,



the phenotypic models are preferable. The experiences with models for stroke and epilepsy demonstrate that the pathomechanism may not be relevant and may be misleading.

The most pressing issue that needs to be addressed involves funding priorities for dystonia. Although multiple animal models have been developed, they have been used most for exploring pathogenesis. Very little effort has been put into exploring treatments. A major reason is that institutions that fund research explicitly favor hypothesis-testing experimental designs that lead to advances in understanding the disease mechanisms or large-scale clinical trials for treatments already recognized as having promise. Drug discovery, especially empiric drug discovery, is not readily classified as hypothesis testing science. If we are to

discover and develop new drugs for dystonia, we will need a very different approach to fund the effort.

### Declaration of interest

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