CHAPTER C4

Mouse Models of Dystonia

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Dystonia is a relatively common neurological disorder with a prevalence of at least 330 per million (Nutt et al. 1988). Dystonia is broadly characterized by simultaneous and sometimes sustained contractions of agonist and antagonist muscles. These co-contractions result in twisting movements and postures that vary among patients (Fahn and Marsden 1994; Jankovic and Fahn 1998). The variety of symptoms categorized as dystonia reflects the heterogeneous biological basis of the disorder. In fact, dystonia may arise as a result of brain injury or insult (secondary or acquired) or occur as a sporadic or inherited disorder (primary or idiopathic); most cases of dystonia are idiopathic.

Animal models of dystonia are of considerable interest as they provide experimental paradigms for elucidating the biological mechanisms underlying this movement disorder. Dystonia can be observed in mice, rats (Lorden et al. 1988), and hamsters (Richter and Loscher 1998). The dystonic rat and hamster models, which are described elsewhere in this volume, have been most intensively studied; the dystonia exhibited by the rat model is chronic, abating only when the animal is at rest, whereas the hamster exhibits episodic periods of dystonia. Functional neuroanatomical mapping studies of the hamster model have revealed abnormalities in the basal ganglia (Richter and Loscher 1998). Both the rat (LeDoux et al. 1993) and hamster models (Richter and Loscher 1998) have also clearly implicated the cerebellum and related regions such as the red nucleus and thalamus in the phenotype. These animal models have provided significant insight into the neuroanatomical regions involved in dystonia.

There are numerous mouse models of dystonia with new models being generated as quickly as human genes are identified. Mouse models of dystonia parallel findings in humans whereby the expression and etiology of the dystonia is heterogeneous. Further, dystonia in mice may arise as a primary inherited disorder or may be acquired through experimental manipulation. The similarities between the mouse and human disorders suggest that the mouse models may prove valuable for understanding the pathophysiology of dystonia.

I. GENETIC MODELS OF DYSTONIA

A. Dystonia Musculorum

1. Background

The dystonia musculorum mutant emerged spontaneously at the Institute of Animal Genetics in Edinburgh and was first described in 1963 (Duchen et al. 1963). The
mutation later proved allelic with the athetoid mutant (\textit{dfr}'), which had arisen at least three times at the Jackson Laboratories in Bar Harbor, Maine (Duchen 1976). Several additional alleles have emerged independently in other mouse colonies, including one in Albany, New York (\textit{dfr}) and another (\textit{dfr}) in Orleans La Source, France (Messer and Strominger 1980; Sotelo and Guenet, 1988).

Dystonia musculorum mice carry a mutation in the \textit{Bpaq1} gene, which encodes a neural isoform of the human bullous pemphigoid antigen, a hemidesmosomal protein (Brown et al. 1995; Brown et al. 1994). The protein plays a role in anchoring and stabilizing the cytoskeletal network within neurons (Dalpe et al. 1998; Yang et al. 1999). The mutation causes loss of neuronal cytoskeletal organization (Dalpe et al. 1998; De Repentigny et al. 2003), axonal swelling (Duchen et al. 1964; Janota 1972) and abnormal axonal transport (De Repentigny et al. 2003) that culminates in axonal degeneration of primary sensory neurons (Duchen 1976; Duchen et al. 1963; Duchen et al. 1964; Guo et al. 1995; Janota 1972; Kothary et al. 1988; Sotelo and Guenet 1988). Additionally, investigators observe postnatal degeneration of muscle spindles that correlates with the onset of the motor disorder, but skeletal muscle appears normal (Dowling et al. 1997). \textit{Bpaq1} mRNA expression is actually much broader than that predicted by the histopathology (Dowling et al. 1997), suggesting that not all neurons are dependent on \textit{Bpaq1} for cytoskeletal maintenance. \textit{Bpaq1} is expressed in pontine, olivary, and sensory neurons that degenerate but \textit{Bpaq1} is also expressed in the optic nerve, olfactory nerve, and sympathetic ganglia, which do not degenerate. Little or no \textit{Bpaq1} is expressed in the basal ganglia, cerebellum, or postnatal motor neurons, although lesions were noted in the striatum of \textit{dystonia musculorum} mice (Messer and Strominger 1980). These mutants also exhibit abnormal myelination in both the peripheral and central nervous system (Bernier and Kothary 1998; Saulnier et al. 2002). At this time, the mechanisms by which dysfunction of the protein and subsequent pathology cause motor dysfunction are unknown.

All strains were reported to have a similar motor phenotype with features resembling torsion dystonia in humans (Duchen et al. 1964; Messer and Gordon 1979; Messer and Strominger 1980; Richter and Loscher 1998). Their writhing and twisting movements with muscle "spasms" leading to abnormal limb postures and severe difficulty with ambulation are carefully described in many reports (Lalonde et al. 1994; Messer and Gordon 1979; Messer and Strominger 1980; Sotelo and Guenet 1988). However, investigators raised the possibility of a severe sensory ataxia in neuropathological studies demonstrating relatively circumscribed lesions of sensory nerves and ganglia, cerebellum, and red nucleus (Duchen et al. 1964). Some reports therefore describe the animals as ataxic (Brown et al. 1995; Janota 1972; Sotelo and Guenet 1988), but most investiga-

2. Motor Disorder (Video)

At rest, the dystonia musculorum mutants appear physically normal. Proximal movements, such as those of the shoulder or hip, are moderately abnormal. More distal movements, such as those of the elbow or knee joints, seem most abnormal. The main abnormality is stiff, twisting, and poorly controlled movements. Many movements are slow and hesitant, though others are relatively quick and fluid. The poor limb control leads to impairments in ambulation. The limbs often take abnormal trajectories during stepping, such as the hind foot retracting above the spine. Because of difficulty with limb control, the mice often ambulate with a swimming technique, in which they lie on the floor and use their limbs to paddle forward. At other times, they ambulate using an inchworm method, where the truncal muscles propel the head and shoulders forward with both fore limbs reaching out. After placing the fore limbs down, the hind limbs are drawn in towards the body. The impaired ambulation causes the animals to spend a significant proportion of time resting motionless, typically with the head and abdomen lying on the floor, the fore limbs folded back along the trunk, and the hind limbs extended caudally. Falling is infrequent since the animals maintain a widened stance with a low center of gravity, and they rarely rear onto the hind limbs. After a fall, the mouse shows an obvious delay in regaining the upright posture because of axial twisting and poor motor control.

3. Comment

The dystonia musculorum mutant demonstrates that a motor syndrome closely resembling generalized torsion dystonia in humans can occur in the mouse. Overall, the majority of abnormal movements are best characterized as dystonic, though some of the movements might also be considered choreoathetoid because they are more rapid and fluid. Though generalized, there is an anatomic gradient of involvement, with distal muscles more severely affected than proximal muscles.

B. P/Q-type Calcium Channel Mutants: Tottering, Leaner, and Knock-outs

The \textit{Caenorhabditis elegans} gene encodes the \(a_{\text{Q}}\) pore-forming subunit of the high voltage-gated P/Q-type calcium channel. Calcium channels are composed of five subunits (\(a_{i}, a_{\gamma}, \beta, \gamma\) and \(\delta\); however, the \(a_{i}\) subunit alone is sufficient to form the structural channel and confer voltage sensitivity. These
channels are characterized by voltage-sensitive activation in response to depolarization resulting in the selective increase in calcium flux into the cell. PVQ-type calcium channels are most often functionally associated with calcium-dependent neurotransmitter release (Charvin et al. 1997; Kim and Catterall 1997; Retig et al. 1996). In humans, mutations of the Ca\textsubscript{ca} gene cause spinocerebellar ataxia type 6, episodic ataxia type 2, and familial hemiplegic migraine (Ophoff et al. 1996; Zhuchenko et al. 1997); dystonia also occurs in humans carrying these mutations (Arpa et al. 1999; Giffith et al. 2002). At least four mouse models currently carry mutations in the Ca\textsubscript{ca} gene that exhibit dystonia: tottering (Ca\textsubscript{ca}t\textsuperscript{−/−}), leaner (Ca\textsubscript{ca}l\textsuperscript{−/−}), and two Ca\textsubscript{ca}l knock-out mice.

1. Leaner Mice

a. Background

The leaner mutation arose spontaneously at the Jackson Laboratories (Yoon 1969). The leaner mutation causes a gross disruption in the \alpha\textsubscript{t} subunit protein, resulting from a G to A point mutation of a splice donor site near the 3' end of the gene (Dove et al. 1997; Fletcher et al. 1996). The mutation produces abnormally spliced mRNA species that produce a dysfunctional channel. Whole-cell recording of leaner mutant Parkinai cells reveal an overall reduction in PVQ-type calcium current density (Dove et al. 1998; Lorenzon et al. 1998). Cell-attached patch recordings demonstrated a reduction in open-probability of leaner channels, explaining the reduction in current density (Dove et al. 1998).

b. Motor Disorder (Video)

In leaner mice, the dystonia is chronic and extreme with episodes of increased severity that are barely detectable over the background motor dysfunction. Historically, investigators characterized leaner mice as ataxic (Heckroth and Abbott 1994; Herrup and Wilczynski 1982; Meier and MacPike 1971; Rhyu et al. 1999; Tsuji and Meier 1971; Yoon, 1969), which suggests falling due to disturbances in balance. However, when the term ataxia is applied to mice, it is often a nonspecific descriptor that encompasses a wide range of gait disturbances. Leaner mice have an extremely debilitating gait abnormality starting at postnatal day 18 resulting from their severe and chronic dystonia. In fact, leaner mice do not fall because they are ataxic; rather they are propelled onto their flanks because the severe dystonia causes stiff extension of the limbs on one side as they attempt to walk. After falling, the mice show a considerable delay in gaining upright posture. Limb tone is increased with a marked reduction in spontaneous activity and extremely slow and stiff movements. The dystonia is generalized with involvement of proximal and distal muscles including the jaw and tongue. Leaner mice do not generally survive past weaning because the severe dystonia limits their ability to obtain and consume both food and water. However, if leaner mice receive softened chow and adequate hydration, they can live a normal lifespan and even breed. As leaner mice age, the dystonia wanes but never entirely remits.

c. Pathophysiology

Neuropathological surveys demonstrate relatively circumscribed degenerative changes of the cerebellum (Heckroth and Abbott 1994; Herrup and Wilczynski 1982). These studies have revealed widespread degeneration of cerebellar granule, Purkinje, and Golgi cells that is most prominent anteromedially (Herrup and Wilczynski 1982; Meier and MacPike 1971). The degenerative process is most severe during the first few months of age, but continues throughout adulthood, leaving less than 20% of Purkinje cells by one year of age. The surviving Purkinje cells ectopically express tyrosine hydroxylase, an enzyme normally associated with catecholaminergic cells (Abbott et al. 1996; Hess and Wilson 1991). It is not yet clear how or if these abnormalities are associated with the dystonia.

2. \alpha\textsubscript{t} Knock-Outs

a. Background

Two strains of Ca\textsubscript{ca}l knock-out mice were generated by targeted disruption (Fletcher et al. 2001; Jun et al. 1999) resulting in the elimination of the \alpha\textsubscript{t} subunit protein and the PVQ-type calcium channel current (Aldea et al. 2002; Fletcher et al. 2001; Jun et al. 1999). The neuropathology in these mice is very similar to leaner mice with late-onset progressive degeneration of the anterior cerebellum (Fletcher et al. 2001).

b. Motor Disorder (As Observed by the Authors)

Juvenile Ca\textsubscript{ca}l null mutants exhibit a motor disorder similar to that of juvenile leaner mutants, with some minor differences. In comparison to leaner mutants, the Ca\textsubscript{ca}l null mutants are much smaller throughout development, have more profound motor impairments, and display much less spontaneous activity. They almost uniformly perish at three to four weeks of age when the transition from suckling to eating solid food occurs in normal mice. With daily parenteral hydration and nutrition, a very small percentage of \alpha\textsubscript{t} null mutants survive to adulthood. The adult Ca\textsubscript{ca}l null mutants again resemble the adult leaner mice, but they have more severe motor impairments characterized by akinesia, bradykinesia, stiff movements, and increased muscle tone. They cannot eat or drink on their own, requiring daily parenteral supplementation for survival. The motor disorder of the Ca\textsubscript{ca}l knock-outs, like that of leaner mice, is most consistent with generalized dystonia.
c. Pathophysiology

Investigators observed an increase in the expression of N-type and L-type calcium channels in the null mutants (Aldea et al. 2002; Fletcher et al. 2001; Jun et al. 1999; Pagani et al. 2004). This abnormality in calcium handling may play a role in the dystonia as described below in the pathophysiology section for tottering mice.

3. Tottering Mice

a. Background

Tottering is an autosomal recessive mutation that occurs spontaneously. The tottering missense mutation is located in the pore-forming domain of the P/Q-type calcium channel (Fletcher et al. 1996). Surprisingly, channel properties of tottering mice reveal only subtle changes, with a slight increase in the non-inactivating component of voltage-dependent inactivation (Wakamori et al. 1998) but a ~40% reduction in whole-cell calcium current density.

Though investigators can identify few gross neuropathological abnormalities in Nissl-stained material from the tottering brain (Green and Sidman 1962; Levitt 1988; Noebels and Sidman 1979), quantitative measures demonstrate subtle decreases in brain volume and the size of cerebellar Purkinje cells (Isaacs and Abbott 1995). Electron microscopic and Golgi-impregnated material reveal few abnormalities, except for shrunken cerebellar Purkinje cells, abnormal Purkinje cell connectivity, and diffuse axonal swellings or torpedoes in older mice (Meier and MacPike 1971; Rhyu et al. 1999). In addition, catecholaminergic measures appear abnormal. There is apparent hyperinnervation of multiple brain regions by noradrenergic fibers, with an associated increase in tissue norepinephrine content (Levitt and Noebels 1981; Noebels 1988). In addition, tyrosine hydroxylase is ectopically expressed in cerebellar Purkinje cells, with relatively normal patterns of expression in the midbrain and locus ceruleus (Abbott et al. 1996; Fletcher et al. 1996; Heckroth and Abbott 1994; Hess and Wilson 1991).

The initial report of the motor disorder described a mild baseline ataxia with intermittent attacks of more profound motor dysfunction that were originally thought to represent motor seizures (Green and Sidman 1962). However, subsequent EEG studies failed to identify any abnormal activity consistently associated with the motor attacks, and these results questioned the classification of the intermittent attacks as epileptic seizures (Kaplan et al. 1979; Noebels and Sidman 1979). Instead, several of these studies describe 6Hz polyspike discharges in association with brief periods of behavioral inactivity suggestive of absence seizures (Heffer et al. 1983; Kaplan et al. 1979; Noebels and Sidman 1979). Although tottering mice exhibit a motor disorder that was originally classified as epilepsy, recent studies suggest that the motor disorder is better described as paroxysmal dystonia.

b. Motor Disorder (Video 5)

Tottering mouse motor attacks are highly stereotyped. The start of an attack is nearly always signaled by the extension of the hind limbs. This initial phase is followed by abduction at the hip and extension at the knee, ankle, and paw with a stiffly arched back, which presses the perineum against the cage bottom. The motor dysfunction then spreads to involve the forelimbs and head, with severe flexion of the neck. During this time, mice assume and maintain twisted and abnormal postures involving the entire body. In the final phase, the mice regain control of the hind limbs, often rearing, while forepaw and facial muscles continue to contract (Green and Sidman 1962). The entire episode lasts thirty to sixty minutes without loss of consciousness.

Movement disorders in humans are sometimes difficult to classify, and these disorders are even more difficult in mice, where normal and abnormal motor behaviors are not well studied. As a result, motor abnormalities in mice are often mislabeled or vaguely classified. Although investigators have described the tottering mouse motor phenotype as myoclonus, convulsions, focal motor seizures, or Jacksonian march, the episodic motor events in tottering mice are better characterized as paroxysmal dystonia rather than epilepsy for several reasons. First, the observed phenomenology, with sustained and asynchronous twisting postures, is more characteristic of dystonia than motor seizures. Second, the duration of thirty to sixty minutes is consistent with dystonia, but quite unusual for a seizure, which typically lasts for only one to sixty seconds. Third, despite apparent generalization with involvement of the entire trunk and all limbs, the motor abnormalities are not associated with epileptiform activity on EEG (Kaplan et al. 1979; Noebels and Sidman 1979).

c. Pathophysiology

The gene defect(s) in the Caenula mutants predicts that abnormalities in calcium handling likely play a role in the mutant phenotype. In fact, calcium channel expression appears to be abnormal in these mutants. Investigators observed a compensatory increase in the expression of N-type and L-type calcium in both the Caenula knock-out mice and in tottering mice (Aldea et al. 2002; Campbell and Hess 1999; Fletcher et al. 2001; Jun et al. 1999; Pagani et al. 2004; Qian and Noebels 2000; Zhou et al. 2003). Consistent with these findings, drugs that block L-type calcium channels block the paroxysmal dystonia in tottering mutant mice (Campbell and Hess 1999). Conversely, L-type calcium channel agonists induce dystonia in tottering mice (Campbell and Hess 1999). This response suggests that an upregulation in the L-type calcium channel subtype, which appears to be a secondary effect of the mutated P/Q-type...
calcium channel, contributes to the expression of the dystonia in totering mice.

In totering mice, the neuroanatomical substrates of the dystonic events were identified using markers of cell activity, such as the immediate early transcription factor c-fos. During a dystonic attack, the cerebellum, including granule cells, Purkinje cells, and neurons in deep cerebellar nuclei, are activated. Further, medial vestibular nuclei, deep cerebellar nuclei, red nuclei, inferior olivary complex, and ventrolateral thalamic nuclei, which are principal relay components of cerebellar circuitry, are also activated during the dystonic episodes. Dystonic events do not induce c-fos expression in the basal ganglia, a region involved in motor control and traditionally associated with dystonia. These findings clearly implicate the cerebellum and related nuclei in the dystonia (Campbell and Hess 1998).

Through lesion studies, investigators again implicated the cerebellum in the expression of the totering mouse paroxysmal dystonia. A genetic approach was used to lesion Purkinje cells, the sole output neurons of the cerebellar cortex, using the pcd mutation. pcd is a recessive mutation that causes all cerebellar Purkinje cells to degenerate (Landis and Mullen 1978). The generation of tgi/tp; pcd/pcd double mutant mice produced totering mice that lacked Purkinje cells. These mice do not exhibit dystonia (Campbell et al. 1999), suggesting that Purkinje cells are an essential link in generating or maintaining dystonia. Further, surgical and chemical lesions of the totering mouse cerebellum, particularly the anterior vermis, are also effective in reducing the duration and frequency of the attacks (Abbott et al. 2000).

d. Comment

These findings suggest that the cerebellum is involved in the expression of dystonia in totering mouse mutants. Although the cerebellum itself does not directly produce or initiate movements, these results suggest that the abnormal signal can ultimately influence the expression of the motor component of dystonic episodes. This notion concurs with the rat (Lorden et al. 1988) and hamster (Richter and Loscher 1998) models of dystonia and functional imaging in humans (Ceballos-Baumann and Brooks 1998; Hutchinson et al. 2000; Kluge et al. 1998; Mazzotta et al. 1998; Odoroff et al. 1998; Playford et al. 1998), where the cerebellum is also implicated.

4. Scn8a Mutants

a. Background

Investigators have identified several mutations at the mouse locus “motor endplate disease” (med), which encodes the gene Scn8a, the Na1.6 sodium channel expressed throughout the nervous system (Burgess et al. 1995). The med mouse mutant arose from an insertion of a truncated LINE element into exon 2 of Scn8a, causing abnormal splicing of exon 1 to an acceptor site within intron 2 and a frame shift that reads a premature stop codon in exon 3 (Kohrmann et al. 1996a). These mice are essentially null mutants and exhibit severe neurological impairment including paralysis, progressive muscle atrophy, and death within the first month (Duchen et al. 1967). The allelic mouse mutant, jolting (medj), is caused by a point mutation that replaces the Ala with Thr at residue 1071 (Kohrmann et al. 1996b). This mutation results in a small shift in voltage-dependent activation of the channel (Kohrmann et al. 1996b) and a relatively mild phenotype consisting of widened stance, unsteady gait, and tremor of the head and neck (Dick et al. 1985). The mouse mutant medj carries a 4 base pair deletion within the 5' splice donor site of exon 3, causing aberrant splicing from exon 1 to exon 4 (Kohrmann et al. 1996a). Most Scn8a mRNA produced in these mutants is abnormally spliced, but a small percentage of transcript is correctly spliced, resulting in very low expression of the protein (Kearney et al. 2002; Sprunger et al. 1999). When the medj mutation is expressed on a C57BL/6J background, the phenotype is nearly identical to the severe lethal phenotype of med mice. However, on a mixed-strain background (C57BL/6J X C3H), many medj mice survive to adulthood and display a phenotype with features resembling dystonia. The enhanced survival is due to the presence of a sodium channel modifier gene (Scmm1) in the C3H mouse strain that doubles the percentage of correctly spliced transcripts; in C57BL/6J mice, this modifier is mutated, rendering it non-functional (Buchner et al. 2003).

b. Motor Disorder (as Reported)

Adult medj mice on a mixed-strain background (C57BL/6J X C3H) exhibit movement-induced tremor of the head and dystonic posturing. Abnormal twisting postures of the trunk and repetitive movements of the limbs are sustained over the course of seconds to minutes (Hamann et al. 2003; Messer and Gordon 1979). Mice cannot ambulate with a coordinated gait due to the persistence of the twisting and posturing. The abnormal movements of the extremities abate with sleep, but axial torsions persist. These mice also exhibit severe muscle weakness and reduced muscle mass (Hamann et al. 2003; Kearney et al. 2002; Sprunger et al. 1999). The profound movement disorder reduces spontaneous locomotor activity. The EEG is normal in these mice, ruling out a possible seizure disorder. In contrast to most other dystonias, the movement disorder of medj mice can be suppressed with phenytoin, a sodium channel blocker (Hamann et al. 2003).

c. Pathophysiology

Many of the physiological studies have focused on the med and medj alleles. There are clear abnormalities in neuromuscular transmission in medj mice, which do not express
the Na+/6 sodium channel. The weakness in these mice results from a failure of evoked transmitter release from motor nerves; this likely causes the loss of muscle mass (Harris and Pollard 1986). A similar defect likely accounts for the weakness observed in med mice. Because the med<sup>−</sup> mice are ataxic, Purkinje cell firing rates were examined in med and med<sup>−</sup> mice. In both mutants, simple-spike firing in Purkinje cells is strikingly reduced (Dick et al. 1985; Harris et al. 1992; Raman et al. 1997). Similar defects occur in cortical pyramidal neurons, neurons of the dorsal cochlear nucleus, and spinocerebellar neurons (Chen et al. 1999; García et al. 1998; Maurice et al. 2001), suggesting that the phenomenon is not specific to the cerebellum.

d. Comment

med<sup>−</sup> mice exhibit a movement disorder very similar to generalized dystonia but some elements of the phenotype are uncharacteristic of dystonia. Generally, dystonia in humans abates with sleep (McGeer and McGeer 1988), whereas the med<sup>−</sup> mice maintain twisted postures. The muscle weakness in these mice is also not common in dystonia. Overall, the mice appear to be dystonic with some atypical features. EMG, which generally reveals co-contraction of agonist and antagonist muscles in dystonia, may help to clarify the nature of the movement disorder.

C. Wriggle Mouse Sagami

1. Background

The mouse mutant known as wriggle mouse Sagami (wri) arose spontaneously at the Ohmura Institute for Laboratory Animals in Japan. Investigators identified the mutation in wriggle mouse Sagami as a point mutation in the Pmca2 gene, a plasma membrane Ca<sub>2+<sub> ATPase (Takahashi and Kitamura 1999). This mutation is allelic with deafwaddler (dfw), a mutant that investigators studied as a model of deafness and vestibular disorders. In fact, stereocilia of the cochlea are completely absent in wriggle mouse Sagami (Takahashi and Kitamura 1999), the cochlea and saccule degenerate, and the mice are completely deaf at one month of age (Takahashi et al. 1999). No gross changes occur within the nervous system itself, but closer inspection reveals a decrease in the number of parallel fiber Purkinje cell contacts and an increase in "bouton-like" structures of Purkinje cells (Inoue et al. 1993). Additionally, the levels of several neurotransmitters are altered in these mutants; nor-pinephrine and serotonin are increased (Ishikawa et al. 1989; Kumazawa et al. 1989) while GABA is increased only in the striatum (Ikeda et al. 1989). The increase in the monoamines may contribute to the motor abnormalities because rianserin, a serotonergic antagonist, and prazosin, a noradrenergic antagonist, reduce the motor signs (Ikeda et al. 1989).

2. Motor Disorder (as Reported)

Wriggle mouse Sagami exhibits a complex motor disorder characterized by jerky movements of the head and neck, occasional limb abduction, and frequent rolling of the trunk, which makes it difficult for the mice to remain upright and to obtain food and water (Ikeda et al. 1989). These mice do not exhibit seizures nor are they weak; tone appears to be increased. Although the movements abate with sleep, the mice maintain an abnormal posture while sleeping, which is atypical of dystonia.

3. Comment

Although wriggle mouse Sagami has been presented as a dystonic mouse mutant, the presence of additional abnormalities such as vestibular defects suggests that dystonia is only a minor component of a more complex phenotype. Since vestibular defects alone may induce a variety of abnormal movements, the use of these mice as a model for dystonia must be interpreted with caution.

D. Fibroblast Growth Factor 14 (FGF14)-Deficient Mice

FGF14-deficient mice are not yet the subject of extensive research, but offer an interesting and unexpected mouse model of paroxysmal dystonia. The function of FGF14 is unknown, but it is expressed in the developing and adult nervous system. In adults, FGF14 mRNA is expressed at high levels in the basal ganglia and cerebellum with lower levels in the hippocampus and cortex (Wang et al. 2002). FGF14-deficient mice develop normally and have an intact nervous system, although the mice show decreased sensitivity to dopamine agonists, suggesting abnormalities of the basal ganglia. These mice are described as ataxic with a widened stance and abnormal gait. In addition, paroxysmal dyskinesia is observed in the younger mutants. Episodes of limb extensions with involuntary rearing and twisting that cause the mice to topple over occur several times a day and last for seven to twelve minutes (Wang et al. 2002). These episodes do not appear to be seizures as no abnormalities were detected with EEG. A video of the paroxysmal dyskinesia exhibited by the FGF14-deficient mice can be viewed at http://www.neuron.org/cgi/content/full/35/1/25/DC1. These mice are intriguing because they implicate dysfunction of several brain regions, including the basal ganglia, cortex, and cerebellum in the motor disorder.

E. Genetic Models: Summary

The ease with which scientists can now genetically manipulate the mouse has spurred the production of mice that carry mutations in genes known to cause dystonia in
humans. These genetic models have etiologic validity, but surprisingly few of these models have face validity. That is, none of the genetic models, with the exception of the α-synuclein mutants, exhibit dystonia, although most exhibit some kind of motor dysfunction (table 1).

The lack of a dystonic phenotype may be attributed to several causes. First, few models are an exact genocopy of the human mutation. Many of the mouse models were generated by transgene insertion, chemically-induced mutation, or homologous recombination to produce null mutants (knock-outs) and thus do not carry the exact mutation that causes the human disease. It is interesting to note that where dystonia is observed in the α-synuclein mutants, a transgene with the precise human point mutation was used to generate the mice (Gomez-Isla et al. 2003; Lee et al. 2002). In contrast, α-synuclein knock-out mice, which are completely deficient in α-synuclein, do not display dystonia and have very mild phenotype (Cabin et al. 2002). Thus, the mutant protein itself may be an important factor in driving the dystonic phenotype, and a precise recapitulation of human mutations in mice may be necessary to reproduce the dystonia. Clearly, the generation of more knock-in models will help to address this question.

Alternatively, the lack of a dystonic phenotype in these genetic models may be attributed to species-specific effects. The mouse brain may be sufficiently different from humans to prevent the expression of dystonia, or mice may not live long enough to fully develop the disease with the accompanying dystonia. Obviously, species-specific attributes are impossible to avoid in genetic models, but this does not mean the models should be discarded nor does it diminish the utility of the models. The genetic models have proven invaluable in understanding the molecular, cellular, and neuropathological phenotypes underlying the genetic disorders.

### II. DRUG-INDUCED MODELS OF DYSTONIA

Genetic models of dystonia provide obvious parallels to human disease and are a rich source for understanding the

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Human disease</th>
<th>Mouse motor phenotype</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA</td>
<td>aarylalcoholase A</td>
<td>metachromatic leukodystrophy</td>
<td>Abnormal rotator performance and progressive ataxia</td>
<td>(D'Hooge et al. 2001)</td>
</tr>
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<td>ATM</td>
<td>ATM</td>
<td>axonia telangiectasia</td>
<td>Supersensitive to amphetamine; stride length asymmetry</td>
<td>(Bilam et al. 1998)</td>
</tr>
<tr>
<td>ATP7B</td>
<td>copper ATPase</td>
<td>Wilson disease</td>
<td>None reported</td>
<td>(Bulaíka et al. 1999)</td>
</tr>
<tr>
<td>DYT1</td>
<td>tonis A</td>
<td>Oppenheim dystonia</td>
<td>Failure to feed in knock-outs; slowed habitation in knock-downs</td>
<td>(Ober and Goodel 2004)</td>
</tr>
<tr>
<td>GCDH</td>
<td>glutaryl-CoA dehydrogenase</td>
<td>glutaric acidemia</td>
<td>Abnormal performance on rotator, beam walking, and propulse inhibition</td>
<td>(Kessler et al. 2002)</td>
</tr>
<tr>
<td>HPFH</td>
<td>GTP cyclohydrolase</td>
<td>Dopa-responsive dystonia</td>
<td>Normal behavior with wasting syndrome after phenylalanine challenge</td>
<td>(Bole et al. 1988)</td>
</tr>
<tr>
<td>HPRT</td>
<td>hypoxanthine phosphoribosyltransferase</td>
<td>Lesch-Nyhan disease</td>
<td>Normal behavior but supersensitive to amphetamine</td>
<td>(Jintagh et al. 1991)</td>
</tr>
<tr>
<td>NPC1</td>
<td>Niemann-Pick type C</td>
<td>Lysosomal disease</td>
<td>Deficiency, abnormal habituation, poor coordination, tremor, gait</td>
<td>(Morriss et al. 1982; Voikar et al. 2002)</td>
</tr>
<tr>
<td>PARKIN</td>
<td>parkin</td>
<td>Parkinson disease</td>
<td>Impaired beam walking and somatosensory function</td>
<td>(Goldberg et al. 2003)</td>
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<td>PLP</td>
<td>proteolipid protein</td>
<td>Pelizaeus-Merzbacher disease</td>
<td>Tremor, seizures</td>
<td>(Biecher and Hoppe 1973, Meier and MacPike 1970)</td>
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<td>PPT1</td>
<td>palmitoyl protein thioesterase</td>
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<td>Spasticity, myoclonus, seizures</td>
<td>(Gupta et al. 2001)</td>
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<tr>
<td>SNCA</td>
<td>α-synuclein</td>
<td>Parkinson disease</td>
<td>Rigidity, dystonia, hindlimb freezing, loss of righting reflex, paralysis</td>
<td>(Gleason et al. 2002; Gomez-Isla et al. 2003; Lee et al. 2002)</td>
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<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
<td>Dopa-responsive dystonia</td>
<td>Mild to marked hypoactivity</td>
<td>(Althini et al. 2003)</td>
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pathophysiology of dystonia. However, all genetic models are fraught with the complications that accompany the development of the nervous system in the context of a mutation, including compensatory changes and cell death. In contrast, drug-induced models of dystonia provide a tool to understand the pathophysiology of dystonia on a background of normal neurological function. The etiology of the dystonia in these models may not directly reflect the etiology of the condition in humans but this does not detract from the value of the model or the knowledge gained from the model. The real value of drug-induced models is that they may help define pathophysiological defects common to many forms of dystonia. Drug-induced models complement the genetic models whereby hypotheses derived from work in genetic models may be tested for their general applicability in the drug-induced models and vice versa. Currently, there are only three well-characterized drug-induced models of dystonia in the mouse; all were developed quite recently.

A. Kainic Acid-Induced Dystonia

1. Background

Abnormal cerebellar function is implicated in human dystonia (Ceballos-Baumann and Brooks 1998; Hutchinson et al. 2000; Kluge et al. 1998; Mazziotta et al. 1998; Odergren et al. 1998; Playford et al. 1998; Eidelberg et al. 1998) as well as dystonia in mice (Campbell et al. 1999; Messer and Strominger 1980; Sprunger et al. 1999), rats (Lorden et al. 1988), and hamsters (Richer and Loscher, 1998). The accumulated evidence for cerebellar involvement in dystonia suggests that artificial disruption of cerebellar signaling should produce dystonia in normal mice. Therefore, the cerebellum became an obvious target for a drug-induced model of dystonia.

2. Motor Disorder (Video 4)

Low doses of the excitatory glutamate receptor agonist kainic acid microinjected into the mouse cerebellum produces dystonia in mice (Pizoli et al. 2002). Kainate microinjection results in the acute expression of gross generalized dystonia without cerebellar cell death and without inducing seizures (Pizoli et al. 2002). After kainate injection, mice display abnormal postures including a stiffly arched back, which presses the perineum against the cage bottom, a dystonic paddling motion of the hind limbs, and sustained twisted abnormal postures that involve most of the body. With moderate doses of kainate, mice assume dystonic postures after being disturbed and dystonia occurs with volitional movement. At higher doses of kainic acid, mice assume sustained abnormal postures for several minutes with involvement of the face, neck, and the rest of the body.

3. Pathophysiology

Human functional imaging and lesion experiments in animal models suggest that cerebellar activation is likely involved in the expression of dystonia. Specifically, the excitatory action of kainate within the cerebellum appears to cause the dystonia in this drug-induced model. Microinjection of kainate elsewhere in the brain does not cause dystonia, and mice lacking cerebellar Purkinje cells exhibit significantly less dystonia than intact mice (Pizoli et al. 2002). Further, cerebellar microinjection of a glutamatergic antagonist does not cause dystonia, suggesting that simply distorting cerebellar signaling is insufficient to produce dystonia. Rather, cerebellar excitation is necessary.

4. Comment

This model demonstrates that dystonia can be provoked in an animal with a normal nervous system. That is, chronic changes in physiology or wiring, which may occur in genetic models, are not necessary to produce dystonia. The model also demonstrates that dystonia occurs with abnormal cerebellar activity, which predicts abnormal activity in the cerebellum in dystonic patients. Indeed, the cerebellum exhibits hypermetabolism that exceeds any other brain region in functional imaging of DYT1 patients (Eidelberg et al. 1998) and other forms of human dystonia (Ceballos-Baumann and Brooks 1998; Hutchinson et al. 2000; Kluge et al. 1998; Mazziotta et al. 1998; Odergren et al. 1998; Playford et al. 1998).

B. Systemic 3-Nitropropionic Acid Intoxication

1. Background

3-Nitropropionic acid (3-NP) is an irreversible inhibitor of mitochondrial complex II. This toxin was first identified in China after individuals who ingested moldy sugarcane developed a movement disorder that included dystonia and chorea (Liu et al. 1992). In fact, the basal ganglia appear to be especially sensitive to the effects of this toxin; systemically administered 3-NP causes selective lesions within the basal ganglia, but not elsewhere in brain. 3-NP intoxication is used as a model of Huntington disease in primates, rats and, more recently, mice.

Mice are relatively resistant to the effects of 3-NP, but Fernagut et al. (2002) demonstrated that an escalating dose regimen delivered to mice over the course of nearly two weeks causes cell death within the basal ganglia. Although the dose regimen is lethal in one-third of mice, in surviving mice, circumscribed lesions occur in the dorsolateral striatum and cell loss is observed in the globus pallidus pars externalis, and the substantia nigra pars reticulata and compacta.
2. Motor Disorder (as reported)

Histopathology in the basal ganglia is accompanied by a complex motor disorder, which varies in severity. Investigators describe a motor syndrome similar to the rat 3-NP model that includes motor slowing, hindlimb dystonia, truncal dystonia, and impaired postural control (Fernagut et al. 2002).

3. Comment

In contrast to kainate-induced dystonia, which directly implicates the cerebellum, this model clearly implicates the basal ganglia in dystonia in mice. Indeed, abnormalities of the basal ganglia are often observed in neuropathological studies and neuroimaging of individuals with dystonia (Jankovic and Fahn 1998). The kainate- and 3-NP-induced dystonia models suggest that the neuroanatomical underpinnings of dystonia are likely heterogeneous, much like the disorder itself.

C. Dystonia Caused by L-Type Calcium Channel Activation

1. Background

Calcium channel agonists, particularly L-type calcium channel agonists, were good candidates for a drug-induced model of dystonia for several reasons. First, descriptions of the motor abnormality produced by systemic administration of the L-type calcium channel agonist Bay K 8644 suggest that the motor phenomenon is likely dystonia. Reports describe twisting movements, impairment locomotion, limb extension, back arching, and tonic-clonic movements (Bianchi et al. 1990; Bolger et al. 1985; Bourson et al. 1989; De Sarro et al. 1992; O’Neill and Bolger 1988; Palmer et al. 1993; Petersen 1986; Shelton et al. 1987). PPL. 64179, another L-type calcium channel agonist provokes a similar motor syndrome (Ranpe et al. 1993; Zheng et al. 1991). Second, although the motor syndrome was characterized as a form of epilepsy, it does not respond to anticonvulsants such as carbamazepine, diphenylhydantoin, phenobarbital, or valproic acid (Bianchi et al. 1990; De Sarro et al. 1992; Palmer et al. 1993; Shelton et al. 1987). Third, the Cacna1a calcium channel mutants clearly implicate calcium dysregulation, including upregulation of L- and N-type calcium channels, in the genesis of dystonia, suggesting that manipulating calcium homeostasis with drugs in normal mice might have similar effects.

2. Motor Disorder (Video 5)

Low doses of systemically administered Bay K 8644 cause slowing of movements with occasional momentary abnormal limb positions. Higher doses cause abnormal severe flexion of the trunk with flexion of the head toward the abdomen, which often causes the mouse to fall. Attempts to move are accompanied by abnormal flexion, extension, or twisting movements of the trunk and limbs; muscle tone is increased. Movements are asymmetric and asynchronous. Generally, activity returns to baseline after ~120 minutes. Tonic-clonic seizures are rarely observed after systemic injection of Bay K 8644 and no EEG abnormalities are observed (Jinnah et al. 2000). However, on EMG, significant increases are observed in resting muscle activity and prolonged movement-related phasic bursting, consistent with dystonia. A similar motor syndrome is evoked by intracerebral injection of Bay K 8644 or systemic administration of PPL. 64179 (Jinnah et al. 2000).

L-type calcium channel agonists also induce self-injurious behavior, including self-biting, stiff or Straub tail, and hypersensitivity to auditory stimuli (Jinnah et al. 2000). Thus, the response to Bay K 8644 is not a pure dystonic disorder, but part of a more complex neurobehavioral syndrome.

3. Pathophysiology

To determine if Bay K 8644 activates specific brain regions, the induction of c-fos was used as a marker of neuronal activation (Jinnah et al. 2003). Despite the extensive distribution of L-type calcium channels throughout the brain, c-fos induction after Bay K 8644 challenge is widespread, but not heterogeneous. Particularly robust activation is noted in the striatum, cortex, hippocampus, locus ceruleus, and cerebellum. The broad distribution of activation suggests that Bay K 8644 may induce dystonia through its action at several different motor regions. Microinjections into specific brain regions will be required to determine if a single region drives the dystonia.

4. Comment

The Bay K 8644 model of dystonia is likely an example of dystonia caused by the simultaneous distortion of motor commands at several levels of motor control. Indeed, for disorders such as DYTI dystonia, where the mutant gene product is expressed in many brain regions (Augood et al. 1999), such abnormal signaling from many brain regions may underlie the expression of dystonia.

III. SUMMARY AND CONCLUSIONS

Investigators generally use animal models to understand the mechanisms underlying a disorder in humans. Several common themes emerge from animal models of dystonia that provide clues for understanding the pathophysiology of dystonia in humans. First, the Cacna1a, Scn8a, and Bay K
8644 mouse models clearly implicate ion channel dysfunction in dystonia. Indeed, ion channel dysfunction induced by either mutation or pharmacologic intervention induces dystonia. Although ion channels are not yet associated with dystonia in humans, the purpose of animal models is to suggest novel approaches and mechanisms for human disease. As such, the suggestion that ion channel dysfunction may drive dystonia is worthy of investigation and presents a novel approach for unraveling the process of dystonia in humans.

Next, although studies in humans have traditionally implicated dysfunction of the basal ganglia in dystonia (Ichinose et al. 1994; Marsden and Quinn 1990; Ondo et al. 1998; Vitek 2002), more recent functional studies in humans have consistently implicated the cerebellum as well (Ceballos-Baumann and Brooks 1998; Hutchinson et al. 2000; Kluge et al. 1998; Mazzotta et al. 1998; Odergren et al. 1998; Playford et al. 1998; Eidelberg et al. 1998). Mouse models clearly implicate dysfunction of both the basal ganglia and cerebellum in dystonia, whereby the models fall into three subtypes. The JGFI4-deficient mice and 3-NP mouse models demonstrate that defects of the basal ganglia can induce dystonia, while Caenorhabditis and 3-nitro-l-arginine-induced dystonia implicate the cerebellum in dystonia. Still other models, including Bay K 8644, Scriba and at, suggest that several dysfunctional brain regions may simultaneously contribute to the expression of dystonia. The concept that dystonia may arise from the basal ganglia or the cerebellum or a complex combination of motor systems appears to have broad general applicability, as both genetic and drug-induced models fall into each of these subtypes. Given the heterogeneous nature of dystonia in humans and the lessons learned from rodent models, it is reasonable to suppose that the site of dysfunction will not be consistent from patient to patient. The mouse models illustrate the complexity of the disorder and suggest that focusing on a single brain region or gene may be counterproductive to understanding general pathophysiological principles of motor dysfunction in dystonia.

References


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