

## Phenotypical Characterization of Genetic Mouse Models of Parkinson Disease

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Parkinson disease (PD) is a debilitating disease primarily characterized by the loss of dopaminergic (DA) neurons in the substantia nigra and the development of Lewy body inclusions. Patients suffer from sensorimotor impairments, including bradykinesia, tremor, and rigidity that worsen over time. Mutations in the genes encoding  $\alpha$ -synuclein, parkin, DJ-1, and UCHL1, can cause familial forms of PD (Bonifati et al. 2003; Kitada et al. 1998; Kruger et al. 1998; Leroy et al. 1998; Polymeropoulos et al. 1997; Singleton et al. 2003; Wintermeyer et al. 2000). Although familial PD is rare, these findings provide a new approach for the study of PD and mark the beginning of a whole new generation of animal models of PD.

Investigators need a careful phenotypical characterization to make the most use of these models. The hope for genetic mouse models of PD is that they will recapitulate the early and late stages of the disease, anatomically and behaviorally, provide insights into the mechanisms causing degeneration, and contribute to developing treatments that prevent or slow disease progression. In this chapter, we describe several new sensorimotor tests that are sensitive to subtle and progressive alterations in the nigrostriatal dopamine system and are abnormal in genetic mouse models of PD. A characterization of a behavioral profile in these mice makes the tests useful for determining optimal time

points for molecular studies and for the preclinical testing of therapeutic interventions.

### I. OVERVIEW OF TRANSGENIC MOUSE MODELS OF PARKINSON DISEASE

#### A. $\alpha$ -Synuclein Mice

The presynaptic protein  $\alpha$ -synuclein is linked to both familial and sporadic PD.  $\alpha$ -synuclein is a major component of Lewy bodies, the pathological hallmark of idiopathic PD, and researchers have found specific mutations in  $\alpha$ -synuclein that cause familial PD (Kruger et al. 1998; Polymeropoulos et al. 1997; Spillantini et al. 1997). Furthermore, increased levels of normal  $\alpha$ -synuclein due to gene duplication also lead to early-onset PD in affected families (Singleton et al. 2003).

Transgenic mice overexpressing wild-type or mutant  $\alpha$ -synuclein show varying degrees of pathological accumulation of  $\alpha$ -synuclein within the brain depending on the promoter used. The first transgenic  $\alpha$ -synuclein mice overexpressed the human wild-type  $\alpha$ -synuclein under the PDGF $\beta$  promoter (Masliah et al. 2000). These mice have increased  $\alpha$ -synuclein expression throughout the brain and

show  $\alpha$ -synuclein inclusions in the neocortex, hippocampus, olfactory bulb, and substantia nigra. At twelve months of age these mice have significantly reduced tyrosine hydroxylase (TH) activity and striatal DA content and display motor impairments on the rotarod. Transgenic mice overexpressing human wildtype and  $\alpha$ -synuclein under the Thy-1 promoter have expression levels throughout the brain that are tenfold higher than human levels (Rockenstein et al. 2002). These mice have extensive accumulation of human wildtype and  $\alpha$ -synuclein in the neocortex, hippocampus, thalamus, and substantia nigra with no abnormal-synuclein accumulation in the spinal cord, neuromuscular junction, or glial cells (Rockenstein et al. 2002).

In contrast, transgenic mice with the human A53T mutation controlled by the Thy 1 promoter have  $\alpha$ -synuclein expression throughout most of the brain and spinal cord with the exception of the substantia nigra (van der Putten et al. 2000). These mice show degeneration in spinal roots and neuromuscular junctions and have  $\alpha$ -synuclein inclusions in the spinal cord and brainstem. Behaviorally, they display rotarod deficits as early as forty days of age.

Similarly, transgenic mice with the A30P mutation under the Thy 1 promoter have expression throughout the brain and an abnormal accumulation of  $\alpha$ -synuclein in the cortex, hippocampus, cerebellum, substantia nigra, and striatum (Kahle et al. 2000). No overt behavioral impairments were observed in these mice. Under the mouse prion promoter, A53T,  $\alpha$ -synuclein expression occurs throughout the brain and spinal cord and inclusions are found in the brainstem, cerebellum, and caudate putamen (Giasson et al. 2002). These mice have a severe motor phenotype consisting of decreased movement, weight loss, hindlimb paralysis, and an inability to feed that eventually leads to death.

Transgenic mice with the A30P mutation under the mouse prion promoter show  $\alpha$ -synuclein expression throughout the brainstem and spinal cord and abnormal accumulation of insoluble  $\alpha$ -synuclein in midbrain, brainstem, and cerebellum (Lee et al. 2002). These mice, too, have a severe progressive motor phenotype that results in death. Under the control of the TH promoter, overexpression of the wild-type or mutated forms of  $\alpha$ -synuclein caused  $\alpha$ -synuclein expression in catecholaminergic cells without loss of TH positive neurons in the substantia nigra or striatal projections, and no detectable behavioral impairments (Matsuoka et al. 2001; Rathke-Hartlieb et al. 2001). However, in doubly-mutated transgenic mice (both A30P and A53T mutations) under the TH promoter, striatal dopamine was reduced by sixteen months of age and amphetamine induced locomotor activity and motor coordination were impaired (Richfield et al. 2002). Although none of the transgenic mice completely recapitulates the features of human PD, some of the models do have nigrostriatal DA alterations consistent with PD.

## B. Parkin Knock-Out Mice

Parkin is an E3 ubiquitin ligase; mutations that cause a loss of parkin function are associated with early onset autosomal recessive PD (Kitada et al. 1998; Lucking et al. 2000; Periquet et al. 2003). Exon 3 deletion in parkin eliminates the parkin protein. In mice expressing this mutation (Goldberg et al. 2003; Itier et al. 2003) no overt loss of TH positive neurons occurs in the substantia nigra or their projections to the striatum, but subtler nigrostriatal DA alterations were uncovered. These parkin "knock-out" mice have increased extracellular striatal DA, reduced synaptic excitability in striatal medium spiny neurons, and sensorimotor impairments (Goldberg et al. 2003). Similarly, in a separate study, another line of mice with a similar mutation had inhibited amphetamine-induced DA release, inhibited glutamate transmission, reduced DA transporter protein, and motor and cognitive deficits (Itier et al. 2003). Despite the lack of accumulation of parkin substrates such as CDCrel-1, synphilin-1, or  $\alpha$ -synuclein in parkin knock-out mice, their DA phenotype makes them useful in the study of PD.

Although the DA phenotypes of these genetic mouse models of PD are not as profound as some of the toxin models, they may provide insight into the early stages of the disease. In PD, there is a presymptomatic phase where patients experience more subtle sensorimotor abnormalities that are difficult to detect until the severity of the disease increases (Di Paola and Uitti 1996). Animals with known genetic mutations associated with PD can be thoroughly assessed behaviorally and anatomically at various ages to uncover potential mechanisms that may ultimately contribute to DA cell death. However, this brief overview of existing genetic mouse models of PD underscores the fact that, although a few models have profound behavioral anomalies likely related to PD (Masliah et al. 2000; Richfield et al. 2002), the majority of mice do not show anomalies in traditional tests such as open field, locomotor activity, or rotarod, or when these are abnormal, the anomalies appear late in life (Masliah et al. 2000; Richfield et al. 2002). This problem seriously limits the usefulness of the mice for preclinical drug testing. A battery of more sensitive sensorimotor tests is clearly necessary to accurately assess the behavior of these mice.

## II. SENSORIMOTOR TESTS FOR DOPAMINE DEFICITS

Excellent non-drug-induced behavioral measures exist for the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD. These measures include tests for limb-use asymmetry, movement initiation, somatosensory neglect, and reaching abilities, among others (Schallert et al. 1982, 1983,

1992; Schallert and Tillerson 2000; Whishaw et al. 1986). The tests discern varying degrees of nigrostriatal DA neuron loss and are used extensively to evaluate the efficacy of various types of transplants and viral vectors (Connor et al. 1999, 2001; Kozlowski et al. 2000; Luo et al. 2002; Mignon et al. 2003; Yang et al. 2002). As in the battery of tests used in rats, rating scales similar to those that assess patients with PD are frequently used in nonhuman primate models of PD (Imbert et al. 2000). With the development of new genetic mouse models of PD, sensitive and reliable sensorimotor tests are needed to accurately assess the function of mice with bilateral deficits.

The most common tests used in mice include activity in the open field and the rotarod test for coordination (for review see Sedelis et al. 2001). Although both of these tests are automated, relatively easy to use, and provide information on sensorimotor function, they often lack the sensitivity needed to detect subtle alterations in the nigrostriatal DA system. For example, parkin-deficient mice with subtle alterations in DA function do not display impairments on the rotarod but do display motor impairments on a challenging beam test (Goldberg et al. 2003). In addition, mice treated with moderate doses of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) do not show impairments on the rotarod but do show significant alterations in gait and impairments on an inverted grid test (Tillerson et al. 2002). Therefore, studies that use only the rotarod for phenotypic assessment may miss more subtle

impairments. The best approach to behavioral characterization is one that includes a battery of tests that detect different aspects of sensory and motor function, including subtle changes in the nigrostriatal DA system (Fleming et al. 2003; Goldberg et al. 2003; Sedelis et al. 2001; Tillerson et al. 2002).

### III. SENSORIMOTOR TESTS IN GENETIC MOUSE MODELS OF PARKINSON DISEASE

Our laboratory has developed a battery of sensorimotor tests to characterize genetic mouse models of PD. The battery includes tests of motor performance and coordination (the challenging beam test), response to sensory stimuli, spontaneous exploratory activity, spontaneous shredding behavior (bin cotton use), and gait analysis. We have assessed several different lines of transgenic mice with varying levels of nigrostriatal dysfunction (table 1) including parkin knock-out mice (Goldberg et al. 2003),  $\alpha$ -synuclein knock-out mice (Abeliovich et al. 2000), and mice overexpressing the human wild-type  $\alpha$ -synuclein under the Thy-1 promoter (Rockenstein et al. 2002).

The battery of tests described in this chapter assesses sensorimotor function in mice. However, whenever researchers characterize genetic mouse models of disease, they must also examine the animal. Researchers should monitor body weight and temperature throughout testing and note any

TABLE 1 Sensorimotor Impairments in Genetic Mouse Models

Mouse Age (months)	Parkin knockout			$\alpha$ -synuclein overexpressor				$\alpha$ -synuclein knock-out			
	2-4	7-9	18	2	4	6	8	2	4	6	8
Beam											
Errors	↑	↑	↑	↑	↑	↑↑	↑↑	=	↑	=	↑
Time	↓	=	=	↑	↑	↑	↑↑	=	=	=	=
Steps	=	=	=	↑	↑	↑	↑↑	=	=	=	=
Sensory Neglect	↑	↑	=	=	=	↑	=	=	=	=	=
Spontaneous											
Activity											
Rearing	=	↓	=	↓	=	=	=	↑	↑	↑	↑
Forelimb Steps	=	=	=	↓	=	↓	=	↑	=	↑	↑
Hindlimb Steps	=	=	=	↓↓	↓↓	↓↓	↓↓	↑	=	=	↑
Grooming	=	=	=	=	=	=	↓	↑	=	↑	↑
Bin Cotton Use		↓			↓		↓↓				↓
Stride Length							=				↓

Summary of Behavioral Results in Various Sensorimotor Tests (Goldberg et al. 2003; Fleming et al. 2003).

↑ = increased compared to age-matched control mice, = profoundly increased compared to age-matched controls and/or compared to their earlier ages. ↓ = decreased compared to age-matched controls. ↓↓ = profoundly decreased compared to age-matched controls and/or compared to their earlier ages. = represents not significantly different from age-matched control mice.

abnormal behaviors, for example if the mouse removes vibrissae or clasps its hind limbs when it is picked up by the tail. Basic neurological assessments are described elsewhere in detail (Crawley 2000; Crawley and Paylor 1997; Fernagut et al. 2003).

### A. Challenging Beam Traversal

After injury or disease, both humans and animals use compensatory strategies to perform tasks accurately, making it difficult for investigators to detect impairments, especially in the early stages of the disease (LeVere 1988; Schallert 1988; Whishaw 2000). Therefore, it is important to challenge the animals to the limit of their abilities to uncover early effects of the mutations. In traditional beam-walking tests, animals must traverse several different beams of narrowing widths (Drucker-Colin et al. 1991). Time to traverse and slips along the beam both indicate alterations in DA function (Dluzen et al. 1995, 2001; Drucker-Colin et al. 1991). With this evidence in mind, we recently designed a challenging beam test that is highly sensitive to subtle sensorimotor dysfunction (Fleming et al. 2003; Goldberg et al. 2003). The test shows some selectivity for PD-causing mutations, as knock-in mouse models of Huntington disease do not display impairments on this test (Fleming et al. 2003; Hickey et al. 2003) at an age when they show other motor anomalies (Menalled et al. 2003). Instead of using several different beams as in previous studies, we constructed one tapered beam. The beam is made of Plexiglas (Plastics Zone Inc., Woodland Hills, CA) and consists of four sections (25 cm each, 1 meter total length), each section having a different width. The beam starts at a width of 3.5 cm and gradually narrows to 0.5 cm by 1 cm increments. Animals are trained to traverse the length of the beam starting at the widest section and ending at the narrowest, most difficult, section. Training is relatively easy and is done over two days. For training and testing, the beam must be placed approximately 10 cm off the ground and the narrow end must lead directly to the home cage. On the first day of training, the animal is placed on the widest part of the beam and the home cage is positioned in close proximity to the animal. As the animal begins to approach the home cage, the cage is gradually moved to the narrow end of the beam. After two assisted trials, animals can typically traverse the entire length of the beam unassisted. Animals then must complete five unassisted runs across the entire length of the beam. On the second day of training, animals must run five trials across the beam. On the day of the test, investigators increase task difficulty by placing a mesh grid (1 cm squares) of corresponding width over the beam surface, leaving approximately a 1 cm space between the grid and the beam surface. Animals are then videotaped and scored while they traverse the grid-surfaced beam for a total of five trials (see Video 1).

### 1. Analysis of the Challenging Beam Traversal Test

An experimenter blind to genotype should view the videotapes in slow motion and rate for errors (slips through the grid), number of steps made by each animal, and time to traverse across five trials. An error is counted when, during a forward movement, a limb (forelimb or hindlimb) slips through the grid and is visible between the grid and the beam surface. An individual animal can make a maximum of four slips per step. By scoring each limb slip individually, the experimenter can measure the severity of the error. For instance, an animal that slips with three or all four limbs through the grid during a step receives a higher error per step score than an animal that slips only one limb through the grid during a step. Slips should not be counted if the animal is not moving forward or if the animal's head is oriented to the left or right of the beam. Error-per-step scores, time to traverse and number of steps are calculated for wild-type and transgenic mice and averaged across all five trials. Error-per-step scores, time to traverse, and number of steps can also be broken down by beam width, illustrating where on the beam transgenic mice may be different from wild-type controls.

### 2. Challenging Beam Data

We have shown that parkin knock-out,  $\alpha$ -synuclein overexpressing, and  $\alpha$ -synuclein knock-out mice display significant impairments on the challenging beam test (table 1).  $\alpha$ -synuclein overexpressing mice display more profound deficits that were progressive compared to both parkin and  $\alpha$ -synuclein knock-out mice.

### B. Response to Sensory Stimuli

Sensory neglect or sensory inattention was first shown to be associated with nigrostriatal damage in rats (Ljungberg and Ungerstedt 1976; Marshall and Gotthelf 1979). Marshall et al. (1971) presented rats treated with the neurotoxin 6-OHDA with various sensory stimuli and measured their responsiveness to each stimulus. Stimuli were presented to various parts of the body including the snout. Subsequently, it has been shown that sensory neglect can be reversed by DA agonists (table 2; Marshall and Gotthelf 1979) and is correlated with DA cell damage in the substantia nigra (table 2; Lees et al. 1985). Similarly, a somatosensory test was designed by Schallert et al. (1982, 1983, 2000; Schallert and Tillerson, 2000) to assess nigrostriatal DA dysfunction in the unilateral 6-OHDA rat. In the first phase of the test, small adhesive stickers are placed on the rat's forelimbs (radial/ulnar region) so that both forelimbs are simultaneously stimulated. Contact and removal times are measured for each fore limb. Typically, animals are consistently slower to contact and remove the stimulus from the contralateral

TABLE 2 Reversal of Sensorimotor Impairments with Dopamimetic Treatments

Behavioral tests	Animal	Model	Reversal			Reference
			L-dopa	APO/AMP	Grafts/viral vectors (DA or TH)	
Beam Walking	Aged vs Young Rats	Haloperidol	Y	Y	Y	1, 2
Inverted Grid Walking	Aged mice	MPTP, Faults correlated with striatal DA content	Y			3
Sensory Neglect	Rats	6-OHDA, Neglect correlated with nigrostriatal DA neurons		Y	Y	4, 5, 6, 7, 8, 9
	Mice	MPTP				10
Locomotor Activity	Mice	MPTP	Y	N		11, 12, 13, 14
	Mice	6-OHDA		Y		15
	Rats	6-OHDA		Y		16
Bin Cotton Use	Mice	DA-deficient			Y	17
		MPTP				18, 19
Stride Length	Mice	MPTP, Correlated with striatal DA content	Y			3
	Mice	Reserpine	Y			20
	Rats	6-OHDA				21

Summary of reversal or improvement in motor function by L-dopa, apomorphine (APO), amphetamine (AMP), dopamine-producing grafts, or viral vectors expressing tyrosine hydroxylase following lesions of nigrostriatal dopaminergic system or dopamine antagonist treatment. Citation (ref): 1 Drucker-Colin et al 1991; 2 Garcia-Hernandez et al 1993; 3 Tillerson et al. 2002; 4 Lees et al. 1985; 5 Schallert et al. 1983; 6 Schallert et al. 1982; 7 Marshall and Gotthelf 1979; 8 Ljungberg and Ungerstedt 1976; 9 Dunnett et al. 1987; 10 Weihmuller et al. 1988; 11 Archer and Fredriksson 2003; 12 Archer et al 2003; 13 Fredriksson et al 1990; 14 Sundstrom et al 1990; 15 Protais et al 1983; 16 Breese et al 1984; 17 Szczytka et al. 2001; 18. Sedelis et al. 2000; 19. Hofele et al. 2001; 20 Fernagut et al 2002; 21 Schallert et al. 1978.

(impaired) side. For the second phase, a somatosensory threshold is established by systematically altering the size of the stimuli. Direct-acting DA agonists can reverse impairments in somatosensory detection (table 2; Schallert and Tillerson 2000; Schallert et al. 1982).

Because genetic mouse models of PD have bilateral deficits, we developed a sensory test adapted from Marshall et al. (1971) and Schallert et al. (1982). In this test, we measure response times to sensory stimuli placed on the snout. Small adhesive stimuli (Avery adhesive-backed labels) are placed on the snout of the mouse and time to make contact and remove the stimulus is recorded. To remove the stimulus, an animal raises both forelimbs towards its face and removes the stimulus with both forepaws. Typically, wild-type mice will make contact and remove a stimulus within ten seconds. Each animal receives two trials and the trials are alternated between mice. All testing is performed in the animal's home cage with cage mates and bedding temporarily removed during testing as both can interfere with stimulus removal. If an animal does not remove the stimulus within sixty seconds then the experimenter removes it, and the trial for the next mouse is initiated. If the animal does not remove the stimulus on either

trial, then the size of the stimulus can be systematically increased until the animal makes contact and removes it (Goldberg et al. 2003).

### 1. Analysis of Sensory Response

Stimulus contact and removal times can be analyzed when animals remove the stimulus in under sixty seconds. If the animal requires larger stimuli to contact and remove the stimulus, then a rank score corresponding to the size of the stimulus can be given to the stimulus and an analysis with nonparametric statistics can be used.

### 2. Sensory Response Data

Both parkin knock-out and  $\alpha$ -synuclein overexpressing mice displayed sensory response impairments whereas  $\alpha$ -synuclein knock-out mice did not (table 1). Parkin mice showed impairments as early as two months of age whereas  $\alpha$ -synuclein overexpressing mice did not develop sensorimotor impairments until six months of age, suggesting a more progressive phenotype in the  $\alpha$ -synuclein overexpressing mice.

### C. Spontaneous Activity

Although activity measures are affected by habituation (Sedelis et al. 2001) and differences can be masked with repeated testing, they have been used extensively in MPTP-treated mice (Sedelis et al. 2001). Hypoactivity in MPTP-treated mice is DA-dependent and can be reversed by L-dopa (table 2; Fredriksson et al. 1990). Spontaneous activity is easily measured using automated activity chambers equipped with photobeam sensors, but it can also be measured without automation. In this case, animals are placed in a small transparent cylinder (height: 15.5 cm and diameter: 12.7 cm) and their spontaneous behaviors are videotaped for three minutes. The cylinder is placed on a piece of glass and a mirror is situated at an angle beneath the cylinder to permit a clear view of motor movements along the ground and along the walls of the cylinder. Four different activity parameters including the number of rears, forelimb and hindlimb steps, and time spent grooming are measured (see Video 2).

#### 1. Analysis of Spontaneous Activity

Videotapes should be viewed and rated in slow motion by an experimenter blind to genotype and experimental manipulation. The experimenter counts forelimb and hindlimb steps when an animal moves either both forelimbs or both hindlimbs across the floor of the cylinder. A rear is counted when an animal makes a vertical movement with both forelimbs removed from the ground. The experimenter also measures time spent grooming within the three minutes.

#### 2. Spontaneous Activity Data

In this test,  $\alpha$ -synuclein overexpressing mice showed significantly reduced spontaneous activity that persisted over time including educations in rearing, forelimb and hindlimb stepping, and grooming (table 1). Parkin knock-out mice showed decreased rearing at seven months of age, however, decreased rearing was not observed in the other ages tested. In contrast to both  $\alpha$ -synuclein overexpressing and parkin knock-out mice,  $\alpha$ -synuclein knock-out mice displayed significant increases in spontaneous activity that were persistent over time and included increases in rearing, forelimb and hindlimb stepping, and grooming (table 1; Fleming et al. 2003). Interestingly, Huntington disease knock-in mice also display increased rearing at an early age similar to  $\alpha$ -synuclein knock-out mice (Menalled et al. 2002; 2003) indicating that rearing is one the earliest motor behaviors to be affected by various forms of basal ganglia dysfunction. However, in contrast to rearing, grooming was never affected in Huntington disease knock-in models, suggesting that anomalies in this behavior may be more specifically related to nigrostriatal dysfunction (Menalled et al. 2002, 2003).

### D. Bin Cotton Use

Orofacial shredding is an important motor behavior involved in nest building, a natural mouse behavior related to thermoregulation and pup survival (Broida and Svare 1982; Crawley 2000; Lynch 1980). Both male and female mice shred material to build nests, and researchers have analyzed shredding behavior to assess nigrostriatal sensorimotor function in rodents (Hofele et al. 2001; Sedelis et al. 2000; Szczypka et al. 2001; Upchurch and Schallert 1983). The behavior requires orofacial and forelimb movements, where animals pull the nesting material apart with their forelimbs and teeth and then break down the material in their mouths and incorporate it into their bedding. These movements are dopamine dependent, are significantly reduced with dopamine antagonists (table 2; Upchurch and Schallert 1983), and impairments can be reversed with increased DA production (table 2; Szczypka et al. 2001). When experimenters place the nesting material in the feeder bin of the cage, animals must rear up and pull the nesting material from the feeder. This adjustment makes the test more challenging than if the nesting material was just placed on the floor of the cage. However, in the case where animals do not pull and shred the nesting material at all, researchers must perform an additional test where nesting material is placed on the cage floor, making it more accessible, and then measure the amount of cotton shredded.

#### 1. Analysis of Bin Cotton Use

Experimenters measure shredding behavior by placing preweighed cotton into the feeder of each mouse's cage for each day of testing. Nests should be removed before placing new cotton into the feeder. Experiments measure the percent of bin cotton use daily for each mouse and compare the amounts between transgenic and wild-type mice.

#### 2. Bin Cotton Use Data

Parkin,  $\alpha$ -synuclein overexpressing, and  $\alpha$ -synuclein knock-out mice displayed varying levels of impairments in bin cotton use (table 1).  $\alpha$ -synuclein overexpressing mice display severe impairments at four and eight months of age. Both parkin and  $\alpha$ -synuclein knock-out mice displayed significant but more subtle impairments in bin cotton use when tested at eight to nine months of age. When cotton was placed in the bottom of the cage and not in the feeder all animals shredded the cotton, and this behavior ruled out loss of nesting instinct.

### E. Gait Analysis

Alterations in gait are a major cause of disability in PD patients (Rao et al. 2003). PD patients typically move in

short shuffling steps that can cause falls and injury (Rao et al. 2003). Researchers can easily measure gait in rodents. In MPTP-treated mice shorter stride lengths correlate with reduced DA content in the striatum and can be reversed with L-dopa (table 2; Eilam et al. 1998; Tillerson et al. 2002). To measure gait, experimenters train animals to walk through a narrow alley leading into their home cage. Once the animals are trained, paper is placed along the alley floor and the animals' forelimbs and/or hindlimbs are brushed with different colors of non-toxic paints. Animals are then placed at the beginning of the alley. As they walk into their home cage they leave their paw prints on the paper underneath (Barlow et al. 1996; Fernagut et al. 2002; Schallert et al. 1978; Tillerson et al. 2002). Stride length is determined by measuring the distance between paw prints. Only strides made while continuously walking (no stopping) should be included in the analysis and the first and last stride lengths should be excluded from the analysis because animals tend to make irregular steps at the beginning and end of the test. Several parameters can be recorded including stride length, stride width, and maximum stride difference (variability between stride lengths).

Gait analysis data showed that  $\alpha$ -synuclein knock-out mice had significantly shorter stride lengths compared to wild-type mice (table 1) at eight months of age. In contrast,  $\alpha$ -synuclein overexpressing mice at eight months of age did not have significantly altered hindlimb gait compared to wild-type mice. Parkin knock-out mice were not tested on gait. In comparison, Huntington disease knock-in mice show gait impairments, but only at a much later age than rearing and locomotor impairments (Menalled et al. 2003).

#### F. Additional Motor Tests

In addition to the tests described here, other tests have been developed that are highly sensitive in MPTP mice and would be useful when phenotyping genetic mouse models of PD. These include the inverted grid test (Tillerson et al. 2002, 2003) and the pole test (Matsuura et al. 1997; Ogawa et al. 1985, 1987; Sedelis et al. 2001). For the inverted grid test, animals are placed upside down on a grid approximately 20 cm above the ground for thirty seconds. In this test, moderate doses of MPTP in mice caused shortened forelimb step length, increased time spent against the wall, and increased forelimb faults compared to controls. These parameters correlate with dopaminergic markers in the striatum that are reversible with L-dopa. For the pole test, researchers placed animals head up on the top of a pole and measured the time to orient the body downward and time to descend. MPTP-treated mice display slower times in both parameters compared to controls and, similar to the inverted grid test, the impairments are reversed by L-dopa (Matsuura et al. 1997; Ogawa et al. 1985, 1987). In addi-

tion, Huntington disease knock-in mice display significant impairments in the pole test (Hickey et al. 2003), indicating it is a useful test for basal ganglia dysfunction.

#### G. Cognitive Tests

In PD patients, motor impairments are the primary symptoms used to diagnose the disorder, but patients also suffer from cognitive impairments and depression (Kuzis et al. 1997; Rao et al. 2003; Uekermann et al. 2003). Cognitive impairments often precede motor impairments and researchers may benefit from analyzing these types of impairments when studying the early stages of PD. Cognitive impairments have been modeled in primate models of PD (Schneider and Roeltgen 1993) and to a lesser extent in rodent models. In primates impairments manifest as delayed matching to sample, and problems with visual discrimination and object retrieval tasks, while rodents show delayed alternation in the T-maze (Tanila et al. 1998) and impaired habit learning in the cross maze (Packard and McGaugh 1996), suggesting cognitive tests could help characterize phenotypes for the genetic mouse models of PD.

#### H. Specificity of Behavioral Impairments in Models of Parkinson Disease

As indicated in table 2, the alterations in sensorimotor function found in the genetic mouse models of PD are similar to those observed in animals with a selective loss of nigrostriatal DA neurons induced by toxin injections and the changes are reversible by DA agonists. However, sensorimotor function can also be altered in other disease models including Huntington disease (Carter et al. 1999; Menalled et al. 2002, 2003) and ataxia telangiectasia (Barlow et al. 1996; Eilam et al. 1998). In the course of our studies we noticed that anomalies in beam traversal are more selective for PD than Huntington disease knock-in models. Interestingly, PD and Huntington disease models show opposite changes in rearing and locomotor activity at the early stages, but as Huntington disease knock-in mice age, they begin to show defects similar to PD mice. These results agree with clinical data showing pervasive akinesia in patients with Huntington disease (Curra et al. 2000; Jahanshahi et al. 1993; Thompson et al. 1988). Furthermore, some studies demonstrated deficits in dopamine function in Huntington disease mouse models, further blurring the line between both diseases (Hickey et al. 2002). This illustrates the major advantage of genetic disease models, where the effects of the mutation in the whole organism can be mimicked, as opposed to toxin models, which produce selective lesions of one pathway, where many more pathways are affected during the disease in humans (Braak et al. 2003; Gesi et al. 2000; Patt and Gerhard 1993).

## IV. CONCLUSIONS

Although the cause of most cases of PD remains unknown, the recently discovered familial forms of the disease have created a new approach for studying PD. Researchers continue to generate mice engineered with genetic mutations similar to those found in familial PD for assessing the long-term effects of these mutations *in vivo*. Researchers must characterize the behavior of these mice to validate their role as a model of PD and to determine optimal therapeutic targets.

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### Video Legends

#### *Spontaneous Activity in the Cylinder*

Animals are placed in a clear plastic cylinder and are videotaped from underneath the cylinder to permit viewing of forelimb and hindlimb movements.

**SEGMENT 1** A wild-type (littermate control) mouse in the cylinder at four months of age.

**SEGMENT 2** An alpha-synuclein-overexpressing mouse in the cylinder at four months of age. Notice the lack of hindlimb stepping compared to the wild-type mouse in segment 1.

#### *Challenging Beam Traversal*

Animals are placed on a tapered beam and time to traverse, number of steps and errors are recorded as the animal moves along the beam.

**SEGMENT 1** A wild-type (littermate control) mouse on the challenging beam at four months of age.

**SEGMENT 2** An alpha-synuclein-overexpressing mouse on the challenging beam at four months of age. Notice the increased number of errors (slips) and amount of time to traverse compared to the wild-type mouse in segment 1.

**SEGMENT 3** The same wild-type mouse as in segment 1, now at six months of age.

**SEGMENT 4** The same alpha-synuclein-overexpressing mouse as in segment 2, now at six months of age. Notice the increased number of errors (slips) and time to traverse compared to segment 2.

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