

# Animal Models of PD: Pieces of the Same Puzzle?

## Minireview

Ted M. Dawson,<sup>1,2,3,5</sup> Allen S. Mandir,<sup>2</sup>  
and Michael K. Lee<sup>4</sup>

<sup>1</sup>The Institute for Cell Engineering

<sup>2</sup>Department of Neurology

<sup>3</sup>Department of Neuroscience

<sup>4</sup>Department of Pathology

Johns Hopkins University School of Medicine  
Baltimore, Maryland 21287

**Parkinson's disease (PD) is a common neurodegenerative disorder with no known cure. The etiology of PD is likely due, in part, to combinations of genetic susceptibilities and environmental factors. In rare familial cases, PD is due to genetic mutations. A number of new genetic and toxin models of PD and advances in older models are yielding important new information about the pathogenesis of PD. This has prompted us to critically review the current animal models for PD and discuss how these models may yield fresh insights into the pathogenesis of PD, as well as new therapeutic opportunities.**

An ideal animal model would recapitulate most, if not all, the features of sporadic PD (Table 1). The degenerative process of PD usually begins in late adulthood. It is relentlessly progressive and initially causes a preferential loss of dopamine (DA) neurons within the substantia nigra pars compacta (SNc), leading to motoric dysfunction (slowness of movement, rigidity, rest tremor, and postural instability). In many cases of PD, cognitive disturbances and degenerative changes in non-DA neurons are prominent features. During the initial stages of PD, excellent symptomatic relief can be achieved with DA replacement therapy. However, as the disease progresses, these medications and other adjunctive therapies become less effective and patients eventually become disabled. In addition to the loss of DA neurons, a key determinant that differentiates this disorder under most circumstances from other neurodegenerative diseases that also exhibit nigrostriatal degeneration is the presence of Lewy bodies (LBs) and Lewy neurites (LNs). LBs and LNs are eosinophilic cytoplasmic proteinaceous inclusions that contain  $\alpha$ -synuclein ( $\alpha$ -syn), ubiquitin-proteasomal proteins, synphilin, neurofilaments, microtubule-associated proteins, synaptic vesicle-associated proteins, and markers of oxidative stress. Ultrastructurally, LBs are composed of 10–16 nm filaments that radiate from the central core, and  $\alpha$ -syn is the major component of these filaments. Another key feature of PD is selective loss or deficits in the activity of mitochondrial complex 1 and increased indices of oxidative stress.

### **Intoxication Models of PD**

Early animal models of PD relied on the depletion of DA by pharmacologic agents such as reserpine (Table 1) (see Beal, 2001, and references therein). These and re-

lated studies led to the discovery that the motor symptoms of PD were due to nigrostriatal DA depletion and the eventual use of DA replacement as the mainstay of therapy for PD. Although DA depleting agents produce parkinsonian signs, their effects are transient, and they do not elicit DA neuronal death.

A popular model using intraparenchymal neurochemical lesioning of the striatum, SNc, or nigrostriatal pathway by 6-hydroxydopamine (6-OHDA) in a variety of species reliably destroys DA neurons and produces parkinsonism (Table 1) (Beal, 2001). The 6-OHDA model has been quite predictive of symptomatic and cell replacement therapies for PD and is of value in investigating regenerative therapies. However, it is an imperfect model, in that it is acute and does not replicate many of the features of PD.

The discovery that the accidental use of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in drug addicts produces parkinsonism in humans has provided many important insights into the pathogenesis of PD (Beal, 2001; Dunnett and Bjorklund, 1999). Thus, it is more than merely just a DA depletion model. Systemic MPTP intoxication in a variety of species satisfies most of the requirements for an ideal parkinsonian model (Table 1), with the exception that it is an acute, nonprogressive insult. However, administration of MPTP in smaller doses over a prolonged period may provide progressive, permanent depletion of nigrostriatal DA. In primates, MPTP intoxication replicates all the clinical signs of sporadic PD and leads to neuronal nonfibrillar inclusions that contain  $\alpha$ -syn (Beal, 2001). DA neuron selectivity of MPTP-induced neurotoxicity stems from the conversion of MPTP to 1-methyl-4-phenyl pyridium (MPP<sup>+</sup>) by monoamine oxidase B and from avid affinity of MPP<sup>+</sup> for the dopamine transporter. Once inside DA neurons, MPP<sup>+</sup> is actively concentrated within the mitochondria where it binds to and inhibits complex 1 of the mitochondrial electron transport chain, thereby leading to oxidative stress and neuronal cell death. These observations spawned the discovery of mitochondrial dysfunction and reduction of complex 1 activity in sporadic PD. Use of MPTP animal models, particularly primate models, led to improved symptomatic surgical and medical therapies in PD. Despite the potential strength of this model, to date, no neuroprotective or neuroregenerative pathway or agent identified in the MPTP model has been shown to be efficacious in PD patients, except for fetal DA neuron transplants. Perhaps the lack of predictive value relates to the acute nature of the MPTP intoxication model, or we do not fully understand the complexity of neuronal degeneration in PD. However, the verdict on the predictability of the MPTP model for neuroprotective and regenerative therapies is still unsettled. In particular, recent use of genetically engineered mice and biochemical reagents have revealed important new information about the molecular mechanisms affecting the degeneration of DA neurons, including the role of reactive oxygen species, excitotoxicity, caspase-dependent and -independent forms of cell death, and the role of microglia (Figure 1). Further-

<sup>5</sup>Correspondence: tdawson@jhmi.edu

Table 1. Key Features of Current Animal Models of PD

Model	Progressive Age-Dependent Loss of DA Neurons	Progressive Age-Dependent Degeneration of Non-DA Neurons	Motor Deficits	L-DOPA Responsive	Mitochondrial Complex 1 Deficiency	LB-Like Inclusions
<b>Intoxication models</b>						
Reserpine	-	-	+	+	-	-
6-OHDA	+	+	+	+	?	-
MPTP	+	-	+	+	+	nonfibrillar $\alpha$ -syn inclusions
Rotenone	+	-	+	+	+	+
Paraquat/maneb	+	-	+	ND	+	nonfibrillar $\alpha$ -syn inclusions
<b>Familial PD models</b>						
<b>Drosophila:</b>						
Pan-neuronal Promoter-Hu WT, A53T, or A30P $\alpha$ -syn	+	-	+	+	?	+
<b>Murine:</b>						
PDGF $\beta$ -Hu WT $\alpha$ -syn	↓TH	?	+	ND	ND	atypical $\alpha$ -syn inclusions
Thy1 $\alpha$ -Hu WT or A53T $\alpha$ -syn	-	+	+	ND	ND	nonfibrillar $\alpha$ -syn inclusions
TH-Hu A30P/A53T $\alpha$ -syn	↓DA	-	+	ND	ND	nonfibrillar $\alpha$ -syn inclusions
Mouse PrP-Hu A53T $\alpha$ -syn	-	+	+	ND	ND	+
<b>Rat:</b>						
AAV-Hu WT or A53T $\alpha$ -syn	+	+	+	+	ND	nonfibrillar $\alpha$ -syn inclusions

Not all familial or intoxication models are listed. Transgenic  $\alpha$ -syn models that do not have significant pathology are not listed (see Lee and Price, 2001, and references therein). Only the pan-neuronal promoter  $\alpha$ -syn transgenic flies are reported (see Feany and Bender, 2000). Abbreviations: ND, not determined.

more, advances in regenerative therapies have arisen from studies of MPTP intoxication, and many of these discoveries are currently in various stages of therapeutic development (Beal, 2001; Dunnett and Bjorklund, 1999).

Several epidemiological studies suggest that pesticides and other environmental toxins may be involved in the pathogenesis of PD (Table 1) (Beal, 2001). The common herbicide paraquat (1,1'-dimethyl-4,4'-bipyridinium) is a putative risk factor for PD based on its

structural similarity to MPP<sup>+</sup>. Systemic administration of paraquat causes DA neuronal loss and striatal dopaminergic denervation and aggregation of  $\alpha$ -syn in rodents (Manning-Bog et al., 2002; Thiruchelvam et al., 2000). The agricultural use of paraquat often parallels the use of the fungicide manganese ethylenebisdithiocarbamate (maneb). Interestingly, in mice, systemic administration of maneb potentiates the DA toxicity of paraquat (Thiruchelvam et al., 2000). The combined use of paraquat and maneb as an animal model for PD is still in the early stages, but it holds particular promise as an important model due to the potential role of environmental toxins in the etiology of sporadic PD.

Rotenone, another agricultural toxin that is used as an insecticide and fish poison, produces parkinsonism in rats when administered intravenously (Table 1) (Betarbet et al., 2000). Rotenone is a mitochondrial complex 1 inhibitor, but it differs from MPTP in that rotenone acts uniformly throughout the brain; thus, DA neurons are uniquely sensitive to mitochondrial complex 1 inhibition. This model is quite remarkable as the rats develop progressive degeneration of nigrostriatal DA neurons and inclusions that stain with antibodies against ubiquitin and  $\alpha$ -syn. These inclusions contain a dense core surrounded by fibrils, similar to LBs. Thus, derangements in mitochondrial complex 1 appear to be directly linked with alterations in  $\alpha$ -syn. Furthermore, the rats have clinical signs reminiscent of PD including bradykinesia, postural instability, unsteady gait, and tremor that respond to the dopamine agonist apomorphine. Although

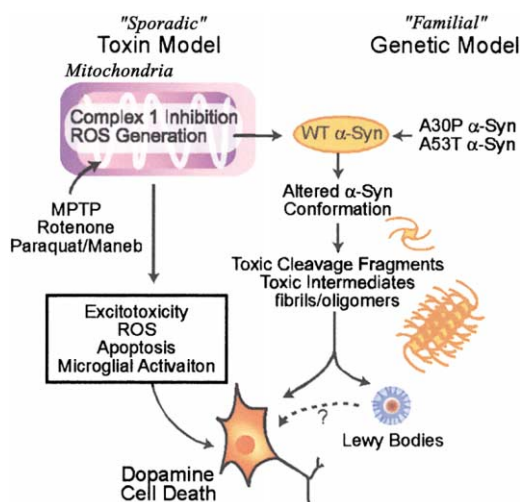


Figure 1. PD Models—Pieces of the Same Puzzle

this model satisfies most of the criteria for an excellent model of PD, its utility may be limited since there is a high mortality rate, and less than 50% of surviving rats treated with rotenone develop consistent lesions.

#### **Genetic Models of PD**

Although the majority of PD is sporadic, a small percentage of PD is familial. Eight loci and mutations in  $\alpha$ -syn (A53T and A30P) and parkin are implicated in the pathogenesis of familial PD (see Lansbury and Brice, 2002, and references therein). Mutations in ubiquitin C-terminal-hydrolase-L1 (UCH-L1) may also play a role in familial PD. The discovery of familial PD-associated mutations provides the opportunity to develop genetic models that may provide novel insights into the pathogenesis of PD and additional models to test therapeutics. The importance of UCH-L1 in neuronal function is indicated by a naturally occurring deletion of the *uch-l1* gene in gracile axonal dystrophy (*gad*) mice that leads to degeneration of sensory axons (see Lee and Price, 2001). No animal models of parkin mutations have been described. A number of animal models of synucleinopathy-induced neurodegeneration have been reported (see Lee and Price, 2001, and references therein).

In *Drosophila*, when human wild-type (WT) and mutant (A53T or A30P)  $\alpha$ -syn is expressed in neurons, there is a remarkably selective age-dependent loss of subsets of DA neurons. The loss of DA neurons is associated with progressive motoric dysfunction that responds to DA replacement therapy. Moreover, the loss of DA neurons is associated with the presence of LB-like, filamentous intracytoplasmic inclusions that contain  $\alpha$ -syn (Feany and Bender, 2000). Thus, the *Drosophila* model is an outstanding model that recapitulates most of the phenotypic and pathologic features of PD. The *Drosophila* model is a powerful and inexpensive tool that can be used to rapidly screen for pharmacologic and genetic interventions that may modify dopaminergic neuronal inclusions and death. Indeed, overexpression of the molecular chaperone Hsp70 prevents DA neuron loss, and interference with endogenous chaperone activity accelerates  $\alpha$ -syn toxicity (Auluck et al., 2002). Hsp70 protected DA neurons without influencing the formation of inclusions, suggesting that LBs may play little if any role in the degeneration of DA neurons. In a *Drosophila* model of Huntington's disease, histone deacetylase inhibitors ameliorate neurotoxicity; thus, candidate therapeutic compounds can be screened in *Drosophila* (Stefan et al., 2001). It is without doubt that the *Drosophila* model will teach us much about the pathogenesis of  $\alpha$ -syn-induced neurodegeneration in flies. However, the challenge will be to verify potential neuroprotective agents and genetic modifiers in human PD.

A number of transgenic mice models overexpressing human  $\alpha$ -syn have been developed (Table 1) (Lee and Price, 2001). While none of the transgenic  $\alpha$ -syn mice accurately model PD, a number of these models exhibit significant synucleinopathy-induced neurodegeneration, thus modeling characteristics of human  $\alpha$ -syn pathology.  $\alpha$ -Syn pathology is emerging as a major pathogenic component of a large spectrum of diverse and related neurodegenerative diseases, designated as synucleinopathies (see Goedert, 2001, and references therein). Only two of the models have alterations in the dopaminergic system as manifested by modest decre-

ments in striatal tyrosine hydroxylase (TH) immunoreactivity or DA levels (Lee and Price, 2001; Masliah et al., 2000; Richfield et al., 2002). Overall, these  $\alpha$ -syn transgenic mice represent powerful tools to investigate the toxicity of  $\alpha$ -syn in vivo and factors that modulate in vivo aggregation of  $\alpha$ -syn.

In contrast to the studies in flies, results from transgenic mice indicate that  $\alpha$ -syn aggregation is mechanistically associated with neuronal dysfunction/degeneration (Figure 1). For instance, studies in the platelet-derived growth factor- $\beta$  (PDGF $\beta$ ) human WT  $\alpha$ -syn mice suggest that  $\beta$ -synuclein ( $\beta$ -syn) inhibits  $\alpha$ -syn aggregation, prevents loss of striatal TH, and improves Rota-rod performance. Thus,  $\beta$ -syn might function as an anti-parkinsonian factor (Hashimoto et al., 2001). Moreover,  $\beta$ -amyloid peptides may contribute to the development of LB disease as human  $\beta$ -amyloid expression in these mice converts nonfibrillar  $\alpha$ -syn inclusions to fibrillar inclusions (Masliah et al., 2001). These biochemical changes are associated with increased loss of DA markers in APP/ $\alpha$ -syn double transgenic mice. Expression of human WT or A53T  $\alpha$ -syn using the Thy-1 promoter also results in  $\alpha$ -syn pathology in transgenic mice with significant degeneration of neuromuscular junctions and axonal degeneration (van der Putten et al., 2000).

Mice expressing human A53T mutant  $\alpha$ -syn, but not WT or the A30P mutant, under the control of the mouse prion protein promoter (PrP) develop an adult-onset neurodegenerative disease with progressive motoric dysfunction leading to death (Giasson et al., 2002; Lee et al., 2002). Thus, A53T mutant human  $\alpha$ -syn appears to have significantly enhanced in vivo toxicity. Neurological dysfunction in the mouse PrP human A53T  $\alpha$ -syn transgenic mice is also associated with age-dependent intracytoplasmic neuronal accumulation of  $\alpha$ -syn and pathological neuronal accumulations of ubiquitin (Giasson et al., 2002; Lee et al., 2002). In affected mouse PrP human A53T  $\alpha$ -syn transgenic mice,  $\alpha$ -syn accumulates in the detergent-insoluble fractions (Giasson et al., 2002; Lee et al., 2002). Significantly, lower molecular mass  $\alpha$ -syn and higher molecular mass aggregates of  $\alpha$ -syn selectively accumulate in the detergent-insoluble fraction (Lee et al., 2002). Lower molecular mass  $\alpha$ -syn and higher molecular mass aggregates of  $\alpha$ -syn only accumulate in areas of the brain undergoing active neurodegeneration, suggesting that proteolytic processing and aggregation of  $\alpha$ -syn contributes to the neuropathology (Lee et al., 2002). The mouse PrP human A53T  $\alpha$ -syn transgenic mice are distinct in many aspects from the other models. For instance, the pathology is more extensive, animals die, and unlike other studies, the  $\alpha$ -syn inclusions contain 10–16 nm wide fibrils similar to human LBs (Giasson et al., 2002). Furthermore, neuropathological studies on individuals with the A53T  $\alpha$ -syn mutation do not develop classical PD but instead exhibit extensive  $\alpha$ -syn aggregates, especially in neuronal processes, similar to the observations in the mouse PrP human A53T  $\alpha$ -syn transgenic mice (Goedert, 2001).

Overall, the results from the various  $\alpha$ -syn transgenic mice studies indicate that formation of toxic  $\alpha$ -syn aggregates are associated with, and may be required for, significant neurodegeneration. It is not clear why none of the  $\alpha$ -syn transgenic models have significant abnormalities in DA neurons, particularly in light of the dra-

matic pathologic changes in *Drosophila* DA neurons and DA-dependent  $\alpha$ -syn toxicity in human neuronal cultures (Xu et al., 2002). It is conceivable that rodent DA neurons are particularly resistant to  $\alpha$ -syn toxicity, perhaps from the relative levels of  $\alpha$ -syn versus  $\beta$ -syn,  $\gamma$ -synuclein, and/or other intrinsic protective factors. Potentially, if sufficient levels of  $\alpha$ -syn expression are achieved in DA neurons via transgenic approaches, a model of  $\alpha$ -synucleinopathy-induced PD should be possible. Consistent with this notion is the observation that stable, virally mediated overexpression of human WT and A53T mutant  $\alpha$ -syn with adeno-associated virus (AAV) in SNc neurons of rats leads to progressive age-dependent loss of DA neurons, motor impairment, and  $\alpha$ -syn positive cytoplasmic inclusions (Kirik et al., 2002).

#### **Do We Have a Good Model For PD?**

Currently, none of the models fully recapitulate all of the key clinical and neuropathologic features of PD. However, because each of the models do recapitulate significant pathological features of PD and represent parts of the same puzzle, combinatorial study of multiple models are warranted to provide a more fully developed view of PD pathogenesis (Table 1, Figure 1). Specifically, the  $\alpha$ -syn transgenic fly and the MPTP intoxication models are ideal for investigating neurodegeneration induced by  $\alpha$ -syn and by mitochondrial complex 1 inhibition, respectively. Both models are amenable to investigating molecular mechanisms of neurodegeneration and screening of candidate therapeutic agents. The MPTP model is also of utility in investigating neuroregenerative and symptomatic therapies.

The  $\alpha$ -syn transgenic mouse models are important reagents to identify and study endogenous mammalian factors that modulate  $\alpha$ -syn aggregation and toxicity. Because it is likely that these "modulators" will also be present in humans, they would be obvious therapeutic targets. The mouse PrP human A53T  $\alpha$ -syn mice may be a powerful model to study pathogenic mechanisms and potential therapies, as this model most closely recapitulates  $\alpha$ -syn pathology. It is also the only mammalian PD model that develops progressive neurodegeneration leading to death, a readily accessible therapeutic endpoint. It will also be important to further refine the  $\alpha$ -syn transgenic mice models to achieve progressive degeneration of DA neurons. These efforts will clearly lead to better understanding about mechanistic relationships between PD and  $\alpha$ -syn abnormalities. Moreover, transgenic mouse models of progressive,  $\alpha$ -syn-dependent degeneration of DA neurons would be a powerful tool to rapidly test the in vivo efficacy of novel therapeutic approaches.

At present, one could envisage investigating molecular mechanisms of pathogenesis in all models with the *Drosophila* and MPTP models being at the forefront of in vivo screening of therapeutic compounds. Positive hits in the *Drosophila* model could be tested in  $\alpha$ -syn transgenic mice and the MPTP model. Positive hits in the MPTP model could be tested in  $\alpha$ -syn transgenic mice and *Drosophila*. Convergence of pathogenic mechanisms and therapies might be particularly attractive to test in humans. Although oxidative stress and mitochondrial complex 1 dysfunction promotes  $\alpha$ -syn pathology, targets identified in either the  $\alpha$ -syn transgenic or MPTP models that don't work in the other model may still be

worth studying in humans, as the pathogenic mechanisms may not be fully interactive. Ultimately, an ideal model would exhibit all the clinical and pathologic features of PD, but this may be a difficult challenge as new insights and unforeseen pathogenic mechanisms of neurodegeneration are bound to arise as additional PD-linked genes are identified, requiring refinement of existing models and the generation of new models.

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