

Review Article

Toxin-Induced and Genetic Animal Models of Parkinson's Disease

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Received 13 October 2010; Accepted 31 October 2010

Academic Editor: Yuzuru Imai

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Parkinson's disease (PD) is a common progressive neurodegenerative disorder. The major pathological hallmarks of PD are the selective loss of nigrostriatal dopaminergic neurons and the presence of intraneuronal aggregates termed Lewy bodies (LBs), but the pathophysiological mechanisms are not fully understood. Epidemiologically, environmental neurotoxins such as pesticides are promising candidates for causative factors of PD. Oxidative stress and mitochondrial dysfunction induced by these toxins could contribute to the progression of PD. While most cases of PD are sporadic, specific mutations in genes that cause familial forms of PD have led to provide new insights into its pathogenesis. This paper focuses on animal models of both toxin-induced and genetically determined PD that have provided significant insight for understanding this disease. We also discuss the validity, benefits, and limitations of representative models.

1. Introduction

Parkinson's disease (PD) is one of the most common chronic neurodegenerative disorders. It is characterized by a variety of motor (bradykinesia, rigidity, tremor, and postural instability) and nonmotor (autonomic disturbances and psychosis) symptoms. Although it can be diagnosed accurately, no therapeutic strategies can cure or completely block the progression of PD. Pathologically, PD is characterized by the severe loss of dopaminergic (DAergic) neurons in the pars-compacta nigra and the presence of proteinaceous α -synuclein inclusions, called Lewy bodies (LBs), which are present in neurons of the central nervous system (specific cortical regions, brain stem, and spinal cord), peripheral autonomic nervous system, enteric nervous system (ENS), and cutaneous nerves [1–3]. Similar to other neurodegenerative diseases, such as Alzheimer's disease, age is the major risk factor for PD although 10% of the people with the disease are younger than 45.

Although PD is regarded as a sporadic disorder, remarkably few environmental causes or triggers have been identified [4–6]. Pesticides and herbicides are the most likely candidates for environmental agents associated with the pathogenesis of PD. On the other hand, PD characteristics are seen in a number of familial motor disorders caused

by different genetic factors. Animal models of neurodegenerative diseases, including PD, have in general been quite instructive in understanding their pathogenesis. Ideally, animal models of PD, whether induced by environmental risk factors (neurotoxins) or genetic manipulations, should faithfully reproduce the clinical manifestations (behavioral abnormalities), pathological features, and molecular dysfunctions characterizing the disease. Unfortunately, animal models rarely mimic the etiology, progression, and pathology of PD completely, and in most cases, only partial insight can be gained from these studies. Despite these difficulties, animal models are considered to be very helpful in the development of therapies to treat PD. In this paper, we discuss recently developed neurotoxin-induced and genetic model animals of PD.

2. Animal Models of PD Induced by Neurotoxins

PD is currently viewed as a multifactorial disease. Environmental exposures, particularly to pesticides, are thought to be involved in the pathogenesis of sporadic PD. Specifically, the herbicide Paraquat (PQ) and the fungicide Maneb (MB; manganese ethylene-bis-dithiocarbamate) have been associated with the incidence of PD [7, 8]. However, a causal

role for pesticides in the etiology of PD has yet to be definitively established. In animal models, PD-like disorders induced by neurotoxins or other chemical compounds have led to a better understanding of the pathophysiology of PD (Table 1).

3. 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

In 1979 and 1983, MPTP was initially identified as a strong neurotoxin when heroin addicts accidentally self-administered MPTP and developed an acute form of parkinsonism that was indistinguishable from idiopathic PD [9, 10]. A detailed neuropathological study of MPTP-induced parkinsonism in humans showed severe neuronal degeneration in the substantia nigra and the absence of LBs [11]. The lack of LBs may have reflected the age of the patient and the duration of exposure to MPTP. The tragic results of MPTP poisoning in the heroin addicts led to the development of MPTP-induced rodent and nonhuman primate animal models of PD, which have proved extremely valuable [12–16]. The MPTP-exposed primates show good response to therapy with L-3,4-dihydroxy-L-phenylalanine (L-DOPA) and dopamine (DA) receptor agonists [15, 16]. However, rats are relatively insensitive to MPTP neurotoxicity compared with primates. Rats given MPTP at doses comparable to those used in mice do not show remarkable neurodegeneration [17, 18]. Only high doses of MPTP cause DAergic neurodegeneration in rats, indicating that complete blockade of the DA receptors is required for them to display signs of parkinsonism. Mice, like rats, are also less sensitive to MPTP than primates [19, 20].

This model also shows pathological changes in the ENS, as observed in PD. In PD, gastrointestinal (GI) dysfunction was hypothesized to depend on neuronal degeneration in the ENS that is similar to that seen in the CNS. Recent studies show that the administration of MPTP results in decreased tyrosine hydroxylase- (TH-) positive enteric neurons in mice, indicating that the MPTP model mice should be suitable for understanding the extranigral pathophysiology of PD [21, 22].

4. 6-Hydroxy-Dopamine (6-OHDA)

Like MPTP, 6-OHDA is a neurotoxin that has been successfully used in induction animal models of PD. 6-OHDA's strong neurotoxic effects were described by Ungerstedt in 1971, in a study presenting the first example of using a chemical agent to produce an animal model of PD [23]. Since 6-OHDA cannot cross the blood-brain barrier (BBB), systemic administration fails to induce parkinsonism. This induction model requires 6-OHDA to be injected into the substantia nigra, medial forebrain bundle, and striatum [24, 25]. The effects resemble those in the acute MPTP model, causing neuronal death over a brief time course (12 hours to 2-3 days).

Interestingly, the intrastriatal injection of 6-OHDA causes progressive retrograde neuronal degeneration in the

substantia nigra and ventral tegmental complex (ST-VTA) [25–27]. As in PD, DAergic neurons are killed, and the non-DAergic neurons are preserved. However LBs do not form. Typically, 6-OHDA is used as a hemiparkinson model, in which its unilateral injection into the substantia nigra causes asymmetric motor behavior (turning, rotation) when apomorphine, a DAergic receptor agonist, or amphetamine, a dopamine releasing agent, is given systemically. In this model, the quantifiable motor behavior is a major advantage for screening pharmacological screening agents for their effects on the DAergic system and for testing cell replacement therapies [28–30].

5. Rotenone

Rotenone is a naturally occurring complex ketone pesticide derived from the roots of *Lonchocarpus* species. It can rapidly cross cellular membranes without the aid of transporters, including the BBB. Rotenone is a strong inhibitor of complex I, which is located at the inner mitochondrial membrane and protrudes into the matrix.

In 2000, Betarbet et al. demonstrated in rats that chronic systemic exposure to rotenone causes many features of PD, including nigrostriatal DAergic degeneration [31]. Importantly, pathological features match those seen in typical PD. For example, many of the degenerating neurons have intracellular inclusions that are morphologically similar to LBs. These inclusions also show immunoreactivity for α -synuclein and ubiquitin, like true LBs [31, 32]. The rotenone-administered model animals also reproduce all the behavioral and pathological features seen in the typical form of human PD. However, rotenone-injected rats without nigrostriatal DAergic neuronal loss demonstrate the same abnormal motor behaviors as those with such pathological features [32, 33]. This finding suggested that the abnormal behaviors of PD could depend, at least partly, on the damage to non-DAergic neurons in the nigrostriatal area. Furthermore, rotenone exposure also causes the loss of myenteric neurons in the rat [34].

6. Paraquat and Maneb

Because of its close structural similarity to 1-methyl-4-phenylpyridinium (MPP⁺, the active metabolite form of MPTP), an herbicide, 1,1'-dimethyl-4,4'-bipyridinium, named paraquat has been suggested as a risk factor for PD [35]. The systemic administration of paraquat to adult mice results in a significant decrease in substantia nigra DAergic neurons, a decline in striatal dopamine nerve terminal density, and a neurobehavioral syndrome characterized by reduced ambulatory activity [36]. These data support the idea that paraquat crosses the BBB to cause destruction of the dopamine neurons in the substantia nigra, like MPP⁺ [36]. The prolonged exposure to paraquat leads to a remarkable accumulation of α -synuclein-like aggregates in neurons of the substantia nigra pars compacta in mice [37]. Chronic exposure to paraquat also reduces the expression of the nicotinic acetylcholine receptor (nAChR) subunit $\alpha 3/\alpha 6\beta 2^*$

TABLE 1: Representative neurotoxin-induced mammalian models of Parkinson's disease.

Neurotoxin	Behavioral and pathological features	Molecular mechanisms
MPTP	(1) Parkinsonism (akinesia, rigidity, and tremor) with acute onset (2) Relatively less potent in rodents (3) Good response to L-DOPA and DA-agonists (4) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region (5) Loss of TH-neurons (-fibers) in ENS (6) α -Synuclein-positive inclusions (7) No typical LBs	(1) Easily crosses the BBB (2) Converted to MPP ⁺ in glial cells (3) Transferred into mitochondria by transporters (4) Inhibits electron transport chain complex I (5) Upregulation of iNOS, NADPH-oxidase, and ROS (6) Microglial activation
6-OHDA	(1) Intracerebral administration (2) Quantifiable locomotor abnormalities (rotation, akinesia) (3) Good response to L-DOPA and DA-agonists (4) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region (5) No typical LBs	(1) Transferred into mitochondria by transporters (2) Inhibits electron transport chain complex I (3) Microglial activation
Rotenone	(1) Parkinsonism (bradykinesia, fixed posture, and rigidity) (2) Good response to L-DOPA and DA-agonists (3) Loss of TH-neurons (-fibers) and DA-content in nigrostriatal region (4) α -Synuclein-positive inclusions, resemblance to true LBs (5) Loss of myenteric neurons	(1) Easily crosses the BBB (2) Inhibits electron transport chain complex I (3) Upregulation of NADPH-oxidase (4) Microglial activation
Paraquat (+ Maneb)	(1) Parkinsonism similar to that of induced by MPTP (2) Loss of DA-content in nigrostriatal region (3) α -Synuclein-positive inclusions with long exposure	(1) Crosses the BBB by neutral amino acid transporter (2) Inhibits electron transport chain complex I (3) Reduction of nAChR-mediated DA release (4) Inhibits complex III (Maneb)

MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA: 6-hydroxy-dopamine; L-DOPA: L-3,4-dihydroxy-L-phenylalanine; TH: tyrosine hydroxylase; DA: dopamine; ENS: enteric nervous system; LB: Lewy body; BBB: blood-brain barrier; MPP⁺: 1-methyl-4-phenylpyridinium; iNOS: inducible nitric oxide synthase; ROS: reactive oxygen species; nAChR: nicotinic acetylcholine receptor.

(the asterisk indicates the possible presence of additional subunits). Normally, the activation of both nAChR subtypes stimulates DA release in the striatum [38–40]. The injection of paraquat selectively reduces the $\alpha 3/\alpha 6\beta 2^*$ -mediated DA release from the striatum in primates [41].

Manganese ethylenebis-dithiocarbamate (Maneb) is an organomanganese fungicide that is broadly used in agriculture and is a putative causative agent for PD. Surprisingly, Thiruchelvam et al. found that the neurotoxic effects of maneb or paraquat on the nigrostriatal DA system in mice are synergistically potentiated in combination [42]. Their report argued that this finding has important implications for the human risk of PD, because the marked geographical overlap in the estimated annual agricultural applications of paraquat and maneb means that people living in these areas may be exposed to the synergistic neurotoxicity of these two agents [42, 43].

7. Pathophysiological Mechanisms of DAergic Neurotoxins

All the representative neurotoxin-induced PD models described above show defective mitochondrial function, manifested by the inhibition of mitochondrial complex I

or III. MPTP is a highly lipophilic agent. After its systemic administration, MPTP rapidly crosses the BBB. Once in the brain, MPTP is converted to 1-methyl-4-phenyl-2,3-dihydropyridium (MPDP⁺) in glial cells (astrocytes) and serotonin neurons by monoamine oxidase B (MAO-B) and then spontaneously oxidizes to MPP⁺ [44, 45]. Thereafter, MPP⁺ is released into the extracellular space. Unlike MPTP, MPP⁺ is a polar molecule that cannot freely enter DAergic neurons. Thus, a plasma membrane transport system is required. MPP⁺ has a high affinity for dopamine transporter (DAT) as well as for norepinephrine and serotonin transporters [46, 47]. Once inside DAergic neurons, MPP⁺ can accumulate in mitochondria and impair mitochondrial respiration by inhibiting complex I in the electron transport chain [44, 48], which induces the generation of reactive oxygen species (ROS). MPP⁺ can also bind to vesicular monoamine transporters (VMATs), which help move selected materials into synaptic vesicles containing DA [49]. MPP⁺ can also remain in the cytoplasm and interact with cytosolic enzymes [50].

Inducible nitric oxide synthase (iNOS) is also involved in the pathogenesis of MPP⁺-induced parkinsonism in animal models. Increased iNOS has also been found in the substantia nigra of autopsied PD patients, indicating that NO overproduction is a feature of the human disease

[51, 52]. Excess NO could contribute to the formation of free radicals, which could damage DAergic neurons, leading to the development of PD symptoms. Mice null for iNOS show a resistance to neuronal damage by MPTP, and iNOS inhibitors protect against the degeneration of DAergic neurons in MPTP-treated mice [53, 54]. Furthermore, microglial cells can be activated by the formation of free radicals and iNOS-mediated damage, and thereby exacerbate the toxicity of MPTP [55–57]. Finally, MPTP can also upregulate NADPH-oxidase in the substantia nigra of mice [56], which is significant because NADPH-oxidase appears to be ubiquitously expressed in all brain regions and metabolizes molecular oxygen, generating superoxide as a product. In fact, MPTP toxicity is diminished in mice lacking functional NADPH-oxidase, indicating a pivotal role for superoxide ions in the neurotoxicity induced by MPTP [56].

The toxicity of 6-OHDA also involves mechanisms of oxidative stress. 6-OHDA can be taken up by DAergic neurons through DAT [58, 59]. Once transported into neurons, 6-OHDA is oxidized like DA. The oxidized molecule generates free radicals inhibits mitochondrial complex I and produces superoxide and hydroxyl radicals [58, 59]. It is not only toxic to the DAergic neurons but can also induce microglial activation [59].

Like MPTP, the pesticide rotenone is very lipophilic, crosses the BBB, and is distributed evenly throughout the brain [59, 60]. It can enter mitochondria, where it inhibits complex I of the electron transport chain with high affinity [59]. Interestingly, the inhibition of microglial activation by an antibiotic, minocycline, can attenuate the neurotoxicity of rotenone [61]. Gao et al. also showed that the neurotoxicity of rotenone is reduced in neuron-glia cocultures from NADPH oxidase-null mice [62]. The DA uptake of the neuron-enriched cultures was not affected by the addition of microglia from NADPH oxidase-null mice, the addition of microglia from wild-type (WT) mice significantly increased the sensitivity of DAergic neurons either from WT or knockout (KO) mice to rotenone neurotoxicity. These data indicate that microglial NADPH oxidase, but not neuronal NADPH oxidase, is responsible for the NADPH oxidase-mediated neurotoxicity of rotenone [62]. Paraquat mainly crosses the BBB through the neutral amino acid transporter [63–65]. Once in the brain, it is selectively taken up by the terminals of DA-containing neurons in the substantia nigra by the DAT, and it inhibits mitochondrial complex I [63]. Maneb contains a major active fungicidal component, manganese ethylene-bis-dithiocarbamate (Mn-EBDC). In a rat model in which Mn-EBDC is directly delivered to the lateral ventricles, Mn-EBDC causes selective DAergic neurodegeneration [66]. Mn-EBDC preferentially inhibits mitochondrial complex III [66].

8. Genetic Animal Models of PD

Although the etiopathogenesis (including environmental factors) of PD is not fully understood, the extensive examination of human postmortem material, the genetic analysis of patients, and the study of experimental animal models

have shed significant light on the molecular mechanisms involved in its progression. However, since the number of patients with familial PD is extremely low compared to the number with sporadic PD, genetic studies in affected human families are very difficult. Therefore, the development of animal genetic models for PD is especially important, and such models provide an opportunity not only to investigate the genetic etiology of PD but also to identify new factors that could be invaluable in terms of diagnosis, drug design, and/or therapy [67, 68]. Even invertebrate animals, for example, *Drosophila melanogaster*, are useful models for surveys of human PD. While their numbers of neurons and glia are obviously much smaller than in rodents and primates, *Drosophila* have the same types of neuron-glia systems, and a great number of genes and molecular transduction pathways are conserved between *Drosophila* and humans.

In recent years, several genetic animal models of PD have been reported, including models for autosomal-dominant (AD) inheritance patterns. The genes manipulated in these models include α -synuclein, leucine rich repeat kinase 2 (*LRRK2*), ubiquitin carboxyl-terminal esterase L1 (*UCHL1*), and high temperature requirement A2 (*HTRA2/Omi*) (Table 2). There are also models of autosomal-recessive (AR) inherited PD, which involve KO or knockdown genes for parkin, DJ-1, and phosphatase and tensin homolog- (*PTEN*-) induced novel kinase 1 (*PINK1*) (Table 3). In addition, we will review a PD mouse model deficient in nuclear receptor-related 1 (*Nurr1*), also named nuclear receptor subfamily 4, group A, member 2 (*NR4A2*), which is a susceptibility gene for familial PD (Table 2).

8.1. α -Synuclein. α -synuclein was the first gene linked to an AD-type familial PD, called Park1. The identification of an α -synuclein mutation in this family revolutionized PD research, since α -synuclein is the main component of LBs, which are observed in the sporadic PD brain. This striking result strongly indicates that genetic and sporadic PD may share similar etiologies and that investigating α -synuclein-mediated pathogenesis in familial PD could uncover important information about sporadic PD. Three missense mutations of α -synuclein, encoding the substitutions A30P, A53T, and E46K, have been identified in familial PD [67–70]. Furthermore, the duplication or triplication of α -synuclein is sufficient to cause PD, suggesting that the level of α -synuclein expression is a critical determinant of PD progression [71, 72]. Even though no direct relationship between sporadic PD and α -synuclein expression has yet been shown, the existence of several polymorphisms in the promoter or 3'-UTR of the α -synuclein gene suggests that its expression level might be a risk factor [73–75].

Human α -synuclein is an abundant 140-amino acid presynaptic phosphoprotein involved in vesicle handling and neurotransmitter release. Mutations in α -synuclein that increase the propensity for misfolding are probably deleterious, because the misfolded forms are toxic, and they induce cell death *in vitro* [76, 77]. Among the variety of abnormal forms that mutant α -synuclein can adopt, protofibrils and fibrils seem to be the most toxic [77]. These demonstrations

TABLE 2: Autosomal-dominant PD models.

Gene	Animal	Manipulation	DA neuron loss	LB-like inclusions ¹	DA-responsive motor deficits ²	References
<i>a-synuclein</i> (<i>PARK1</i>)	Nematode	Transgenic	Yes [§]	No	Yes	[79, 80]
	Fly	Transgenic	Yes	Yes	Yes	[78]
	Mouse	Transgenic	No	Yes [§] (PrP promoter)	Yes [§] (PDGF β promoter)	[81–91]
	Rat	Transgenic	Yes	No	Yes	[92–95]
	Monkey	Transgenic	Yes	No	ND	[96]
<i>UCHL1</i> (<i>PARK5</i>)	Mouse	Transgenic	Yes	No	Yes	[105, 106]
<i>LRRK2</i> (<i>PARK8</i>)	Nematode	Transgenic	Yes	ND	ND	[116]
	Fly	Transgenic	Yes	No	Yes	[113–115]
	Mouse	Transgenic	No	No	Yes	[117–119]

DA, dopamine; LB, Lewy body; ND, not determined; PrP, prion; PDGF β platelet-derived growth factor β .

¹LB-like inclusions by definition contain filamentous α -synuclein.

²ND could include some degree of behavioral impairment in spontaneous and locomotor activity and in response to sensory stimulation.

[§]Controversial. The opposite result has also been shown.

TABLE 3: Autosomal-recessive PD models and other causative genes of PD.

Gene	Animal	Manipulation	DA neuron loss	LB-like inclusion ¹	DA-responsive motor deficits ²	References
<i>Parkin</i> (<i>PARK2</i>)	Nematode	Knockout	No	No	No	[124]
	Fly	Knockout	Yes	No	Yes	[125, 126]
		Transgenic	Yes	No	Yes	[131, 132]
	Mouse	Knockout	No	No	ND	[127–130]
		Transgenic	Yes	Yes	ND	[133]
<i>PINK1</i> (<i>PARK6</i>)	Fly	Knockout	Yes	No	Yes	[135, 136]
	Mouse	Knockout	No	No	ND	[137–139]
<i>DJ-1</i> (<i>PARK7</i>)	Fly	Knockout	Yes	No	Yes	[144–148]
	Mouse	Knockout	No	No	ND	[149–151]
<i>HtrA2/Omi</i> (<i>PARK13</i>)	Fly	Knockout	No	No	No	[153]
	Mouse	Knockout	No	No	ND	[154, 155]
<i>Nurr1</i> (<i>NR4A2</i>)	Mouse	Knockout	Yes	No	ND	[158–160]

DA, dopamine; LB, Lewy body; ND, not determined.

¹LB-like inclusions by definition contain filamentous α -synuclein.

²ND could include some degree of behavioral impairment in spontaneous and locomotor activity and in response to sensory stimulation.

of α -synuclein toxicity *in vitro* led to the creation and extensive analysis of many α -synuclein-based animal models of PD.

Although flies (*Drosophila*) and nematodes (*C. elegans*) do not have complex nervous systems compared to vertebrates and do not express endogenous α -synuclein, they are useful for identifying genetic and pharmacological modifiers of α -synuclein and its product. In *Drosophila*, the overexpression of WT and mutated (A30P, A53T) human α -synuclein causes the age-dependent loss of dorsomedial DAergic neurons, an accumulation of LB-like filamentous inclusions with α -synuclein immunoreactivity, and compromised locomotor activity (climbing ability) [78]. In *C. elegans*, α -synuclein overexpression leads to accelerated DAergic neuronal loss and motor impairment [79, 80]. However, the neurons of these nematodes do not contain notable synuclein-containing inclusions.

Many different mouse lines that overexpress α -synuclein under various promoters have been generated in the last ten

years, and most have been described in recent reviews [81–83]. Mice expressing α -synuclein containing two mutations (A30P + A53T) under the TH promoter show progressive declines in locomotor activity and the loss of substantia nigra neurons and striatal DA content [84, 85]. Similarly, mice overexpressing WT human α -synuclein under the neuron-specific platelet-derived growth factor β (PDGF β) promoter show reduced TH immunoreactivity and DA content in the striatum and impaired motor performance [86]. Mice overexpressing WT human α -synuclein under another neuron-specific promoter, Thy1, show strong widespread expression in cortical and subcortical neurons, including the substantia nigra pars compacta, but no glial, spinal, or neuromuscular pathology [87–89]. These mice have an increased sensitivity to mitochondrial damage from low doses of MPTP [89]. Mice in which the mouse prion promoter (mPrP) is used to drive the expression of α -synuclein A53T show α -synuclein aggregation, fibrils and truncation, α -synuclein phosphorylation, ubiquitination, and progressive

age-dependent neurodegeneration, just as in humans [90, 91].

Several viral vectors, primarily lentiviruses and adeno-associated viruses (AAVs), have been used to drive exogenous α -synuclein. Because viral vector delivery requires stereotactic injections within or near the site of the neuronal cell bodies in the substantia nigra pars compacta, rats are generally used for these studies although the model has been reproduced in other rodents [92–95]. The overexpression of human WT or A53T mutant α -synuclein by AAVs in the SNc neurons of rats causes the progressive age-dependent loss of DA neurons, motor impairment, and α -synuclein-positive cytoplasmic inclusions [92]. Kirik et al. also overexpressed WT or A53T mutant α -synuclein in marmosets [96], in which the α -synuclein protein was expressed in 90%–95% of all substantia nigra DA neurons. The transduced neurons showed evidence of severe pathology, including α -synuclein-positive cytoplasmic inclusions, granular deposits, and loss of the TH-positivity.

It is particularly notable that the phenotypic outcome of α -synuclein overexpression in mice heavily depends on the promoter used to drive transgene expression. Unfortunately, most of these models fail to accurately mimic PD in that there is no progressive loss of DA neurons. The loss of TH-positive cell bodies in the substantia nigra does not necessarily indicate cell death. Despite the lack of overt degenerative pathology in the DA-positive neurons, obvious locomotor abnormalities due to degeneration of the nigrostriatal system and a lack of DA responsiveness are observed in the various mouse α -synuclein models. Thus, most of these lines are excellent models of α -synuclein-induced neurodegenerative disorders, such as PD.

Although mutated α -synuclein causes human familial PD, α -synuclein's physiological roles in PD are not fully understood. In KO mice of α -synuclein, neuronal development and the formation of presynaptic terminals are normal [97]. Moreover, double KO mice that lack α - and β -synuclein exhibit normal basic brain functions and survive to adulthood [98]. Thus, the loss of α -synuclein function is unlikely to play a role in the pathogenesis of α -synuclein-induced neurodegeneration. Meanwhile, α -synuclein KO mice show reduced rearing activity in the open field, decreased DA content in the striatum, and a decrease in the reserve pool of vesicles in the hippocampus [97, 99]. These results indicate that α -synuclein may play a regulatory role *in vivo*, possibly in the fine tuning of synaptic plasticity and/or vesicle maintenance. Interestingly, several lines of α -synuclein-null mice have a complete or partial resistance to the MPTP [100, 101]. Dauer et al. showed that this resistance is not due to abnormalities of the DA transporter, which appears to function normally in α -synuclein null mice [100]. These reports indicate that α -synuclein is not obligatorily coupled to MPTP sensitivity, but can influence MPTP toxicity on some genetic background.

8.2. UCHL1. A rare AD-inherited form of PD, PARK5, is caused by a missense mutation in the *UCHL1* gene. *UCHL1* constitutes 1%–2% of the brain proteins and functions

in the ubiquitin-proteasome system. The ubiquitin hydrolase activity of *UCHL1* is important for freeing reusable ubiquitin monomers. The missense mutation in PARK5 causes an Ile93Met substitution in the *UCHL1* protein (*UCHL1*Ile93Met), and this mutant was initially shown to have decreased ubiquitin hydrolase activity [102]. Interestingly, *UCHL1* is detected in LBs in sporadic PD cases [103]. These findings initiated a debate on whether the Ile93Met mutation causes a gain of function (toxicity) or loss of function (deficiency).

The gracile axonal dystrophy (*gad*) mouse is an AR-mutant that shows sensory ataxia at an early stage, followed by motor ataxia. Saigoh et al. showed that these mice exhibit spontaneous intragenic deletion of the *UCHL1* gene and do not express the *UCHL1* protein [104]. These mice do not show obvious pathological changes in the nigrostriatal DA pathway; in particular, there is no loss of DA cell bodies in the substantia nigra. Setsuie et al. generated *UCHL1*Ile93Met-overexpressing mice and reported a reduction in the DAergic neurons of the substantia nigra and of the DA content in the striatum [105]. These mice show behavioral and pathological phenotypes of parkinsonism at 20 weeks of age. Moreover, recently, Yasuda et al. performed a viral vector-mediated α -synuclein injection into the substantia nigra of the *UCHL1*Ile93Met transgenic mice [106]. These mice show a significantly enhanced loss of DA-positive cell bodies in the substantia nigra and of DA content in the striatum. The neurotoxicity is enhanced by PARK5-associated *UCHL1*Ile93Met mutant, but not influenced by the loss of *UCHL1* WT protein *in vivo*, indicating that the *UCHL1*Ile93Met toxicity results from a gain of function.

8.3. LRRK2. The *LRRK2* mutation is another type of AD-PD, called *PARK8*. *LRRK2* is a large protein containing a serine/threonine kinase and a GTPase domain that is localized to membranous structures [107]. The frequency of the common *LRRK2* Gly2019Ser mutation was 1% in patients with sporadic PD and, interestingly, 4% of patients with hereditary PD [108]. The risk of PD when the *LRRK2* Gly2019Ser mutation was present was 28% at age 59 years, 51% at 69 years, and 74% at 79 years. The motor symptoms and non-motor symptoms of *LRRK2*-associated PD are more benign than those of idiopathic PD. In autopsied tissue, the LB pathology was present in a representative *LRRK2* G2019S case, indicating that *LRRK2* and α -synuclein share some pathogenic mechanisms [109]. Yet, *LRRK2* may play a role in neuronal outgrowth and guidance, and its precise physiological function remains to be clarified [110].

dLRRK is a *Drosophila* orthologue of *LRRK2*, and it shows elevated expression in DA neurons of the head [111, 112]. Liu et al. overexpressed constructs with mutations similar to those found in patients (G2019S), in *Drosophila* [113]. The neuronal expression of *LRRK2* or *LRRK2*-G2019S produces an adult-onset selective loss of DAergic neurons, locomotor dysfunction, and early mortality. However, the phenotype caused by the G2019S-*LRRK2* mutant is more severe than that caused by the expression of equivalent levels of WT *LRRK2*. Treatment with L-DOPA improves

the mutant LRRK2-induced locomotor impairment but does not prevent the loss of TH-positive neurons. Some fly models that overexpress other LRRK2 mutations, such as I1122V, Y1699C, and I2020T, show similar results, in terms of an age-dependent impairment of locomotor activity that improves with DA stimulation, and the loss of DA neurons [113–115]. Moreover, in transgenic *C. elegans*, DA marker loss is greater in those expressing G2019S LRRK2 than WT LRRK2 [116].

Transgenic mice made using bacterial artificial chromosome (BAC) technology and expressing WT LRRK2, or the R1441G or G2091S mutation exhibit mild axonal pathology in the nigrostriatal DA projection [117, 118]. However, the conditional overexpression of neither WT LRRK2 nor its G2019S mutation causes degeneration of the DA-containing neurons [119]. Interestingly, although the LRRK2 conditional transgenic mice show minimal nigrostriatal pathologies, they exhibit a progressive age-dependent motor impairment that is improved by DA stimulation. LRRK2 involvement in the pathogenesis of PD may be limited, and other genetic and/or environmental factors are probably required to trigger DA neuronal degeneration.

LRRK2 KO mice are viable, have no major abnormalities, and live to adulthood, and there is no significant difference in the susceptibility of LRRK2-deficient and WT mice to MPTP [120]. In *LRRK2-KO Drosophila* models, differing results on the pathology of the DA neurons have been obtained [111, 121]. Lee et al. showed that *LRRK* loss-of-function mutants exhibited severely impaired locomotive activity [111]. Moreover, DAergic neurons in *LRRK* mutants showed a severe reduction in tyrosine hydroxylase immunostaining and shrunken morphology. Conversely, Wang et al. demonstrated that mutants lacking *dLRRK* kinase activity are viable with normal development and life span as well as unchanged number and pattern of DAergic neurons [121]. Nematode deletion mutants indicate that LRRK2 is dispensable for the development and maintenance of DA neurons [122].

8.4. *Parkin*. *Parkin* covers approximately 1.3 Mb of genomic DNA and is the causative gene for representative AR juvenile PD (*PARK2*). Mutations in *parkin* are not only a cause of familial PD but are also seen in 20% of young-onset sporadic PD cases [123]. *Parkin* is an E3 ubiquitin ligase that functions in the ubiquitin-proteasome system. The loss of *parkin* function is believed to result in abnormal accumulations of *parkin*'s substrates. Springer et al. demonstrated that *pdr-1* (the nematode *parkin* homolog) mutants are viable and display no obvious morphological defects or alterations in motility, egg-laying behavior, brood size, or life span under standard growth conditions [124]. Moreover, the authors did not detect any effect of the mutations on the survival of the DA neurons in the worms. However, overexpression of the α -synuclein A53T mutation in *pdr-1* mutants leads to developmental arrest and lethality, indicating this *C. elegans* model recapitulates *parkin* insolubility and aggregation similar to several AR juvenile PD-linked *parkin* mutations [124].

Drosophila parkin-null mutants exhibit a reduced lifespan, locomotor defects (flight and climbing abilities), and

male sterility [125, 126]. The locomotor defects derive from the apoptotic cell death of muscle subsets whereas the male sterile phenotype derives from a spermatid individualization defect at a late stage of spermatogenesis. Mitochondrial pathology is the earliest manifestation of muscle degeneration and a prominent characteristic of individualizing spermatids in *parkin* mutants. These mutants also display a decrement in the TH level and degeneration of a subset of DA neurons in the brain [126]. Several *parkin*-null mice have been generated and display motor and cognitive deficits including reduced locomotor activity and decreased spontaneous alteration in the T-maze; however, they show no substantial DAergic behavioral abnormalities [127–130]. Pathologically, KO mice exhibit slightly abnormal DA nigrostriatal and locus coeruleus noradrenergic regions [128, 129].

The overexpression of human mutant *parkin* in *Drosophila* causes an age-dependent, selective degeneration of DA neurons accompanied by progressive motor impairment [131, 132]. *Parkin-Q311X* mice also exhibit multiple late-onset and progressive hypokinetic motor deficits [133]. Stereological analyses revealed that the mutant mice develop age-dependent DA neuron degeneration in the substantia nigra and a significant reduction of the striatal DA level, accompanied by a significant loss of DA neuron terminals in the striatum. These results indicate that *parkin* mutants may play a pivotal role in the dominant-negative etiological mechanisms of PD.

8.5. *PINK1*. *PINK1* is another causative gene for the AR inherited PD called *PARK6*. *PARK6* is the second most frequent early-onset AR PD. *PINK1* is located in mitochondria and is a putative mitochondrial kinase, because it contains a conserved serine/threonine kinase domain with an N-terminal mitochondrial-targeting motif [134]. Thus, the PD-causative mutations of *PINK1* may cause loss of function. Park et al. and Clark et al. generated and characterized loss-of-function *Drosophila PINK1* mutants [135, 136]. These flies exhibit male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology, and increased sensitivity to multiple stresses, including oxidative stress.

Park et al. showed an age-dependent decrease in DA levels and a mild loss of DA neurons in these *Drosophila* mutants [135]. Notably, the *PINK1* mutants share marked phenotypic similarities with *parkin* mutants. *Parkin* overexpression is able to rescue the mitochondrial defects found in *PINK1*, although the double mutants do not show an enhanced phenotype. *PINK1* overexpression does not rescue *parkin* phenotypes. Together, the data indicate that *parkin* and *PINK1* function, at least partly, in a common pathway, and *PINK1* acts upstream of *parkin*. Whereas *PINK1*-deficient mice show age-dependent mitochondrial dysfunction, increased sensitivity to oxidative stress, decreased evoked DA release, and DA receptor agonist-responsive impairment of striatal plasticity, the number of DA neurons, the level of striatal DA, and the level of DA receptors are the same as in WT animals [137–139]. These phenotypes are similar to those of *parkin*-KO mice.

8.6. *DJ-1*. Deletion or point mutations in *DJ-1* have been identified in early onset AR PD (*PARK7*). *DJ-1* plays a role as an antioxidant and chaperone, and it is expressed ubiquitously in the cytosol, mitochondrial matrix, and intermembranous space [140]. In vitro, downregulation or KO of the endogenous *DJ-1* increases cells' vulnerability to oxidative stress and proteasome inhibition, implicating it in the cellular response to oxidative stress [141–143]. *Drosophila* possesses two different orthologs of the human *DJ-1* gene, named *DJ-1 α* and *DJ-1 β* . While loss-of-function *DJ-1 β* mutants have normal numbers of DA neurons, classical genetic analyses and RNAi experiments have yielded contradictory results regarding the function of *DJ-1 α* in DA neuron maintenance [144–148]. However, DA neuron loss cannot be detected in *DJ-1 α /DJ-1 β* double-deletion mutants, which are also viable, fertile, and have a normal life span. Some studies have reported a loss of DA neurons upon acute RNA silencing of *DJ-1 α* [147, 148].

Similar to α -synuclein and parkin KO mice, *DJ-1* KO mice do not show major DA-agonist-responsive behavioral abnormalities or the loss of nigrostriatal DA neurons [149–151]. In particular, although the levels of striatal DA and DA receptors are unchanged, the evoked dopamine release from striatal slices is clearly reduced, most likely as a consequence of increased reuptake. *DJ-1* mutant mice also show an increased sensitivity to MPTP [150]. This is rescued by restoring the *DJ-1* expression in mutant mice, further indicating a role for *DJ-1* in the oxidative stress response.

8.7. *HtrA2/Omi*. *HtrA2/Omi* has been identified as the causative gene for a rare inherited PD, *PARK13*. *HtrA2/Omi* has a PDZ domain in addition to a serine protease domain and is localized to the mitochondrial intermembrane space by its mitochondria-targeting sequence. Whitworth et al. have demonstrated a genetic interaction between *HtrA2/Omi* and *PINK1*, described below, by investigating the eye phenotype of double mutant flies [152]. Their study revealed that *HtrA2/Omi* acts downstream of *PINK1* and is independent of the *parkin* gene. Yet, Yun et al. indicated that *HtrA2/Omi* null fly mutants show neither mitochondrial morphological defects nor DAergic neuronal loss [153]. They also generated a *Drosophila HtrA2/Omi* mutant analogue to the human mutation G399S, which was identified in *PARK13* patients. *HtrA2/Omi* G399S retains a significant, if not complete, function of *HtrA2/Omi*, compared with protease-compromised versions of the protein, indicating that *HtrA2/Omi* is unlikely to play a pivotal role in PD pathogenesis or as an etiological factor. The targeted deletion of *HtrA2/Omi* in mice increases their sensitivity to stress-induced cell death [154, 155]. Animals lacking *HtrA2/Omi* display a progressive movement disorder similar to progressive akinesia, a rigidity syndrome, showing lack of coordination, decreased mobility, bent posture, tremor, and a decreased number of TH-positive striatal neurons [155].

8.8. *Nurr1* (*NR4A2*). *Nurr1* is a member of the nuclear receptor superfamily and is involved in the differentiation and development of nigrostriatal DA neurons. Le et al.

identified two mutations in *Nurr1* associated with Parkinson disease (–291Tdel and –245T → G), which map to the first exon of *NR4A2* and affected one allele in 10 of 107 individuals with familial Parkinson disease [156]. Mutations in *Nurr1* alter the transcription of *TH* and the DA transporter, suggesting that alterations in *Nurr1* may cause chronic DA alterations that could increase susceptibility to PD [157]. *Nurr1* is essential for the development of the ventral mesencephalic DA neurons, because homozygous *Nurr1*-KO mice do not develop DA neurons in the substantia nigra and die soon after birth [158]. Heterozygous *Nurr1*-KO mice exhibit a significant decrease in rotarod performance and locomotor activities [159]. These phenotypes are associated with decreased DA levels in the striatum, decreased numbers of DAergic neurons, and a reduced expression of *Nurr1* and *DAT* in the substantia nigra. Moreover, Le et al. reported that heterozygous *Nurr1*-KO mice show a significant decrease in the total number of TH-positive neurons in the substantia nigra and reduced DA in the striatum after MPTP administration [160]. Thus, these mice show a progressive DA phenotype that bears some resemblance to that found in α -synuclein-overexpressing and mutant mice. Therefore, *Nurr1*-knockdown mice may provide a good model for investigating the later stages of PD characterized by severe DA neuron loss.

9. Concluding Remarks

The symptoms of PD become apparent after more than 80% of the DA neurons have died. The rate of substantia nigral cell loss is assumed to be about 2,500 per year in normal people. The loss of DA function can be accelerated by exposure to neurotoxins and by molecular (genetic) abnormalities, leading to a fast and significant decrease in the number of DA neurons. Consequently, these pharmacological and/or genetic insults can cause early onset of PD. This scenario indicates that critical pathological changes could be initiated one or two decades prior to the onset of PD.

As described above, whether the causative factor is a toxic compound or a mutated gene, we have no perfect animal models of PD. So far, the neurotoxin-induced vertebrate models of PD are suitable for investigating disease-modifying therapies, since they have already proved predictive. Several genetic animal models of PD are useful for understanding the early processes of degeneration in the nigrostriatal DA system. In particular, transgenic α -synuclein animals are valuable for researching general toxicity effects and the mechanisms of α -synuclein pathology, as well as for confirming potential therapeutic strategies. Recently, causative mutations and risk factors for PD have been identified in more genes. The homozygous loss of function of *glucocerebrosidase* (*GBA*) causes Gaucher's disease whereas its heterozygous loss of function increases the risk of developing sporadic PD [161]. *ATP13A2* is causative for a juvenile onset AR hereditary PD with dementia (*PARK9*) [162]. Animal models of these mutations have not been described, but once they are available, they will undoubtedly shed new light on the mechanisms of PD.

Conflict of Interests

The authors declare no conflict of interest.

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