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Animal Models of Parkinson's Disease

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Doi: <http://dx.doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch5>

Abstract: Parkinson's disease (PD) is a heterogenous disease with a varying age of onset, symptoms, and rate of progression. This heterogeneity requires the use of a variety of animal models to study different aspects of the disease. Neurotoxin-based approaches include exposure of rodents or non-human primates to 6-OHDA, MPTP, and agrochemicals such as the pesticide rotenone, the herbicide paraquat, and the fungicide maneb. Acute exposure to neurotoxins induces motor deficits and rapid nigro-striatal dopaminergic cell death by disrupting mitochondrial function and/or increasing oxidative stress, while chronic administration of neurotoxins induces progressive models which can include alpha-synuclein (α -synuclein) aggregates. Genetic-based approaches to model Parkinson's disease include transgenic models and viral vector-mediated models based on genes linked to monogenic Parkinson's disease, including SNCA, LRRK2, UCH-L1, PRKN, PINK1, and DJ-1, as well as manipulation of dopaminergic transcription factors. SNCA mutations, overexpression, and introduction of α -synuclein preformed fibrils induce toxic protein aggregates and variable nigro-striatal neurodegeneration and motor deficits, depending on the specific model. Species, genetic background of a

In: *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Stoker TB, Greenland JC (Editors). Codon Publications, Brisbane, Australia. ISBN: 978-0-9944381-6-4; Doi: <http://dx.doi.org/10.15586/codonpublications.parkinsonsdisease.2018>

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strain, and environment affect the display of symptoms and neurodegenerative hallmarks of animal models. These models can be combined to study the interplay between genetics and environment and untangle the heterogeneity and mechanisms underlying Parkinson's disease. In this chapter, we discuss the strengths and limitations of mouse, rat, and non-human primate models of Parkinson's disease.

Keywords: Animal models; Genetic models; Neurotoxic models; Parkinson's disease; Non-human primates; Mouse, Rat

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized by loss of nigro-striatal dopaminergic neurons and aggregation of the α -synuclein-rich inclusions called Lewy bodies and Lewy neurites. The characteristic symptoms of PD are progressive motor deficits, including tremor, bradykinesia, akinesia, rigidity, postural instability, and gait difficulties. Non-motor symptoms, including depression, anxiety, sleep disturbance, cognitive decline, and anosmia are also prevalent in PD patients, and often occur prior to the onset of motor symptoms (1).

Although around 10% of all PD cases have a monogenic origin, the majority are idiopathic, with multiple contributing environmental and genetic risk factors. Similar to the etiology, the clinical phenotype of PD is heterogeneous. While motor and non-motor symptoms are clinically detectable, the brain pathology in humans can only be confirmed by examining post-mortem tissues (2). Thus, there is a great need for experimental models to deepen our understanding of this multifaceted disease and expand the currently limited treatment options. The heterogeneity of both PD etiology and pathology requires a diverse range of models that can replicate different aspects of PD in animals (2).

Two main approaches are used to model PD in experimental animals: neurotoxins and genetics. Neurotoxins can model dopaminergic neurodegeneration arising from environmental factors that have been implicated in PD. They generally induce a strong and rapid cell loss in the substantia nigra pars compacta (SNpc), elicit motor symptoms and behavioral changes, but lack the formation of Lewy bodies (3). By contrast, genetic-based models not only demonstrate variable cell loss and motor symptoms but can also exhibit α -synuclein pathology, depending on the specific model. Genetic mutations or changes in gene expression can be modeled using transgenic animals or be induced by viral transfection. Both neurotoxins and genetic approaches are applied in different animal species to model PD. This chapter provides an overview of the currently available animal models of PD.

SPECIES-SPECIFIC CHARACTERISTICS

There are three animal groups that are commonly used for modeling human diseases, and PD is no exception: rodents, non-human primates (NHP), and

non-mammalian species. Each group has its own advantages and limitations that determine the suitability for a given experiment. Understanding species-specific differences therefore facilitates experimental design as well as interpretation of behavioral observations and pathophysiology.

Rodents

Rodents are extensively studied across biomedical fields because they are convenient to care for in laboratory conditions and have associated robust experimental protocols, including different forms of drug administration, generation of transgenic strains, and behavioral assessments. The majority of 23,000 animal studies of PD published since 1990 involve rodents (Figure 1). An advantage of rodent PD models is that nigro-striatal dopaminergic degeneration correlates to motor deficits in mice and rats. These can be observed and measured with a series of behavioral tests most of which involve measuring movement, grip, or strength of the front paws. Behavioral tests in rodents include the open field test for a general assessment of locomotor activity, the stepping test to measure akinesia, and the pole test to measure bradykinesia (4, 5). Strength is measured by grip strength and grip coordination tests (5). It is difficult to directly measure rigidity in rodents, but the performance on the rotarod test accounts for multiple factors such as balance, strength, and coordination (5).

Rodents with unilateral lesions will display asymmetric motor behavior where deficits in contralateral limb use can be measured and compared to the ipsilateral limb as an internal control. Drug-induced circling behavior is a more dramatic measure of a unilateral lesion (5, 6). In these tests, administration of amphetamine stimulates the release of dopamine in the intact contralateral side, resulting in ipsilateral rotation, while apomorphine causes contralateral rotations due to dopamine hypersensitivity of the lesioned side. Tremor and posture are often described qualitatively, and tremor monitors can be used to record shaking frequency (5). Gait can be assessed with paw print, but this is rarely done (5). Many of these motor tests can also be used to assess dyskinesia.

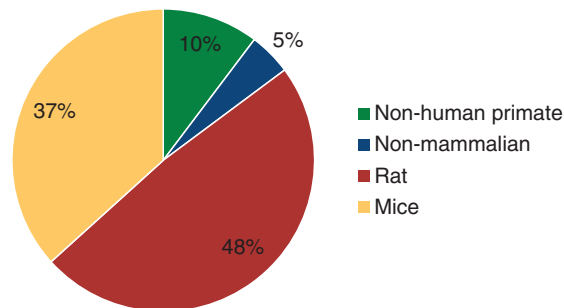


Figure 1 Proportion of animal models used in 23,000 research articles on Parkinson's disease published from January 1990 to June 2018. Numbers of original article publications were obtained from following searches on Web of Science: "Parkinson AND (mice OR mouse)"; "Parkinson AND rat"; "Parkinson AND (primate OR monkey)"; "Parkinson AND (non-mammalian OR drosophila OR elegans)."

To study non-motor symptoms in rodents, the preferred method is using a model such as a partial nigro-striatal lesion, which does not cause concurrent motor deficits that may affect the test results. Sleeping, drinking, and eating patterns are monitored to assess sleep disturbance and weight loss (7). To model neuropsychiatric symptoms, a panel of complementary tests can be used, where the tail suspension test or the forced swim test is used to model depression or behavioral despair (7). Excessive grooming is a stereotypy signaling anxiety or compulsive behavior, and reduction in mouse species-specific nest building behavior can be used to model motivation and goal-oriented tasks (5).

Non-human primates

Studies of NHP give valuable insights into PD pathology owing to their anatomic and genetic similarity to humans (8). Compared to rodents, NHP are larger, have a longer life span, require more demanding care, incur higher costs, and involve more complex ethical considerations. Based on publications, only 10% of animal studies on PD are performed in NHP (Figure 1) and these are often reserved for preclinical evaluation of therapies (8). NHP models are currently based on neurotoxic or viral vector-mediated PD pathology, which generally induces parkinsonian symptoms and behaviors that are similar to those in humans. Therefore, a Unified Parkinson's Disease Rating Scale (UPDRS)-like measure can be used to assess the severity of the phenotype (9). However, unlike the clinical scale used for PD patients, these assessments are not standardized worldwide.

The NHP used most commonly in PD studies include macaques (10) followed by common marmosets. These species are convenient to use because of their smaller size, high reproductive efficiency, and relative ease of care and housing in laboratory conditions (11). Squirrel monkeys, African green monkeys, and baboons have also been used to model PD (10). PD models in the old world species such as macaques exhibit Levodopa-induced dyskinesia, similar to human chorea and dystonia, which are better distinguished than in new world monkeys such as marmosets (10). Other examples of NHP species-specific behavior include marmosets' jumping in the tower test to assess akinesia, and the righting reflex in the hourglass test to assess axial rigidity (12). Pre-diagnostic non-motor symptoms affecting sleep or social behavior have been preferentially studied in macaques since they are diurnal and better replicate human sleeping patterns, in contrast to rodents which have higher nocturnal activity (13). Social behavior changes, such as increased aggressiveness, can be assessed by monitoring facial expressions in female macaques (14).

In addition to behavioral assessments, NHP can be used for neuroimaging studies (15). These are especially valuable during preclinical drug trials, because these can be compared to patients during clinical phases (16). Thus, NHP models have many advantages but are demanding in terms of resources.

Non-mammalian species

Non-mammalian models, including drosophila and *Caenorhabditis (C.) elegans*, have been used in a small proportion of experimental PD studies over the past decades (Figure 1). These models, however, have several advantages such as easily generated genetic manipulations, a rapid reproductive cycle, low costs of maintenance, and well-defined neuropathology and behavior.

A variety of transgenic drosophila and *C. elegans* models have been generated (17, 18). Due to the low cost and rapid completion of experiments, these models can be used for large screens for drug discovery. Furthermore, *C. elegans* models have the advantage of a fully mapped connectome possessing only 302 neurons, out of which only 8 are dopaminergic. Drosophila's larger connectome containing 135,000 neurons is also currently being mapped. Therefore, these models can be used to study fundamental principles governing cellular, genetic, and network changes resulting from dopaminergic loss. Since studies in non-mammalian species are, so far, used in a small fraction of PD models and can be challenging to translate to humans, this chapter will focus on the most commonly used neurotoxic and genetic PD models in rodents and NHP.

NEUROTOXIC MODELS

Several PD models are based on degeneration of dopaminergic neurons induced by local or systemic administration of neurotoxins. The first to be discovered was 6-hydroxydopamine (6-OHDA) (19), initially used in the periphery to degenerate sympathetic nerves (20), and later in the brain to model PD. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered through cases of chemically induced parkinsonism after failed synthesis of the opioid drug 1-methyl-4-phenyl-4-propionpiperidine (MPPP) (19). Reports of an increased risk of PD in populations exposed to pesticides for agricultural purposes (21) have resulted in additional neurotoxin-based PD models and studies on environmental risk factors of PD (Table 1).

MPTP

MPTP is a commonly used neurotoxin for modeling PD and can be administered acutely or chronically by different routes (22). It easily crosses the blood–brain barrier (BBB) within minutes due to its lipophilic nature. Glial cells take up MPTP, where it is converted first to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP⁺) by monoamine oxidase-B (MAO-B) followed by spontaneous oxidation into the toxic agent 1-methyl-4-phenylpyridinium (MPP⁺) (23). Once released back into the parenchyma through the organic cation transporter 3, MPP⁺ is transported selectively into dopaminergic cells through the dopamine transporter (DAT) and accumulates in the cytoplasm and in vesicular monoamine transporter (VMAT⁺) vesicles (24). MPP⁺ blocks mitochondrial complex I, which reduces ATP production, increases oxidative stress, and eventually causes cell death and neuroinflammation (23). This mechanism of action has contributed to the study of dopaminergic neurodegeneration, mitochondrial dysfunction, oxidative stress, and neuroinflammation in PD.

MPTP has been used to model PD in mice and NHP. However, rats are resistant to moderate doses of MPTP, while higher doses increase the mortality rates (26). The reliability and accessibility of the mouse MPTP model makes it a popular choice in PD research. The extent of the lesion depends on the dosage, route of administration, and the mouse strain used (33). Large lesions are useful to model late stages of PD for preclinical studies in drug discovery and development of other treatment strategies, such as deep brain stimulation (DBS), cell transplantation,

TABLE 1

Summary of characteristics, uses, and limitations of neurotoxic models

PD model	Characteristics	Uses	Limitations
MPTP Neurotoxin: Inhibition of complex I (23)	Acute dose: (10, 22) - No α -synuclein aggregates - Rapid and strong dopaminergic neurodegeneration - Strong motor deficit Subacute/chronic dose: (10, 25) - Progressive model - α -synuclein aggregates - No dopaminergic neurodegeneration - No motor deficit	- Common preclinical model - Systemic injection (bilateral parkinsonism) - Used in mice and NHP (24)	- Rats are resistant (26) - Functional recovery in mice and NHP (5, 10)
6-OHDA Neurotoxin: Inhibition of complex I and oxidative stress (19)	Injection site-dependent lesion: (23) - No α -synuclein aggregates - Rapid and strong dopaminergic neurodegeneration - Strong asymmetric motor deficits	- Common preclinical model - Intracranial injection into SNpc, striatum, or medial forebrain bundle - Hemiparkinsonism (19)	- High mortality with bilateral injection (24)
Rotenone Pesticide: Inhibition of complex I	- α -synuclein aggregates - Moderate dopaminergic neurodegeneration - Some motor deficits (27)	- Model environmental risks - Evaluate risk of pesticides	- Toxicity in humans remains controversial (28)
Paraquat and maneb Herbicide and fungicide: Oxidative stress (29)	- α -synuclein aggregates - Some dopaminergic neurodegeneration - Some motor deficit (30, 31)	- Model environmental risks - Evaluate risk and interaction of pesticides	- Toxicity in humans remains controversial (28) - High doses of paraquat cause pulmonary fibrosis (32)

NHP: non-human primates, SNpc: substantia nigra pars compacta.

and gene therapy (24). Most commonly, mice are administered an acute dose of MPTP by intraperitoneal (i.p.) injection which causes a loss of dopaminergic neurons, specifically in the SNpc rather than nearby regions such as the ventral tegmental area (VTA) (3). The neurodegeneration occurs within hours and stabilizes within 7 days. Four injections of an acute dose (up to 20 mg/kg) with 2-hour intervals cause 90% of striatal dopamine depletion and 70% loss of dopaminergic neurons in the SNpc but no α -synuclein aggregates (22).

MPTP-induced dopaminergic degeneration in mice correlates with motor deficits. However, these deficits can recover within a few days post-acute injection, which creates limitations on the duration of behavioral studies (5). The extent of

degenerative effects of MPTP and functional recovery seems to be partially determined by the mouse strain used, that is, the genetic background (34). The MPTP model has also been used in studies on gut microbiota dysbiosis in PD. Fecal microbiota transplants from MPTP-treated mice reduced striatal dopamine levels and induced motor impairments in non-wild-type mice, while transplants of normal microbiota alleviated PD-like symptoms in MPTP-treated mice (35).

As an alternative to the acute mouse model, repeated daily i.p. injections of subacute doses result in delayed nigro-striatal neurodegeneration and progressive build-up of α -synuclein inclusions (25, 36). The progressive nature of prolonged MPTP exposure in mice resembles PD, including neuroinflammation, α -synuclein inclusion pathology, and molecular mechanism preceding cell death. By contrast, these models do not show apparent motor deficits.

MPTP is the gold standard for PD models in NHP (24). Most commonly, bilateral parkinsonism is induced through multiple low-dose systemic injections (10), but hemiparkinsonism can also be induced by intracarotid infusion (37). Usually, 1–2 mg/kg of MPTP is administered systemically over days to months. Symptoms develop over several months, and are then stable for another few months (10). A chronic MPTP model in NHP also exists, comparable to the subacute MPTP mouse models. This is induced in NHP by daily doses of MPTP of 0.2 mg/kg over 2–3 weeks and is used to model the progressive pre-symptomatic dopaminergic cell loss seen in early stages of PD (10). Just like with the MPTP mouse model, NHP recover function a few months post-injection (10). However, another round of MPTP exposure can be given to prolong the experiment.

Thus, in NHP, MPTP can cause dose-dependent dopaminergic degeneration in the SNpc and putamen as well as α -synuclein aggregates that closely mimic PD pathology, and the extent of the lesion determines the severity of motor deficits (3). The neuropathology and symptoms are, however, more pronounced in PD patients (10). The presence of tremor correlates with the dopamine depletion in the basal ganglia and spontaneous firing, as seen in PD patients. Also, similar to PD patients, administration of L-dopa initially rescues motor deficit in NHP, but prolonged use elicits dyskinesia. Bilateral MPTP parkinsonism also displays some extra-nigral pathology, such as anosmia (38) and sleep disturbance (39). Thus, the resemblance of MPTP models in NHP to PD in humans makes them suitable for preclinical research of therapeutic strategies that have previously been validated in rodents.

6-OHDA

6-OHDA is an analogue of dopamine and norepinephrine. It is produced endogenously through the hydroxylation of dopamine metabolites and has been found in the human caudate nucleus (6, 24). 6-OHDA does not cross the BBB and needs to be administered directly into the brain to induce neurodegeneration. It enters dopaminergic and noradrenergic neurons through monoamine transporters; therefore, the injected solution must contain selective noradrenergic reuptake inhibitors (e.g., desipramine) to selectively target dopaminergic neurons (6). 6-OHDA induces cell death through oxidative stress by inhibiting mitochondrial complex I and by producing reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (19). This reaction is catalyzed by iron, and toxicity can be prevented by iron chelating agents, such as

vitamin E and MAO-B inhibitors (selegiline) (19). The specifics of 6-OHDA neurotoxicity remain poorly understood, but many antioxidant agents have neuroprotective effects.

Intracerebral 6-OHDA injections in rodents and NHP allow the targeting of the SNpc, striatum, or medial forebrain bundle (23), and the generation of unilateral, hemiparkinsonian models (19). Bilateral injections often cause adipisia, aphagia, seizures, and high mortality (24). In rats, SNpc injections of 6-OHDA result in large dopaminergic degeneration within 24 hours and 90% striatal dopamine loss within a couple of days (6, 23). However, injected in the striatum, 6-OHDA causes a retrograde degeneration of nigro-striatal neurons over a span of 1–3 weeks (23). Unilateral injections lead to asymmetric motor deficits and rotational behavior, also used to assess L-dopa-induced dyskinesia (6, 23). The unilateral models allow the use of the non-lesioned ipsilateral limbs as an internal control.

NHP require serial intracerebral 6-OHDA injections, which can result in post-surgical complications (15). In marmosets, nine striatal injections of 8 μ g 6-OHDA induced rotational behavior indicative of unilateral nigro-striatal lesion, but symptoms recovered after 10 weeks. Doubling the number of injections delayed functional recovery to 17 weeks post-surgery. 6-OHDA NHP models do not display α -synuclein inclusions but have been used to study hyperactivity of the subthalamic nucleus, dyskinesia, anhedonia, and depressive behaviors (41). The 6-OHDA NHP models are also used to develop treatments, for example, DBS, neuroprotective agents, transplantation, and gene therapies (42, 43).

Pesticides and herbicides

The most recent and most debated neurotoxins used for modeling PD are pesticides and herbicides (44). Correlations between agro-chemical exposure in populations and increased risk for PD have been difficult to conclude due to a lack of details on exposure to particular agents (28). Emphasis has been made on rotenone, paraquat, and maneb as possible environmental causes of PD. So far, these pesticides and herbicides have been mostly used in rodents to try to model PD.

Both rotenone and paraquat are thought to cause dopaminergic degeneration by inducing oxidative stress. Rotenone probably acts through complex I, while paraquat exerts its toxicity through cellular redox cycling (29), despite being structurally similar to MPP⁺. It is still unclear how paraquat penetrates the BBB despite its high polarity (PQ²⁺). It seems that penetration is slow and inefficient as PET scans on macaques showed that the BBB prevents paraquat from penetrating the brain (45), and low levels of paraquat are seen after systemic exposure to rats (46). PQ²⁺ is not transported by DAT (47) but is converted to a monocation radical PQ⁺ through redox cycling. PQ⁺ seems to be the active agent penetrating the dopaminergic neurons through DAT and exerting cytotoxicity, as shown in cultured cells and mice (48). Despite the unclear mechanisms of action, rotenone and paraquat models are used to induce parkinsonian pathology and to study inhibition of inflammatory and oxidative stress pathways in adult rodents.

The rotenone rat model has gained some reliability as a pesticide model of PD due to its ability to cause dopaminergic degeneration, α -synuclein inclusions, and motor deficits (27). Daily i.p. injections of 3 mg/kg of rotenone for 6–10 days are sufficient to induce 45% loss of dopaminergic neurons in the SNpc, striatal

dopamine depletion, and motor symptoms such as bradykinesia, postural instability, and rigidity (49). Rotenone can also induce non-motor symptoms such as sleep disturbances in rats (50).

Chronic paraquat administration in mice induces some dopaminergic cell loss in SNpc but does not cause dopamine depletion in the striatum or clear motor deficits (51). High doses of paraquat are not suitable for modeling PD, since they cause pulmonary fibrosis which could have secondary effects on behavior (32).

Pesticide models can increase the understanding of how environmental factors can affect PD risk. The primary research focus is on the mechanisms of action and the interaction with other risk factors, for example, genetic risk, age, and environmental exposure to other agro-chemicals. The combination of the herbicide paraquat and the fungicide maneb can elicit selective dopaminergic cell loss, dopamine depletion in the striatum, aggregation of α -synuclein, as well as PD-like motor and non-motor symptoms in rodents. Repeated administration of 10 mg/kg paraquat and 30 mg/kg maneb to mice twice a week over the span of 6 weeks exacerbate parkinsonian pathology compared to each compound alone (30). The response to this combined exposure, however, varies between individuals from the outbred Sprague Dawley rat strain as a result of peripheral effects and/or genetics (31).

GENETIC MODELS

Genetics plays an important role in PD pathogenesis (see chapters 6 and 7). Disease-causing mutations have been identified through linkage analyses in familial PD, while genetic risk factors for idiopathic PD have been identified through association analyses in patients and controls (52). Monogenic PD can be inherited through autosomal dominant or autosomal recessive mutations with variable penetrance. However, *de novo* mutations of these genes can also be seen in patients with no family history of PD.

SNCA was the first gene to be linked to familial PD and together with the finding that the encoded protein α -synuclein is aggregated in Lewy bodies, this led to a breakthrough in PD research (53, 54). Since then, a lot of research has focused on α -synuclein models and pathology. The identification of additional monogenic PD mutations has been used to study the effects of mutated proteins and to develop new reliable animal models (Table 2). Most genetic models have only been effective at reproducing some of the PD hallmarks (Table 3). Development of new genetic tools, however, allows for the generation of new genetic-based models. One such example is viral vectors that can be used to introduce wild-type (WT) or mutated genes for targeted expression of a disease-associated protein.

α -synuclein and transgenic models

Aggregated α -synuclein protein is a main component of Lewy bodies in PD patients. The encoding gene, SNCA, was the first to be linked to familial PD, and was named PARK1 (Table 2). Three autosomal dominant SNCA point mutations that are fully penetrant have been identified: A53T, A30P, and E46K (62, 73).

TABLE 2**Summary of familial PD mutations that have been replicated in animal models**

Locus	Gene	Mutation	Protein	Inheritance
PARK1	SNCA	A30P, A53T, E46K	α -synuclein	Dominant
PARK2	PRKN	Various mutations, exonic deletions, duplication and triplication	Parkin	Recessive
PARK4	SNCA	Duplication and triplication	α -synuclein	Dominant
PARK5	UCH-L1	I93M and S18Y	UCH-L1	Dominant
PARK6	PINK1	G309D, exonic deletions	PINK1	Recessive
PARK7	DJ-1	Homozygous exon deletion, L166P	DJ-1	Recessive
PARK8	LRRK2	G2019S, R1441C/G, and others	Dardarin	Dominant

SNCA: α -synuclein, UCH-L1: ubiquitin carboxyl-terminal hydrolase isozyme L1, PINK1: serine/threonine-protein kinase PINK1, DJ-1: protein/nucleic acid deglycase DJ-1, LRRK2: leucine-rich repeat serine/threonine-protein kinase 2.

TABLE 3**Characteristics, uses, and limitations of genetic models for Parkinson's disease**

PD model	Characteristics	Uses	Limitations
SNCA transgenic rodents Point mutations (A53T, A30P, E46K) and overexpression of α -synuclein (ASO)	Mice (55) and rats (56) - Widespread α -synuclein aggregation - No dopaminergic neurodegeneration - Some motor deficits	- Models familial mutations of SNCA - Study α -synuclein function/propagation and synucleiopathies	- May affect development (57) - No transgenic NHP
Viral transfection of α-synuclein Adeno-associated virus and lentivirus vectors	Mice, rats, and NHP: (58) - α -synuclein aggregates - Moderate dopaminergic neurodegeneration - Moderate motor deficits	- Administered into SNpc - Study α -synuclein function, propagation, and synucleiopathies - Potential preclinical use	- Potential vector toxicity and interference of viral vector
α-synuclein preformed fibrils Seeding of α -synuclein aggregates	Mice, rats (59), and NHP (60): - Widespread α -synuclein aggregation - Mild dopaminergic neurodegeneration - Lack of motor deficits in rats and NHP, some in mice	- Administered to the striatum (59) - Study α -synuclein function/propagation and synucleiopathies - Can be combined with α -synuclein viral vector (61)	- Slow/weak onset of pathology (59)

TABLE 3

Characteristics, uses, and limitations of genetic models for Parkinson's disease (Continued)

PD model	Characteristics	Uses	Limitations
LRRK2 (WT, G2019S, R1441C) Transgenic and viral vector-mediated	Mice and rats: (3, 62) - Affects inclusion formation - Little dopaminergic neurodegeneration - Most lack motor deficits	- Study the role of LRRK2, its interaction with α -synuclein, and toxicity of mutant LRRK2	- Large gene size prevents the use of AAV vectors (58) - No NHP models
UCH-L1 I93M mutation	Mice: (63) - No α -synuclein aggregates - Dopaminergic neurodegeneration - Mild motor deficits	- Study role of UCH-L1 - Used in combination with α -synuclein and MPTP to study interaction	- Relevance to pathogenesis of PD is debated (23)
Parkin, PINK, DJ-1 Transgenic KO, silencing, or overexpression using viral vectors	Rodents: (62) - Affects aggregation in α -synuclein models - Most give no dopaminergic neurodegeneration - Lack clear motor deficits	- Study role of Parkin, PINK1, and DJ-1 - Used in combination with ASO or MPTP models to study interaction (64)	- Large number of identified mutations (65) - No NHP models
Nurr1 (66), EN1 (67), Pixt3 (68), SHH (69), MitoPark (70) Transcription factor deficiencies	Mice: - No α -synuclein inclusions - Dopaminergic neurodegeneration - Motor deficits	- Often heterozygous KO or targeted to dopamine neurons (DAT-Cre mice) - Study development and maintenance of the nigro-striatal system	- Not specific for PD - Nurr1 and Pixt3 models also used for schizophrenia (71, 72)

SNCA: α -synuclein, ASO: α -synuclein overexpression, LRRK2: leucine-rich repeat serine/threonine-protein kinase 2, WT: wild-type, UCH-L1: ubiquitin carboxyl-terminal hydrolase isozyme L1, PINK1: serine/threonine-protein kinase PINK1, DJ-1: protein/nucleic acid deglycase DJ-1, Nurr1: nuclear receptor-related 1, EN1: engrailed-1, Pixt3: paired like homeodomain 3, NHP: non-human primate, SNpc: substantia nigra pars compacta, KO: knockout.

Many have made efforts to replicate familial PD by generating transgenic mice carrying these mutations. However, these transgenic models do not display clear dopaminergic neurodegeneration or parkinsonian motor deficits (55). Nonetheless, they show altered neuronal function and α -synuclein aggregation. Mutated α -synuclein also disrupts cellular processes such as autophagy in A30P and A53T *in vitro* models (74) and is associated with the formation of tau fibrillary tangles in E46K transgenic mice (75). Truncated forms of α -synuclein are believed to be more toxic than full-length human α -synuclein, and transgenic mice expressing truncated α -synuclein show reduced number of nigro-striatal neurons due to cell loss during early development (57). Although these models are useful to elucidate the poorly understood α -synuclein function and the relation of PD to other synucleinopathies, the clinical relevance of this model for PD is questionable since the transgene affects early developmental stages instead of modeling late onset

neurodegeneration. As with other transgenic models, there is also a risk of compensatory mechanisms or additional deficits during development.

SNCA duplication or triplication (PARK4) is linked to PD with a dominant inheritance pattern (Table 2), showing that increased levels of WT α -synuclein can cause Lewy body pathology and PD. Interestingly, common polymorphisms in the SNCA promoter affecting gene expression levels are also associated with the risk of developing idiopathic PD (76). α -synuclein overexpression (ASO) can be modeled by knock-in transgene expression of human α -synuclein in rodents (77). ASO rat and mouse models affect both the development and maintenance of dopaminergic neurons and are useful models for early PD, and to study pathological cascades arising from α -synuclein. The spread of α -synuclein aggregates in these models depends on the integration site, the promoter used to drive SNCA transcription, and on the genetic background (78). The Thy1 promoter provides widespread SNCA expression and the formation of α -synuclein aggregates in the brain. Thy1-ASO mice also display some motor symptoms and olfactory deficits but lack dopaminergic neurodegeneration (79). The Thy1-ASO model unfavorably expresses α -synuclein in motor neurons in FVB mice but not in the C57Bl/6 background (77). In rats, the BAC-driven expression of E46K α -synuclein lacks dopaminergic neurodegeneration but displays α -synuclein aggregation, altered dopaminergic metabolism in the striatum, and evidence of oxidative stress (56).

Viral vector-mediated models

In addition to transgenic strains, α -synuclein overexpression can be induced by viral vectors. The advantages of this method include targeting of the nigro-striatal system, the induction of pathology in adulthood, and the possibility to adjust dosage (58). Recombinant adeno-associated virus (rAAV) and lentivirus (LV)-based vectors have been used to transfer SNCA in rodents.

Different AAV serotypes have been used for human α -synuclein transgene overexpression in the rat SNpc (59). Many have used AAV2 (59); however, the AAV6 serotype gave the largest dopaminergic cell loss (80% after 8 weeks) and profound motor deficits (80). Using a LV vector also provides α -synuclein aggregation but no clear dopaminergic cell loss or behavior changes with any of the α -synuclein genotypes (58). In addition to the viral vector, the genetic variant of the α -synuclein transgene influences the PD phenotype. rAAV-mediated expression of WT or A53T mutated α -synuclein seem to elicit a comparable pathology, while the expression of A30P mutated α -synuclein results in weaker and delayed pathology in rats (58, 81).

Efforts have been made to produce an equally strong rAAV- α -synuclein model in mice as in rats; however, most mouse models display weak neurodegeneration and no clear α -synuclein inclusions. Nevertheless, rAAV2/7- α -synuclein transduction in the SNpc produced strong dose-dependent dopaminergic neurodegeneration after 8 weeks and motor deficits after 12 weeks (82).

Viral vector-mediated α -synuclein models have also been applied to NHP. In the first model, rAAV2/2 vector was used to deliver WT or A53T α -synuclein (83). Similar to mice, after 16 weeks, marmosets showed some variability (30–60%)

in nigral dopaminergic cell loss, with matching dopaminergic striatal depletion and mild motor deficits (83). This model did not show functional recovery by 42 weeks post-injection. A second rAAV1/2-A53T α -synuclein NHP model has been produced and replicated in macaques (84). The macaque model displayed about 30% loss of dopaminergic nigro-striatal neurons, about 50% DA depletion, and 40% reduction of DAT, resulting in ON-OFF display of bradykinesia.

If viral vector-mediated α -synuclein models in NHP demonstrate robust motor deficits, they can become important preclinical models, complementing the current standard of MPTP-induced NHP models. The viral models have the advantage of producing α -synuclein pathology and can lead to the development of treatments targeting α -synuclein toxicity. A potential drawback of viral vector-based models is the unfavorable interaction with subsequent viral transductions used in gene therapy. Exposure to the first viral vector may change the response to future exposure to viral vectors, altering transfection and the reliability of experimental results.

Preformed fibril models

Evidence of α -synuclein toxicity, seeding, and anatomical spread (85) has led to the development of models directly introducing α -synuclein protein fibrils into the brain. α -synuclein is produced as a soluble protein that forms higher-order structures, including oligomers, fibrils, and filaments. Oligomeric structures seem to be the most toxic and aggregate into typical Lewy body inclusions. Mutations and truncations of α -synuclein increase its misfolding, aggregation, and toxicity (86). Administration of exogenous α -synuclein preformed fibrils (PFF), also called seeding, triggers formation of endogenous α -synuclein aggregates (59, 87). This prion-like propagation has been used to produce PD models with widespread bilateral α -synuclein inclusions to study their role in pathogenesis. In both mice and rats, introduction of α -synuclein inclusions in the striatum causes neuronal dysfunction, mitochondrial damage, and eventual retrograde degeneration of nigro-striatal dopaminergic neurons (88, 89). However, mice display more robust bilateral motor deficits than rats (59). The combination of viral vector-mediated overexpression of SNCA and seeding with PFF in rats induces progressive dopaminergic neurodegeneration and motor deficits alongside α -synuclein aggregation (61).

Two NHP models with α -synuclein fibrils have been published to date: macaques administered Lewy bodies extracted from PD patients into the striatum (60) and marmosets administered mouse A53T α -synuclein into the caudate and putamen (90). The macaques lost striatal dopaminergic fibers by 4 months and displayed neurodegeneration in the SNpc after 17 months. The marmosets also showed signs of nigro-striatal dopaminergic neurodegeneration. Overall, both of these experiments demonstrate a benefit in modeling PD using α -synuclein propagation, however, a limitation is that neither species showed clear motor deficits (91). The seeding of specific preformed structures of α -synuclein is a promising method to further explore the function, propagation, and role of α -synuclein in PD pathogenesis.

Autosomal dominant mutations: LRRK2 and UCH-L1

LRRK2 was identified as a monogenic cause of PD in 2004 (92). LRRK2 mutations linked to PD display autosomal dominant inheritance, but with incomplete and varying penetrance, depending on the population. This means that not all mutation-carriers will develop PD (93). The most common LRRK2 mutations are G2019S and R1441C/G.

Most LRRK2 transgenic mouse and rat models have been unsuccessful in replicating PD hallmarks (3, 62). Some success has been achieved with BAC-LRRK2-R1441G mice, which display some motor deficits and axonal pathology in the striatum, but lack clear dopaminergic neurodegeneration and formation of α -synuclein inclusions (94). A combination of transgenic models can enhance PD-like pathology, and overexpression of LRRK2 in A53T α -synuclein transgenic mice promoted dopaminergic degeneration and α -synuclein aggregation (95).

Due to the large size of the LRRK2 gene, a limited number of viral models have been generated using herpes simplex virus (HSV) and adenoviral vectors (58). In rodents, transfection of LRRK2-G2019S is more toxic than WT LRRK2, causing more neurodegeneration and formation of inclusions (96). Injection of HSV-LRRK2-G2019S in the mouse striatum has been shown to induce degeneration of about 50% of the dopaminergic neurons in the SNpc (97). LRRK2 models do not robustly replicate all PD hallmarks but are useful to understand the interplay between different genetic mutations and environmental factors and to untangle the mechanisms behind their functions in PD. Mutations in LRRK2 have not yet been used in PD models in NHP, but LRRK2 kinase inhibitors have been proposed as a potential therapeutic option in PD, and their effect has been explored in macaques following MPTP exposure (98).

The pathogenic effects of UCH-L1 mutations have been debated due to their rarity in PD patients (23). However, transgenic mice with mutated UCH-L1 display a mild reduction in locomotion, reduced number of dopaminergic neurons in the SNpc, and dopamine depletion in the striatum, without α -synuclein inclusions (63). Mutant UCH-L1 also exacerbates pathology in MPTP and ASO mouse models (64).

Autosomal recessive mutations: PRKN, PINK1, and DJ-1

Autosomal recessive mutations in PRKN (Parkin), PINK1, and DJ-1 have been linked to familial PD. There are over 100 known mutations of PRKN and it is the most commonly mutated gene in early onset PD (50% of familial and 20% of idiopathic early onset cases) (65). PINK1 is the second most commonly mutated gene in early onset PD, present in 1–7% of the cases, while DJ-1 mutations are uncommon (81). Rare individuals who have two mutations in one of these three genes appear to have complete penetrance (62, 65, 99).

Mutations in all three genes give loss-of-function, and therefore knockout models have been generated. However, none of the knockout models display dopaminergic cell loss or motor deficits (62). Some DJ-1 and PRKN models display changes in dopamine neurotransmission and mitochondrial dysfunction (100–102). Interestingly, overexpression of mutated PRKN-Q311X leads to age-dependent dopaminergic neurodegeneration, α -synuclein aggregation, and some motor deficits, suggesting a gain of pathological function (103). Backcrossing

of DJ-1 nullizygous mice onto a C57Bl/6J background generates a new PD phenotype (104). These mice display strong early unilateral dopaminergic neurodegeneration that progresses into bilateral pathology in 15-month-old mice, demonstrating the age- and strain-dependent nature of some PD models.

Silencing gene expression of PINK1 or Parkin in adult mice using viral vectors has not yielded better PD models compared to knockout (58, 105), possibly because of less efficient silencing (86). However, both knockout and viral vector-mediated knockdown of PINK1 in mice rendered dopaminergic neurons more sensitive to MPTP (58, 105). PINK1 knockout mice, but not PINK1 silencing, enhanced neurodegeneration in the ASO model (106). Inactivation of PINK1 using RNA interference knocked down 71% of PINK1 in striatum and 68% in SNpc (86). These mice exhibited higher sensitivity to MPTP-induced neurodegeneration. Knockdown of DJ-1 also enhanced toxicity in the MPTP mouse model and could be rescued by reintroducing the gene with viral vectors (107). These examples of combinations of genetic and neurotoxic models highlight the complex interplay between multiple factors in PD pathology.

The overexpression of autosomal recessive genes, where loss-of-function is linked to PD, could become sources of treatments. For example, overexpression of PRKN via rAAV vectors had protective effects in the striatum of rAAV- α -synuclein-treated NHP (108).

Transcription factors

Nuclear receptor-related 1 protein (Nurr1), engrailed 1 (EN1), pituitary homeobox 3 (Pitx3), sonic hedgehog (SHH), and c-Rel are all transcription factors that play a role in the development and maintenance of the dopaminergic nigro-striatal system (3). Nurr1 knockout mice fail to differentiate dopaminergic neurons (109) and heterozygous Nurr1-deficient mice show a progressive dopaminergic cell loss (3, 66). DAT-Cre transgenic mice with Nurr1 knockout in dopaminergic neurons display rapid striatal dopamine depletion, loss of SNpc dopaminergic neurons, and motor deficits (110, 111). Meanwhile, silencing Nurr1 in dopaminergic neurons by using rAAV vectors gives progressive retrograde dopaminergic degeneration. However, none of the Nurr1-deficient models display α -synuclein aggregates.

Heterozygous deficiency of EN1 gives rise to a progressive retrograde degeneration with dystrophy of striatal dopaminergic axons as early as at 4 weeks, and clear dopaminergic cell loss by 16 weeks accompanied by motor deficits and depressive-like behavioral changes (67, 112, 113). The phenotype is seen in EN1-heterozygous SwissOF1 mice, but not in C57Bl/6 with the same mutation. The neuroprotection in C57Bl/6 is multigenic and could be useful for studying risk factors for idiopathic PD (114).

Knockout of c-Rel creates mice with disrupted production of cell survival factors (SOD2 and Bcl-xL). These mice have clear dopaminergic cell loss in the SNpc, dopamine depletion in the striatum, age-dependent motor deficits, and α -synuclein aggregation in the SNpc (115). Pitx3 knockout mice, also called aphakia mice, have abnormal dopaminergic systems. They display strong dopaminergic degeneration in the SNpc, 90% striatal dopamine depletion (68, 116), and clear motor deficits that are reversed with L-dopa administration (117).

SHH has a vital role in the embryonic development of dopaminergic projections in the midbrain (118) and ablation of SHH in dopamine neurons causes dopaminergic and cholinergic neurotransmitter dysfunction, progressive neuronal loss, and motor deficits (69). The MitoPark model disrupts mitochondrial function in dopaminergic neurons by knocking out TFAM in DAT-Cre mice (62). These mice display progressive dopaminergic degeneration, motor impairments, and formation of inclusions that lack α -synuclein (41, 70, 119).

Transgenic mice with deficiencies in dopaminergic transcription factors have a role in modeling PD but display other dopaminergic pathologies that undermine their specificity. *Nurr1* and *Pitx3* models have been suggested being applicable to cognitive disorders such as attention-deficit hyperactivity disorder (ADHD) and schizophrenia due to their involvement in other dopaminergic pathways (71, 72). Other transcription factors like SHH, TFAM, and NF- κ B are relevant in the survival of many non-dopaminergic cells. These models can thus be useful to study defects in development and maintenance of dopaminergic neurons but are not specific to PD-related pathologies.

CONCLUSION

None of the animal models described above perfectly mimic the neuropathology of PD and the models cannot replicate the clinical syndrome. However, the wide variety of rodent, NHP, and non-mammalian models allows targeted studies of different pathological mechanisms of PD. The heterogeneity of these models can be seen as an advantage, since a clinical PD diagnosis reflects a heterogeneous group of patients with differences in onset, progression, symptoms, and neuropathology. The complex etiology of the disease, with interindividual variations in environmental and genetic risk factors, reflects the heterogeneity of PD and is seen both in idiopathic and monogenic cases. For example, some patients with DJ-1, Parkin, and LRRK2 mutations do not form Lewy bodies (62, 104). Diversifying the animal models can help understand various subtypes of PD and develop personalized treatments.

Current animal models of PD focus on degeneration of dopaminergic nigro-striatal neurons and formation of α -synuclein aggregates, but many lack one or the other. Classical neurotoxins (MPTP and 6-OHDA) (24) in rodents and NHP, and transcription factor deficiencies—*Nurr1* (92), *Pitx3* (68), SHH (69), MitoPark (102)—in mouse models cause an extensive loss of dopaminergic neurons in the SNpc and clear motor deficits but no α -synuclein aggregation. In contrast, mutated α -synuclein transgenic mouse models induce α -synuclein aggregation without neurodegeneration (55). Other models have struck a balance between the aggregation of α -synuclein and progressive degeneration of nigro-striatal neurons. These include chronic administration of MPTP in mice and NHP (10, 25), pesticide and herbicide rodent models (30, 31, 49), seeding of PFF in mice and NHP (59, 91), c-Rel knockout mice models (115), PRKN-Q311X overexpression (103), transfection of AAV- α -synuclein in rodents and NHP (80, 82, 84), and HSV- LRRK2 G2019S transfection in mice (97).

Furthermore, combined models can enhance PD hallmarks by hitting disease-associated pathways at several levels. For example, seeding of PFF in rats combined

with rAAV- α -synuclein transfection leads to enhanced α -synuclein aggregation and neurodegeneration (61). Many transgenic models (mutated LRRK2 [3] and UCHL1 [63]; DJ-1, PINK1, and Parkin knockout [62]) show some functional disruption in the nigro-striatal system but have been ineffective at inducing a robust display of neurodegeneration and α -synuclein pathology. However, UCHL1 mutations, DJ-1 knockout and PINK1 knockout, exacerbate ASO and MPTP mouse models (64, 86, 107).

When choosing an animal model for PD, genetics of the selected species also needs to be taken into consideration, since the genetic background of mouse strains has been shown to alter the phenotype of different PD models. One example of strain effects on a monogenic PD model is the strain-dependent α -synuclein toxicity in Thy1-ASO mice (77). Functional recovery in MPTP-exposed mice is also determined by the strain used, highlighting the importance of interaction of environmental factors with the genetic background (34). Attempts to map the genetic factors underlying strain-dependent susceptibility to PD-like phenotypes include linkage analysis in mice treated with MPTP (120) and paraquat (121) as well as in EN1-hemizygous mice (114). In addition, backcrossing DJ1-null mice to the C57Bl/6J background generated a progressive unilateral to bilateral neurodegeneration in a subset of mice that segregated with exonic variants in five genes (104). Genes identified as conferring strain-dependent susceptibility to PD models are strong candidates as risk-modifying genes in PD.

Functional recovery in neurotoxic models and the lack of comparable phenotypes in mice carrying mutations that cause PD in humans highlight the difficulty of replicating PD in animal models. There is also a lack of models that include non-motor symptoms, which significantly affect the quality of life of patients with PD. This questions the relevance of animal PD models to human pathology. However, the development of PD models in NHP that have greater anatomic and genetic resemblance to humans, and new genetic technologies open new avenues towards creating models that display a complex pathology more similar to what is seen in PD patients (11). In addition, the panel of non-mammalian, rodent and NHP models offers the possibility to selectively study specific aspects of PD pathology. New technologies together with further refinement and combination of existing models could thus generate an array of animal models that can deepen our understanding of PD and help translate research into treatment.

Conflict of interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this manuscript.

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