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Research Paper

Aging, Parkinson's Disease, and Models: What Are the Challenges?

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Parkinson's disease (PD) is a chronic, neurodegenerative condition characterized by motor symptoms such as bradykinesia, rigidity, and tremor, alongside multiple nonmotor symptoms. The appearance of motor symptoms is linked to progressive dopaminergic neuron loss within the substantia nigra. PD incidence increases sharply with age, suggesting a strong association between mechanisms driving biological aging and the development and progression of PD. However, the role of aging in the pathogenesis of PD remains understudied. Numerous models of PD, including cell models, toxin-induced models, and genetic models in rodents and nonhuman primates (NHPs), reproduce different aspects of PD, but preclinical studies of PD rarely incorporate age as a factor. Studies using patient neurons derived from stem cells via reprogramming methods retain some aging features, but their characterization, particularly of aging markers and reproducibility of neuron type, is suboptimal. Investigation of age-related changes in PD using animal models indicates an association, but this is likely in conjunction with other disease drivers. The biggest barrier to drawing firm conclusions is that each model lacks full characterization and appropriate time-course assessments. There is a need to systematically investigate whether aging increases the susceptibility of mouse, rat, and NHP models to develop PD and understand the role of cell models. We propose that a significant investment in time and resources, together with the coordination and sharing of resources, knowledge, and data, is required to accelerate progress in understanding the role of biological aging in PD development and improve the reliability of models to test interventions.

Introduction

Parkinson's disease (PD) is a chronic, neurodegenerative condition affecting approximately 10 million people worldwide. While ~5% of PD is thought to be familial, the vast majority of PD cases have an unknown cause (sporadic PD [sPD])[1]. The most common cause of early-onset PD is mutations in the *PRKN* gene, whereas mutations in *LRRK2* (leucine-rich repeat kinase 2) are a common

cause of late-onset PD, clinically similar to sPD. Many risk factors have been identified that influence the onset and penetrance of sPD. These include single nucleotide polymorphisms in *LRRK2, GBA1*, and *SNCA*, as well as other genes, exposure to pesticides, head trauma, and old age.

PD is characterized by a complex array of both motor and nonmotor symptoms. Motor symptoms often include a resting tremor, rigidity, akinesia (or bradykinesia), and postural instability. The onset of these motor symptoms varies between patients and can often be preceded by nonmotor symptoms, which have historically been understudied[1]. Nonmotor symptoms include autonomic dysfunction, constipation, incontinence, sleep abnormalities, sensory disturbances (loss of olfaction), cognitive impairment, and depression. Each patient with PD has a unique disease onset and course, making it difficult to diagnose and predict progression[1]. However, clinical rating scales and several novel prediction tools are increasing our understanding of PD as a multisystemic and heterogeneous disease[2–5]. Historically, PD was diagnosed at death upon postmortem examination revealing loss of the dopaminergic (DA) neurons (often labeled with tyrosine hydroxylase [TH], a rate-limiting enzyme in dopamine synthesis) in the substantia nigra (SN) and the presence of Lewy body inclusions. It is thought that the loss of DA neurons underlies the core motor symptoms observed in patients (resting tremor, akinesia, and bradykinesia). Loss of other neuronal populations, including, for example, noradrenergic, serotoninergic, and cholinergic neurons, could underlie some of the nonmotor symptoms, although there is insufficient quantitative data on the extent of actual cell loss in regions other than the SN in PD. Lewy bodies are intracellular proteinaceous inclusions containing many proteins, with α-synuclein being a major component. These inclusion bodies, containing misfolded or aggregated α-synuclein, are found not only in the SN but also in other brain regions, and a growing literature suggests a potential spread of PD pathology via expansion of α-synuclein fibrils, perhaps even beginning in the gut and progressing to the central nervous system (CNS)[6]. Braak and colleagues proposed a staging of PD pathology based on Lewy body inclusions and the brain regions affected[7]. According to Braak staging, pathology begins in the olfactory system and lower brainstem, spreading up to medullary structures. In stages 1 and 2, more Lewy neurites are visible rather than Lewy bodies. Lewy neurites are thread-like aggregates containing α-synuclein, rather than the globular structures of Lewy bodies. At stage 3, the pathology reaches the SN, with loss of DA neurons in the SN and more Lewy body formation. In stage 4, severe cell loss of predominantly DA neurons is observed in the SN, and the pathology begins to spread to the neocortex, and at the final stage of the disease, Lewy bodies are also observed in the cortex[7]. Although this is only one method of staging PD, it is a useful paradigm to compare animal models of PD to the clinical and pathological features seen in humans. The pathology of PD is not limited to these features, with astrogliosis and other signs of inflammation also being prominent features[8].

The loss of SN DA neurons, also revealed by the loss of neuromelanin in this brain region, appears to be preceded by the loss of DA axon terminals in the caudate and putamen (striatum). This is accompanied by drastic reductions in the levels of DA itself and changes in its metabolites (most notably 3,4-dihydroxyphenylacetic acid [DOPAC]) in PD patient brains. The loss of terminals detected by positron emission tomography imaging using fluorodopa or dopamine transporter (DAT) or vesicular monoamine transporter 2 (VMAT2) ligands is one of the most readily detectable pathological features in PD patients and can be used to track disease progression longitudinally[9].

PD and Aging

Aging is the major risk factor for PD, as shown by the prevalence of PD, which increases sharply with age. A meta-analysis of 47 studies shows that the incidence rises from 41 per 100,000 in individuals 40–49 y old to 1,903 per 100,000 in those over the age of 80[10]. Many of the pathological changes that occur in the brain with age resemble those seen in a pre-Parkinsonian state. It has been estimated that the number of DA neurons in the SN declines with age in healthy individuals more so than in other regions of the brain, suggesting that DA neurons may be more vulnerable to the effects of aging[11]. About 10% of older people without clinically defined PD show Lewy body pathology[12]. In healthy rhesus monkeys, there is an age-related decline in TH staining in the ventral SN, which is the area most affected by PD[13], and the decrease in TH staining is associated with an increase in intracellular α-synuclein in neurons of the SN[13].

Mechanistically, mechanisms dysregulated during aging overlap with those driving PD pathogenesis, including mitochondrial dysfunction, autophagy, inflammation, and cellular senescence, which are all considered hallmarks of aging[14,15]. Decreased mitochondrial complex I protein expression and activity has been shown in tissues from individuals with PD, including the midbrain, cortex, muscle, and fibroblasts[16]. Strikingly, the environmental toxicants rotenone and paraquat, which damage mitochondria, are sufficient to cause a PD-like phenotype and neuropathological changes in rodents similar to those observed in humans afflicted with PD[17]. Genes associated with familial PD, such as *SNCA*, *PINK1*, *PRKN*, and *LRRK2*, all impact mitochondrial function, directly or indirectly[18–24]. Protein degradation through the ubiquitin proteasome system and autophagy is reduced with age, and such dysfunction has been implicated in PD[25]. Impaired proteostasis may occur downstream of mitochondrial dysfunction as it requires adenosine triphosphate (ATP), and, in turn, impaired proteostasis can contribute to the accumulation of damaged mitochondria, which requires autophagy for clearance. In addition, DA metabolism generates a significant amount of reactive oxygen species (ROS), which damage proteins and mitochondria, further contributing to brain aging. The accumulation of damaged proteins and impaired proteostasis could contribute to greater neuronal loss in the SN. ROS also contributes to lipid peroxidation and oxidative DNA damage in the mitochondrial and nuclear genomes. Indeed, postmortem analysis of PD brains reveals increased oxidative damage to proteins, lipids, and DNA[26,27].

Genotoxic, proteostatic, and mitochondrial stress can all drive cellular senescence characterized by a stable cell cycle arrest, loss of cell function, and the production of proinflammatory and tissue remodeling factors called the senescence-associated secretory phenotype[28]. The number of senescent astrocytes increases with age and with PD[29].

Both the aged and PD brains present a state of low chronic inflammation with changes in astrocytes and microglia, which can affect the adjacent neurons[29] and is believed to contribute to neuronal loss. Removal of senescent cells by the ablation of p16+ cells using a prodrug system in a mouse model of PD induced by paraquat improves outcomes[29], suggesting a causal relationship between senescence and PD. The causal relationship between mechanisms of aging and PD pathology has also been reported in *Caenorhabditis elegans*. Putting an *lrrk2* mutation into a long-lived worm (expressing a mutant insulin growth factor 1 receptor, *daf-2*) prevented PD features such as loss of DA neurons and improved DA-dependent deficits[30]. Although these

observations suggest that aging biology plays a role in PD, the precise mechanisms and how well the pathways leading to dysregulation of these mechanisms overlap are currently unclear. The rate of loss of DA neurons with age is slower than their rate of loss in PD organisms, however, suggesting that other factors are at play.

Here, we review the available evidence on the role of aging in the pathogenesis of PD, focusing primarily on phenotypic tests using *in vitro* and *in vivo* mammalian systems. We highlight the barriers to studying aging in PD and propose recommendations for further work.

Patient-Derived Cell-Based Models of PD

Patient-derived cells are an extremely useful tool to study PD, in particular to model sPD. Blood cells and fibroblasts can be easily isolated from patients with PD and utilized to study the underlying cellular mechanisms related to PD. Patient-derived cells retain some of the aging-related changes of their donors; however, the characterization of many aging changes is limited. Cells from PD patients have mitochondrial abnormalities as well as alterations in the autophagy/lysosome pathway compared to cells from healthy individuals; many of these changes are in the same direction as age-related changes but are more severe. Indeed, in cells from PD patients with familial PD, such as those caused by *PRKN* or *LRRK2* mutations, changes are relatively homogeneous in these key organelles/pathways[19,20,31–40].

Cellular reprogramming has enabled researchers to investigate PD-relevant mechanisms in the cell types most affected by PD. Classical reprogramming into induced pluripotent stem cells (iPSCs) and subsequent differentiation into a DA-enriched population of neurons has been undertaken by numerous research groups (reviewed here[41,42]). These reprogrammed and differentiated DA neurons recapitulate many of the cellular mechanisms associated with PD, including mitochondrial dysfunction, lysosomal abnormalities, α-synuclein pathology (particularly increased levels of phospho-α-synuclein), and susceptibility to α-synuclein preformed fibril (PFF) seeding[42–47]. In addition, for the proportion of neurons that successfully differentiate from iPSCs, markers of apoptosis and neuron viability differ between PD and healthy control donors[48,49], indicating PD patient-derived neurons are more susceptible to cell death during differentiation. This preferential neuron cell during differentiation could be viewed as strength as it recapitulates the neuron death observed in PD patients; however, those neurons that are lost during differentiation could be in fact those neurons that need to be studied to understand the neuronal death pathways active in PD. Hence, further studies investigating that population of vulnerable cells throughout differentiation would be warranted. Furthermore, DA, DA metabolites, and expression of genes controlling DA synthesis and sequestration (DOPAC and homovanillic acid) differ even between PD patients displaying varying severity of disease[49]. These changes in DA metabolites and neuronal complexity are similar to those reported from several *in vivo* rodent models of PD (discussed below). iPSCs can be differentiated into nonneuronal cells as well, revealing defects in many of the same pathways in glial cells derived from PD patients, although these are less extensively studied compared to DA neurons[50,51].

The reprogramming of iPSCs reverses many aging-related changes including DNA methylation, reverting to a more embryonic phenotype[14,52,53]. Some aging-related features can be re-attained after several months of differentiation *in vitro* or attained by introducing genes that are known to accelerate aging[54,55]. However, it is still unclear if *in vitro* aging accurately reflects *in vivo* aging.

Other reprogramming methods seek to maintain the aging features of the donor. Direct reprogramming from fibroblast to neuron has been reported to maintain several aging features, including epigenetic methylation status, telomere length, telomerase activity, and the expression of several age-related genes of the donor[56–58]. A limited number of studies show defective mitochondrial and lysosomal pathways in these directly reprogrammed neurons[48,59]. The reproducibility of this technique is problematic, as the reprogramming of cell states is not perfectly homogeneous across batches of neurons. To address this, one can reprogram fibroblasts to intermediate cell types such as neural progenitor cells, which can then be banked and subsequently differentiated into astrocytes, oligodendrocytes, or neurons, an approach first utilized in amyotrophic lateral sclerosis research[60]. The differentiated cells retain many features of aging, including alterations in nuclear envelope integrity, telomere length, and the expression of several age-related genes[61]. Furthermore, this method yields relatively pure DA neurons, with ~95% expressing TH and DAT and robust alterations in mitochondrial function, particularly without the need for additional stressors[23,33,62].

Coculture and organoid culture systems are also being investigated to model the complexity of native tissues. These systems have the potential to better model age-related changes as extrinsic factors are better accounted for, which require the interplay of multiple cell types. However, research in this area is somewhat in its infancy, and further work is needed to define which age-related changes are retained and interact with PD-relevant pathways in these organoid systems. It remains unclear whether these culture systems are able to model some of the basic features that likely contribute to SN DA neuron vulnerability (e.g., their extensive axonal connectivity)[63,64]. Finally, there are aspects of aging that cannot be fully modeled by patient-derived cells, in particular those that require complex interactions and the circulatory system, such as immune and inflammatory mechanisms. Therefore, approaches that employ multiple models (e.g., patient-derived cells and animal models of PD) will likely lead to a more complete picture of the underlying mechanisms that contribute to PD pathogenesis.

In Vivo Models of PD

PD researchers utilize a number of experimental models that have been developed over the years. They come essentially in four flavors: pharmacological (e.g., reserpine), toxic (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP] or rotenone), genetic (e.g., transgenic rodents), and proteostatic (e.g., exposure to synuclein). We will not describe pharmacological models, as they are transient and are discussed in more depth elsewhere[65]. We will focus on toxic models subcategorized into neurotoxins (6-hydroxydopamine [6-OHDA] and MPTP), pesticides (rotenone, paraquat, trichloroethylene [TCE]), and endotoxins (lipopolysaccharide [LPS]) with more permanent effects. For a more in-depth description of the models, we refer the reader to ref. [66]. Finally, among the proteostatic models, there has been the development of nontransgenic α-synuclein models involving the injection of preformed α-synuclein fibrils. Each model provides insight into the underlying causes and mechanism(s) of the disease and offers different approaches to test new strategies to treat PD. Some investigators prefer classification as etiologic models, which encompass

all gene-based models, versus *pathogenic models*, which include toxin models and those involving genetic mutations. More in-depth reviews of this classification can be found in ref. [67].

To model PD in animals, a variety of mouse, rat, and nonhuman primate (NHP) systems have been developed and reproduced in multiple labs. Mice and rats are relatively inexpensive and more practical in comparison to NHPs. Even though rats display better reproducibility in terms of behavioral readouts in comparison to mice, the biggest limitation to the use of rats is the general lack of tools for molecular analysis and aging (e.g., antibodies). NHPs have some advantages as they display clinical features (e.g., sleep disturbances, social/cognitive symptoms, and gastrointestinal [GI] disturbances) more similar to those observed in human disease following exposure to MPTP (reviewed in ref. [68]). Moreover, the anatomical organization of the adult NHP striatum is similar to that of a human, and, unlike rodents, NHP DA neurons contain neuromelanin. The following section will provide an overview and highlight the advantages and disadvantages of the main mammalian animal models of PD. It is important to understand the limitations of each model, and aging has been accounted for in the various models. The following sections and tables do not include every animal model but focus on the more established and reproducible animal models used by the PD research community.

Toxin Models

Neurotoxins

Toxins such as 6-OHDA or MPTP are typically used to model the loss of DA neurons and the denervation of the striatum that is known to occur in PD. However, a major limitation of these neurotoxins is that they do not mimic the multisystemic nature of PD as they selectively target DA neurons due to their uptake through the DAT and therefore are not ideal candidate models to study changes in the GI track. Overall, depending on the dosing protocol, these toxins can cause either progressive or rapid loss of nigral DA neurons, neuroinflammation, oxidative stress, and motor deficits, as summarized in **Table 1**.

6-OHDA is an analog of DA and norepinephrine (NE) and cannot cross the blood–brain barrier (BBB). It must be injected into the brain (typically in the SN, medial forebrain bundle, or striatum) to produce DA neuron loss. The cellular mechanism by which 6-OHDA causes cell loss is thought to be by increasing free radical production and inhibiting complexes I and IV of the mitochondrial respiratory chain. Many different injection protocols have been developed (e.g., injecting 6-OHDA bilaterally or unilaterally) and produce differing effects on DA neuron loss and behavior. See refs. [69] and [70], for a complete review on 6-OHDA and the different 6-OHDA protocols.

There are several considerations when using 6-OHDA as a model of PD. First, the requirement of administration of 6-OHDA directly into the nigrostriatal pathway. Second, as 6-OHDA is readily taken up by both DA and NE transporters, to achieve selective DA neuron loss, an NE reuptake inhibitor, such as desipramine, must be administered. Finally, the time course for 6-OHDA-induced DA cell death can be very rapid, which is not consistent with the slow, progressive nature of the human disease nor does 6-OHDA cause the formation of insoluble α-synuclein aggregates.

Unlike 6-OHDA, MPTP can be given systemically. Due to its lipophilic nature, MPTP rapidly crosses the BBB and is taken up by astrocytes, where it is metabolized by monoamine oxidase-B (MAO-B) to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+), which spontaneously oxidizes into the highly toxic metabolite, 1-methyl-4-phenylpyridinium (MPP+)[71,72]. Surprisingly, MPP+ is not toxic to astrocytes but is highly toxic to DA neurons. MPP+ is released into the parenchyma through the cation transporter 3 and rapidly taken up by DAT and then VMAT2. MPP+ readily crosses the inner mitochondrial membrane and inhibits mitochondrial complex 1 of the electron transport chain (ETC). This impairs ATP production and causes the accumulation of ROS, eventually leading to DA degeneration[73,74]. Interestingly, MPP+ is taken up by DA neurons in both the SN and ventral tegmental area (VTA), but seems to be more toxic to the DA neurons of the SN compared to the VTA[75–77]. This may be because SN neurons are more vulnerable to bioenergetic challenges compared to VTA DA neurons[63].

Despite its effectiveness for modeling PD in mice and NHPs, rats are relatively resistant to MPTP at moderate doses, and it is lethal at higher doses[78,79]. Typically, MPTP is administered acutely or chronically to C57BL/6 mice, as this is the most sensitive strain to MPTP. Depending on the dosing paradigm, MPTP can cause sizable SN lesions[79–82]. However, it is important to recognize that depending on the dosing protocol, MPTP can cause phenotypic suppression of TH, rather than true DA neuron loss[83]. Therefore, similar to all animal models for PD, when assessing DA neurodegeneration, it is crucial not only to quantify TH-positive neurons but also to include a secondary neuronal marker such as Nissl[83]. Like the 6-OHDA model, MPTP does not cause accumulation of endogenous α-synuclein accumulation in SN DA neurons, which is a hallmark of the disease[80], nor does it cause GI dysfunction. Even though mice exposed to MPTP do not display a behavioral phenotype reminiscent of PD, the MPTP model has been extremely useful for elucidating mechanisms of cell death in DA neurons.

Environmental toxicants: Pesticides and herbicides

Paraquat is structurally similar to the active metabolite of MPTP, MPP^+, and can reliably provoke a progressive loss of nigrostriatal DA neurons. The maximum neuronal loss induced by paraquat is considerably less than that induced by MPTP (~30% vs. 50%[84–87]). It is unclear, whether paraquat reduces striatal TH-positive fibers or depletion of striatal DA release[88]. However, a major strength of the paraquat model is that the loss of DA neurons in the SN is both age- and dose-dependent with a greater loss in older animals[89]. Paraquat can provoke the formation of Lewy body-like inclusions[90]. Paraquat can also trigger both motoric and nonmotoric disturbances, including reduced locomotor activity[88,91,92] and diminished performance on a forced swim and open field test[93–95]. This is interesting because forced swim and open field measure affective disturbances. This is a particular strength of the model, as PD patients are known to suffer from depression[96]. Systemically administered paraquat is thought to cross the BBB in mice through a neutral amino acid transporter and have a half-life of one month[97,98]. However, whether paraquat is able to cross the BBB in NHPs is still unclear[99]. Like 6-OHDA and MPTP, paraquat can accumulate in mitochondria, but it mediates toxicity through a different mechanism. Paraquat acts mainly as a redox cycler, stimulating ROS production by accepting electrons from complex I for redox cycling, which, in turn, generates superoxide anions and subsequently other species of ROS[97]. Paraquat is known to cause pulmonary and renal dysfunction; however, to date, the GI system has not been

Table 1. Summary of characteristics of neurotoxin and environmental models.

Neurotoxin and Environmental Toxicants-Based Models	Species	Displayed Characteristics	Limitations
6-hydroxydopamine—Stereotactic injection to SN, MFB, striatum[233]	Rat	Nigrostriatal damage (SN cell body, striatum terminals, striatum DA)[234]	Administered directly into the nigrostriatal pathway
	Mice	Motor deficits (L-DOPA or apomorphine responsive)[70]	Selective DA neuron loss seen only in the presence of NE reuptake inhibitor
			Acute loss of DA neurons
			No Lewy body formation
			No extranigral pathology
1-methyl-4-phenyl-2,3-dihydropyridinium—i.p., i.m., intracarotid infusion, chronic (osmotic minipumps)[80]	Mice	Selective DA neuron death—apoptotic[235] (Chronic treatment), necrotic[236] (acute treatment), reduction in striatal dopamine levels	No endogenous α-synuclein accumulation in SN DA neurons in mice[80]
	NHP	Motor imbalance, tremor, rigidity, slowness of movement, postural instability, and freezing in NHP monkey model[237,238]	No Lewy body formation in NHP[239,240]
			Failure to capture behavior phenotype reminiscent of PD in mice
			Rats are resistant to MPTP treatment[241]
			Functional recovery in mice[242] and NHP[243]
Paraquat—i.p.	Mice	Age and dose dependent loss of DA neurons in the SN[89]	Excluded from the brain by BBB in NHP[99]
	NHP	Formation of Lewy body-like inclusions[90]	High dose causes pulmonary fibrosis and mortality[244]
		Reduced locomotory activity, diminished performance on a forced swim test and open field[86,92,93,95]	
Rotenone—Infusion via osmotic minipumps, i.p. injection	Mice	Selective nigrostriatal degeneration, early and sustained activation of microglia and iron accumulation in SN[109]	Variability in lesion size and strain sensitivity in rats[110]
	Rat	α-synuclein positive cytoplasmic inclusions in nigral DA neurons[113], lysosomal and protein degradation deficits[108]	Systemic toxicity and mortality[245]
		Motor symptoms such as bradykinesia, postural instability and rigidity in rats[110]	
		Nonmotor symptoms—sleep disturbances[114], GI disturbances and α-synuclein accumulation in the myenteric plexus[115,245]	
TCE—dosing for 6–12 weeks, i.p., oral gavage	Mice	Loss of DA neurons in SN	Insufficient (≤50%) loss in dopaminergic neurons
		Glial dysfunction, mitochondrial dysfunction, oxidative stress, α-synuclein accumulation[102–104]	No loss in dopamine—Requires long-term exposure
			Advanced technical expertise for oral gavage

BBB, blood–brain barrier; DA, dopamine; GI, gastrointestinal; i.m., intramuscular; i.p., intraperitoneal; L-DOPA, levodopa or l-3,4-dihydroxyphenylalanine; MFB, medial forebrain; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, norepinephrine; NHP, nonhuman primate; PD, Parkinson's disease; SN, substantia nigra; TCE, trichloroethylene.

extensively characterized in this model. Therefore, it is unclear if paraquat causes GI deficits.

TCE is a chlorinated solvent used as a degreaser and chemical feedstock. TCE is pervasive in the environment and is linked epidemiologically to PD[100,101]. TCE treatment causes a slow and progressive Parkinsonian phenotype in mice and rats which is accompanied by glial inflammation, mitochondrial dysfunction, oxidative stress, and accumulation of α-synuclein[102–104]. In mice, a significant loss of SN DA neurons was reported with 400 mg/kg/day dosing for eight months in mice[104]. Studying the cellular mechanisms at earlier timepoints after dosing in this prolonged dosing model may be an important way to investigate the cellular

mechanisms active during the preclinical phase of sporadic late-onset PD. In five-month-old rats exposed to TCE for six weeks, a dose-dependent loss of SN DA neurons was reported following 500 and 1,000 mg/kg/day dosing[102]. Moreover, daily dosing for six weeks of a lower dose of TCE (200 mg/kg) was sufficient to achieve SN DA degeneration in older (12-month-old) rats[103]. These older rats also display marked oxidative stress, endo-lysosomal impairment, and α-synuclein accumulation within the surviving SN DA neurons[103]. There are very little data looking at the gut microbiome or GI dysfunction in rodents exposed to TCE. However, there is a single study where mice exposed to TCE at a dose equivalent to environmental or occupational exposures for 154 or 259 days in drinking water resulted in disturbances in the gut microbiome, which were associated with an increase in proinflammatory cytokines[105].

Rotenone is a naturally derived compound, mainly used in fishery management to eradicate fish populations[106]. Like paraquat, chronic exposure to rotenone is associated with a higher incidence of sPD, strengthening the rationale for use of rotenone to model the disease in animals. Similar to MPTP, rotenone is a highly lipophilic compound that easily crosses the BBB and acts to inhibit mitochondrial complex 1 of the ETC. In addition to promoting oxidative stress, rotenone can cause other histopathological features resembling PD not observed with either 6-OHDA or MPTP. It causes dose-dependent systemic toxicity and mortality. The most reliable route of administration for rotenone to produce features of sPD is systemic delivery into the intraperitoneal cavity (2–3 mg/kg/day)[107–109]. Depending on the dosing regimen and route of administration, rotenone can cause dorsolateral lesions in the striatum in ≥12-month-old rats that are associated with a reduction in DA levels; this loss is not seen in animals ≤7 months of age[110]. The DA neurons in the SN are highly sensitive to rotenone in comparison to the DA neurons in the VTA[63]. Rotenone causes a 45% loss of DA neurons in the SN, whereas the VTA seems relatively spared in comparison[111–113]. This enhanced nigral sensitivity and the fact that rotenone causes endogenous α-synuclein accumulation within surviving DA neurons, increased nigral reactive microglia, and motor symptoms such as bradykinesia, postural instability, and rigidity in rats[108,110,113] further strengthens the validity of the use of rotenone to model some aspects of sPD. Rotenone can also induce nonmotor symptoms such as sleep disturbances in rats[114], GI disturbances, and α-synuclein accumulation in the myenteric plexus[115].

Despite the strengths of the rotenone model, particularly the age dependency, it has limitations. Lewis rats are the most sensitive to rotenone, while other strains produce unreliable and highly variable lesions. Until recently, rotenone has been unreliable in mice, regardless of age. A recent study using young mice dosed them with rotenone for 14 days and then left an additional 14 days yielded nigral DA degeneration accompanied by neuroinflammation[116].

The main features of the described environmental toxin models are summarized in **Table 1**.

Endotoxins: LPS

Central LPS administration: LPS is a gram-negative bacterial endotoxin that activates toll-like receptor 4 (TLR-4). Injecting LPS into the SN results in a strong proinflammatory response and the loss of DA neurons[117,118]. The SN is more sensitive to LPS in comparison to other brain regions, as it is prone to neuroinflammation. It remains unclear why the SN is more sensitive; it may be due to the higher number of microglia in the SN compared to other brain regions[119]. A single intranigral injection of LPS can induce microglial activation, a loss of astrocytes within 2 days, and a loss of DA neurons[120]. High doses of LPS can even result in motor impairment, α-synuclein, and ROS accumulation in addition to SN DA neurodegeneration[121,122].

Peripheral LPS administration: A single systemic dose of LPS in adult mice can cause progressive SN DA degeneration and α-synuclein alterations in the gut, despite not crossing the BBB[123–126]. It has been postulated that increased peripheral production of the proinflammatory cytokine tumor necrosis factor (TNF-α) following LPS administration crosses the BBB and induces microglia activation. Chronic intranasal administration of LPS causes behavioral deficits, microglial activation, SN DA degeneration, and α-synuclein aggregation[127,128].

A summary of the main features of the LPS endotoxin model are summarized in **Table 5**.

Genetic Models of PD

α-synuclein (SNCA) transgenic animal models

The *SNCA* gene was the first gene identified as a genetic cause for familial PD. A53T and A30P missense mutations, as well as *SNCA* duplication or triplication, cause early-onset PD. The function of α-synuclein remains unclear. However, the protein is expressed at very high levels in neurons and found to be enriched in axon terminals. It has been suggested to regulate the neurotransmitter release[129,130]. In addition to familial PD, α-synuclein likely plays a role in sPD given that it is the main component of Lewy bodies and Lewy neurites and has been associated with the genetic risk of developing PD through genome-wide association studies. Therefore, many groups have dedicated considerable effort to generating transgenic mouse or rat models either overexpressing wild-type (WT) or mutant *SNCA* (A53T, A30P) to try and understand how α-synuclein impacts DA function and neuron survival (see **Table 2**). A plethora of α-synuclein transgenic mouse models have been developed over the years (see ref. [131], for a comprehensive review). The degree of pathology and motor impairments greatly depends on the genomic integration site, the promoter used to drive human SNCA transcription, and the genetic background. While some of these models cause accumulation of insoluble α-synuclein inclusion bodies[132–136], and some display deficits in DA vesicle clustering and DA neuron firing[137], only the recently characterized N103 mouse model results in degeneration of DA neurons in the SN[136]. Inclusion bodies also accumulate in brain regions other than the SN[134,136]. The lack of degeneration of the DA neurons in most of these transgenic models has made it difficult to determine if these models are successfully modeling specific aspects of early-onset PD as patients with *SNCA* mutations or duplications present with.

A novel transgenic mouse model overexpressing the A53T α-synuclein mutation in mice using the DAT promoter in tetracycline-regulated transgenic mice has also been generated. These mice develop motor deficits, which are associated with a loss of DA neurons in the SN. Interestingly, this pathology was associated with a decrease in DA release and impaired mitophagy[138].

There is a growing hypothesis, initiated by the work of Braak, who demonstrated that α-synuclein accumulation in PD begins in the enteric nervous system and traffics to the CNS via the vagus nerve. Braak hypothesized that α-synuclein from the gut reaches the vagus nerve during the early stages of PD and gradually

Table 2. Summary of genetic models.

Genetic Mouse Models	Species	Displayed Characteristics	Limitations
α-synuclein-based models			
Overexpression human WT α-synuclein (Thy-1, PDGF promoter)[246]	Mice	Widespread α-synuclein overexpression[246]	No TH+ neuron loss in dorsal SN
		Deficits in DA release[247]	Overexpression of SNCA may affect development
		Early & progressive sensorimotor deficits[248,249]	Motor deficits present at two-months
		Increased microglial reactivity in SN[250]	Can be used to model familial mutations of SNCA
		Progressive autonomic dysfunction[133]	Overexpression of SNCA doesn't exacerbate paraquat-induced SN DA loss[251]
		α-synuclein inclusions in the heart and enteric nervous system[133]	
Overexpression Human WT[137,252], A30P[252] α-synuclein (bacterial artificial chromosome promoter)	Mice	Widespread α-synuclein overexpression[137]	Can be used to model familial mutations of SNCA
		Modest SN DA loss and gait disturbances	No loss of SN DA neurons at 18-months[252]
		Deficits in DA release and DA neuron firing[137]	
		Alterations in DA vesicular clustering[137]	
		WT and A30P α-synuclein exacerbates MPTP effects[252]	
Point mutations (A53T prp[253], A30P Thy-1[254])	Mice	Severe motor deficits[253]	No loss of SN DA neurons[253,254]
		A30P α-synuclein	Widespread α-synucleinopathy in the brain stem and spinal cord[253,254]
		Progressive motor deficits and cognitive decline[254]	A30P tg mice used to model Dementia Lewy body
		Amygdala pathology[254]	Overexpression of A30P did not exacerbate TCE-induced SN loss of DA neurons[255]
		Modest SN loss of DA neurons[255]	
Parkin, PINK1, DJ-1 KO, VPS35 KI[256]	Mice	Progressive loss of DA SN neurons (VPS35KI)[167]	Used to study familial forms of PD
		Motor defects (VPS35KI)	No SN loss of DA neurons (Parkin, PINK1 KO)
		Tau positive pathology (VPS35KI)	Minimal brain pathology
			Lack of α-synuclein pathology
Parkin/PINK1/ DJ-1 KO[257]	Rats	PINK1 and /or DJ-1	Used to study familial forms of PD
Reviewed elsewhere[258]		Modest motor impairment	Parkin KO rats do not display α-synucleinopathy
		Increase in striatal DA and 5-HT content	
		Approximately, 50% loss of SN DA neurons	
		Mitochondrial dysfunction	
		α-synucleinopathy[259]	
		Parkin KO	
		Mitochondrial dysfunction and oxidative damage	
MitoPark[260]	Mice	Loss of SN DA neurons and striatal TH+ terminals	Does not recapitulate PD
		Accumulation intraneuronal inclusions	Short lifespan (~45 weeks)
		SN DA neuronal loss	
		Severe motoric deficits	
		Can be used to study mitochondrial dysfunction	

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Table 2. Continued.

Genetic Mouse Models	Species	Displayed Characteristics	Limitations
LRRK2-based models (reviewed elsewhere[261])			
Overexpression of human	Mice, rats	Progressive motor impairment[146,147]	Most transgenic models do not cause a reduction in SN DA neurons
WT, G2019S, R1441C/G		Accumulation of autophagosomes[147]	No α-synucleinopathy
		Impaired striatal DA release[146,148]	No GI dysfunction
		Cognitive deficits[146]	
Knock-in G2019S[149], R1441C	Mice	Progressive α-synucleinopathy[150]	No loss of SN DA neurons or striatal TH-intensity
		Dysfunctional DA release and DA transporters[150]	No motor impairments
		Increased LRRK2 kinase activity	
		Mitochondrial dysfunction[149]	
		G2019S mice have region specific mitophagic deficits[262]	
Overexpression of G2019S Adenoviral[263,264]	Rats	Modest SN loss of DA neurons	Technically challenging to generate stable adenoviral construct and can cause immunological response
		Dystrophic neuritic processes	Does not model all aspects of sPD
Other Animal models			
GBA1 D409V KI[151]	Mice	Reduction in glucocerebrosidase activity and accumulation of glycolipids	No loss of SN DA neuron, neuroinflammation, α-synucleinopathy
			No motoric phenotype

5-HT, 5-hydroxytryotamine; DA, dopamine; GI, gastrointestinal; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PDGF, platelet-derived growth factor; SN, substantia nigra; sPD, sporadic Parkinson's disease; TCE, trichloroethylene; TH, tyrosine hydroxylase; WT, wild type.

traffics from the hindbrain to the forebrain as the disease progresses[139]. In support of the Braak hypothesis, aged Fischer 344 rats display aggregated α-synuclein in the intestinal submucosal plexus[140]. Using an established weekly oral protocol for bacterial exposure[141] in these rats resulted in α-synuclein deposition in the myenteric plexus and submucosa and neuroinflammation and α-synuclein accumulation within the striatum and hippocampus[142]. Moreover, using Thy-1 h WT α-synuclein (antisense oligonucleotide) transgenic mice, researchers demonstrated that gut-brain signaling by gut-microbial molecules that impact neuroinflammation and α-synuclein aggregation is required for the hallmark motor and GI dysfunction observed in this mouse model of PD[143].

LRRK2

LRRK2 was identified as a monogenic cause of PD in 2004 and displays an autosomal-dominant inheritance pattern, but with incomplete and varying penetrance. In addition, *LRRK2* is considered a genetic risk gene for sPD. The G2019S mutation is the most common PD-associated mutation. Genetic mutations associated with PD cause an increase in LRRK2 kinase activity. Overall, transgenic mouse and rat models that overexpress or knock in (KI) a PD-related LRRK2 mutation (e.g., R1441C/G or G2019S) have been largely unsuccessful at replicating the hallmark features of PD (DA neurodegeneration and α-synuclein inclusion bodies)[144,145]. However, subtle changes have been observed in these models, including changes in dopamine metabolites and in mitochondrial and lysosomal functions[146–150]. Taken together, these studies suggest these transgenic mice may be useful to study gene × environment interactions as well as the functions of LRRK2, which may enable these models to be utilized to study late-onset PD. A summary of the characteristics of the most used models is shown in **Table 2**.

GBA1^{D409V} KI mice

Mutations in the *GBA1* gene, which encodes the lysosomal hydrolase glucocerebrosidase, are associated with sPD. Mutations in *GBA1* and *LRRK2* are considered as the highest genetic risk factors for developing sPD. With over 300 mutations in *GBA1* identified, sPD patients with a *GBA1 mutation* typically have a more aggressive form of the disease. Therefore, elucidating the role that GBA1 plays in sPD is crucial. Recently, a transgenic mouse model was developed in collaboration with The Michael J. Fox Foundation characterizing the *GBA1* D409V point mutation[151]. These mice have a dose-dependent reduction in glucocerebrosidase (GCase) activity in the hippocampus and SN[151,152]. Unfortunately, these mice lack α-synuclein accumulation in the nigrostriatal pathway and do not show any loss of DA neurons in the SN[151]. Mice with a heterozygous GBA1 D409V mutation were recently reported to have no overt phenotype and have unaltered spread of α-synuclein fibrils[153]. However, mice carrying an L444P mutation show increased susceptibility to MPTP[154], and A53T α-synuclein mice haploinsufficient for GBA show an exacerbated phenotype[155].

MitoPark mouse model

The MitoPark mouse model, initially described in 2007, consists of a selective deletion in the mitochondrial transcription factor M (TFAM) within DAT-positive (DAT$^+$) neurons[156]. This deletion results in mitochondrial dysfunction that is limited to DA neurons. Interestingly, despite this limited mitochondrial

dysfunction, MitoPark mice have characteristic features that resemble PD in humans, including a significant drop in mitochondrial gene expression (within six weeks after birth), motoric deficits, and nigrostriatal DA degeneration[156–158]. As the expression of the mutation is restricted only to DA neurons, the utility of this animal is limited. However, deficits in non-DA systems involving circadian rhythms[159] and GI motility have been reported[160]. The most significant limitation of this model is the drastically shortened lifespan of 45 weeks (11 months). This shorter lifespan, while useful for therapeutic investigations, may not fully capture mechanisms driving the slow progression of the age-related disease phenotype in human PD, which raises the concern of failures in subsequent clinical human trials of therapeutic interventions developed using this model. However, this model may have utility in investigating specific mechanisms involved in early-onset PD which are yet to be explored.

VPS35 mouse model

The vacuolar protein sorting 35 (*VPS35*) gene encodes the cargo subunit of the retromer complex. Due to its essential function in regulating protein breakdown and recycling, it has been implicated in numerous neurodegenerative diseases[161]. VPS35 and the retromer are essential for normal cellular function and viability; full deletion in mice results in embryonic death by day 10[162]. Mutations in the *VPS35* gene cause an autosomal-dominant form of PD (*PARK17*) with clinical symptoms comparable to those observed in sPD[163,164]. In particular, a single heterozygous missense mutation, Asp620Asn (D620N), is pathogenic with ~1.3% frequency in familial cases and 0.3% in sPD[165,166]. Various *in vivo* models have been generated to study the D620N mutation on VPS35 function and PD pathology[161]. The D620N mutation results in either a toxic gain-of-function or a dominant-negative mechanism, or possibly a combination of both. The phenotypic assessment of a germline D620N *VPS35* KI mouse model reported neuropathological hallmarks of PD, including age-related motor defects, progressive degeneration of SN DA neurons, increased DA release, and widespread axonal damage and tau-positive (hyperphosphorylated) pathology throughout the brain[167,168]. However, these mice fail to develop the α-synuclein neuropathology characteristic of PD. This is surprising, as a direct relationship between VPS35 dysfunction and α-synuclein accumulation has been established[169]. In addition, the D620N VPS35 KI model also failed to show enhanced α-synuclein pathology when crossed with human A53T-α-synuclein transgenic mice or mice injected with α-synuclein PFFs[167].

DJ-1/PAK7 mouse model

DJ-1, a small (20 kDa), highly conserved protein of 189 amino acids, was linked to early-onset, familial types of PD in 2003[170,171]. DJ-1 is well recognized for its role as an oxidative stress sensor; in addition to PD, DJ-1 is implicated in other age-related disorders such as cancer and type 2 diabetes[172–174]. Even though DJ-1 KO mice display age- and task-dependent motoric deficits, including hypoactive behavior in the open field assay and deficits in adhesive tape removal coupled with striatal neurotransmission deficits, these mice fail to show SN DA neurodegeneration[175,176]. There are conflicting data in the literature regarding the age-dependent accumulation of markers of oxidative stress in these mice[177,178]. Intriguingly, when a subgroup of DJ-1-KO mice were fully backcrossed onto a C57BL/6 background, they showed a severe early-onset (eight-week) unilateral loss of SN DA neurons but not VTA DA neurons, which gradually progressed to bilateral nigrostriatal degeneration at later ages. This age-dependent loss of SN DA neurons was accompanied by a loss of DA neurons in the locus coeruleus (LC) as well as modest motor deficits at specified time periods[179]. In summary, even though loss-of-function mutations in DJ-1 cause familial PD, current transgenic rodent models failed to find integral neuropathological changes reminiscent of PD. It is possible that the shortened lifespan of mice in comparison to humans can explain the absence of profound SN DA neurodegeneration; investigation of cellular mechanisms in these mice well before death may contribute to our knowledge of the mechanisms leading to early-onset PD in humans.

PINK1/Parkin mouse model

The PTEN-induced kinase 1 (PINK1), a serine threonine kinase, and Parkin, an E3 ubiquitin ligase, work in coordination to target mitochondria for autophagic degradation via a process known as mitophagy. Since the discovery that autosomal recessive mutations in the *PARK2* (Parkin) and *PARK6* (PINK1) genes cause early-onset PD in humans, multiple groups have generated systemic KO mouse models of these genes[180–183]. Parkin models target different exons of the Parkin gene. The first transgenic animal model was a systemic Parkin KO (premature stop codon inserted into exon 4) mouse, which displayed slight motor/behavioral deficits, increased extracellular DA, abnormal mitochondrial respiration rates, and higher oxidative damage within SN mitochondria[184,185]. They do not, however, display the characteristic loss of DA neurons in the SN. Similar findings were reported for subsequent systemic Parkin KO models targeting exons 2, 3, and 7, wherein they caused modest motor impairments without concurrent loss of SN DA neurons[186,187]. It should be noted that the Parkin KO mouse model targeting exon 7 displayed a loss of NE in LC neurons in both young and older animals[188,189]. Even though knocking out Parkin in rodents does not result in significant DA neuron loss as seen in PD patients with a recessive Parkin mutation, these transgenic models are still valuable to study the role mitophagy and mitochondrial dysfunction play in PD, in particular in relation to early-onset PD caused by *Parkin* mutations. PINK1 KO rats showed progressive neurodegeneration with about 50% DA cell loss observed at eight months of age and a two- to threefold increase in striatal DA and serotonin content at eight months of age. These mice also exhibited significant motor deficits starting at four months of age. Interestingly, the Parkin KO rats displayed a normal phenotype without any neurochemical or pathological changes.

Mice homozygous for the PINK1 null allele are viable, and, similar to the Parkin models, they do not exhibit a loss of striatal DA content or DA neurons[190,191]. However, PINK1 KO mice exhibit diminished DA release and other alterations in striatal DA neuron physiology[192]. In addition, loss of PINK1 resulted in reduced mitochondrial function and Ca^{2+} storage capacity in mice[193]. In an attempt to better understand and replicate the disease pathology, systemic PINK1 KO models were genetically crossed with other familiar PD genetic models. Unfortunately, the triple combination cross consisting of systemic knockout of DJ-1, PINK1, and PARKIN also did not show DA neurodegeneration or loss of LC neurons[194]. Genetic crossing of the PARKIN KO with a transgenic α-synuclein model resulted in mitochondrial abnormalities; however, these mice did not experience DA neurodegeneration[195]. Adeno-associated viral-mediated overexpression of α-synuclein in the SN of PINK1 KO mice was found to result in

enhanced DA neurodegeneration as well as in significantly higher levels of α-synuclein phosphorylation at serine-129 at four weeks postinjection in comparison to adeno-associated virus (AAV)-α-synuclein injected mice[196].

Regulator of G protein signaling 6 (RGS6)-deficient mice

RGS6 is a member of the RGS protein family and is required for SN DA neuron survival in adult mice[197]. RGS6 KO mice display an age-dependent loss of DA neurons in the striatum and α-synuclein accumulation. This loss of nigrostriatal neurons correlates with motoric deficits[198].

A summary of the main features of genetic models of PD is described in **Table 2**. A schematic representation of PD-associated genes and their mutational variants used to generate disease models is shown in **Figure 1**.

α-Synuclein Proteostatic Models

A summary of the main α-synuclein proteostatic models is shown in **Table 3**.

Viral-vector-mediated animal models

α-synuclein overexpression can be induced by viral vectors. Depending on the serotype, promoter, titer, and time of incubation, viral-mediated overexpression of WT or mutant human α-synuclein results in a progressive loss of DA neurons over the course of 8–24 weeks[199–203]. There are several advantages to

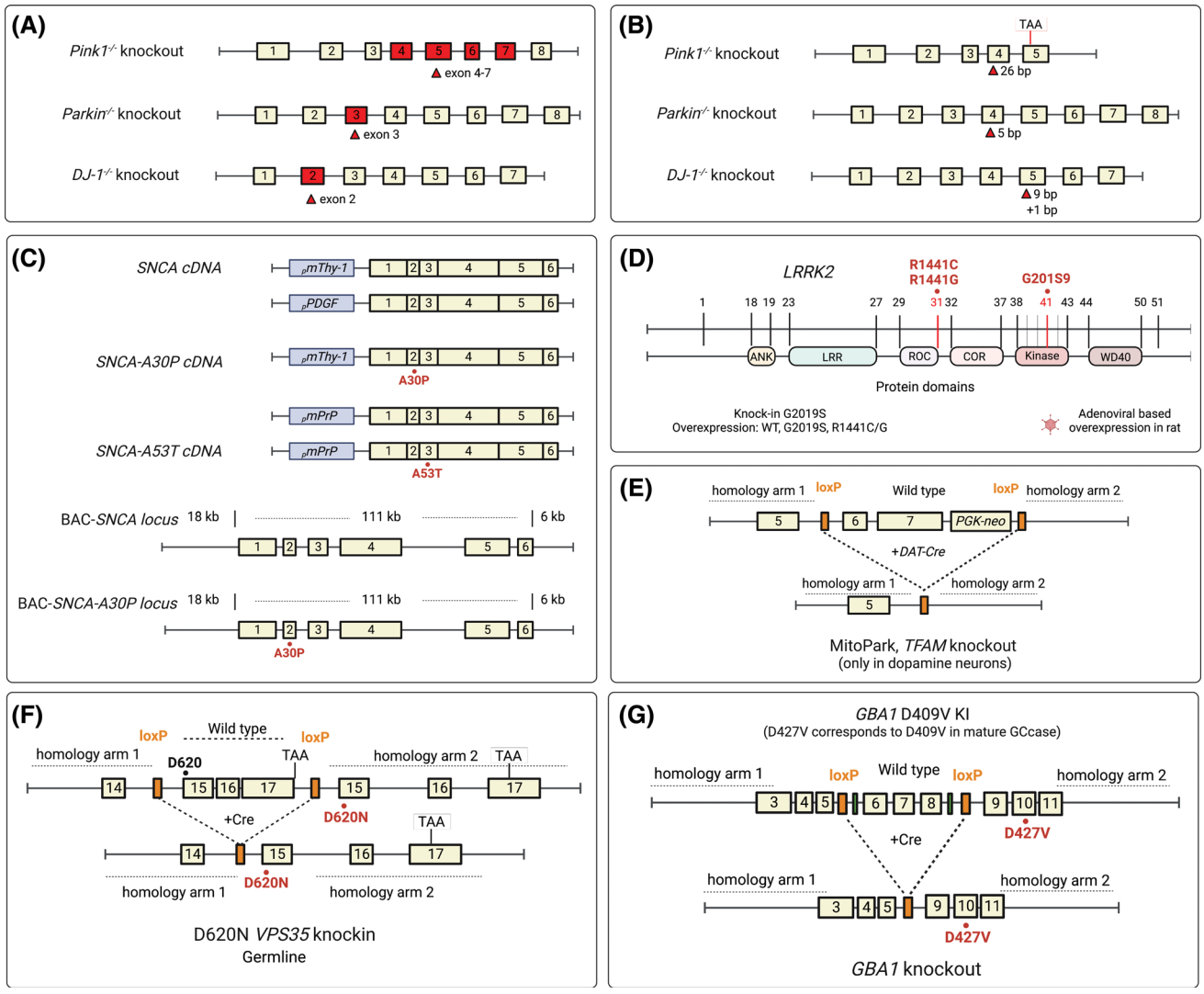

Figure 1. Schematic representation of Parkinson's disease-associated genes and their mutational variants used to generate disease models (see Table 2). *PINK-1*, *Parkin*, and *DJ-1* variants used for knockout generation in (A) mice (B) rats. (C) Overexpression of human α-synuclein (*SNCA*) and variants under control of promoters: mouse thymus cell antigen 1 (*mThy1*), platelet-derived growth factor (PDGF), and mouse prion protein (mPrP). (D) Overexpression of human *LRRK2* and variants: G2019S and R1441C/G. (E) Dopamine transporter (DAT)-cre mice (recombinase expression only in dopamine neurons) and mice with a loxP-flanked mitochondrial transcription factor A (*Tfam*) allele were crossed to produce MitoPark mice. (F) The conditional D620N knock-in (KI) mice were developed by replacing endogenous exon 15 (with a D620N mutant version) and introducing a loxP-flanked wild-type (WT) minigene with VPS35 exons 15–17. Upon Cre-mediated recombination D620N VPS35 is expressed from the endogenous allele. (G) The glucocerebrosidase (*GBA1*) D409V KI mutation was introduced in the mice Gba1 gene through the constitutive KI of a Gba1 D427V point mutation, as the D427V mutation corresponds to the D409V mutation in the mature GCase protein. An additional feature of this model is the insertion of loxP sequence flanking exons 6–8, which after Cre recombination allows constitutive knockout of *GBA1*.

Table 3. Summary of α-synuclein proteostatic models.

Proteostatic Models			
Viral transfection of α-synuclein (AAV and lentiviruses) Reviewed elsewhere[265,266]	Mice, rats, NHP	Extent of α-synucleinopathy is dependent on serotype	Vector toxicity
		Progressive accumulation of α-synuclein aggregates in SN DA neurons	Transduction efficiency can vary
		Progressive SN loss of DA neurons	Packaging capacity is ~4.7 Kb, roughly half the packaging limits of lentiviral and adenoviral vectors
		Motor deficits	Cannot be used to study all aspects of sPD
Exogenous α-synuclein preparation (preformed fibrils[267,268], brain extracts) reviewed elsewhere[206]	Mice, rats, and NHP	Progressive Lewy-body like pathology	Challenging to generate pure preformed fibrils.
		Modest and progressive neuronal loss	Different PFF strains cause different biological effects.
		Behavioral deficits on rotor rod	Validation of successful preparation is crucial
		Increased neuroinflammation[269]	
		Non-CNS injection can cause widespread brain pathology	
		A good model to study α-synuclein propagation[212,270]	

AAV, adeno-associated virus; CNS, central nervous system; DA, dopaminergic neurons; NHP, nonhuman primate; PFF, preformed fibrils; SN, substantia nigra; sPD, sporadic Parkinson's disease.

using a viral vector system over creating a transgenic mouse line. This approach can efficiently deliver genome particles to mature neurons and avoid any developmental remodeling. It is also possible to selectively target specific cell types (e.g., glia vs. neurons), depending on the promoter and the vector used. Finally, this approach can be applied to aged animals. AAV vectors are typically injected unilaterally, which allow the uninjected hemisphere to be used as an internal control.

As with any animal model, viral-vector-mediated overexpression of α-synuclein has challenges. Viral-mediated overexpression of α-synuclein does produce reliable DA neurodegeneration and α-synuclein inclusion bodies. However, this approach does require a specialist technique and can be time-consuming. Verification of the injection site for every animal is necessary. Inserting a fluorescent reporter protein (e.g., green fluorescent protein [GFP]) into the construct can help verify the injection site, but it can also be toxic to DA neurons and cause phenotypic suppression of TH[204]. It is possible to avoid the use of a fluorescent tag by using an empty vector[204,205]. However, this approach does not allow control for nonspecific toxicity due to protein overload.

PFF animal models

Another approach to study α-synuclein is the administration of exogenous α-synuclein PFFs typically into the striatum, or SN, which is referred to as seeding (reviewed in ref. [206]). The α-synuclein PFF model relies on manual injection(s) of the recombinant form α-synuclein protein. PFFs are aggregates that have been sonicated to produce short fibrils—50 nm or smaller will yield most pathology; anything larger will greatly reduce the pathology. This protocol reliably causes the templating of endogenous WT α-synuclein into pathological species characterized by phosphorylation at S129 (pS129 α-synuclein), beta-sheet formation, and aggregation. One of the advantages of this model is that it allows for flexibility, meaning different forms of α-synuclein PFFs can be introduced (e.g., mouse vs. human α-synuclein or mutated α-synuclein), targeting any desired brain region(s) or peripheral organ. This allows the researcher to model distinct aspects of PD. The uses of the PFF model in PD have been extensively reviewed elsewhere[207]. Essentially, the presence of either human or rodent α-synuclein PFFs triggers endogenous α-synuclein phosphorylation, ubiquitination, and aggregation and results in a prion-like propagation of α-synuclein inclusions that can result in retrograde nigrostriatal (from the striatum injection site to the cell bodies in the SN) DA neuronal degeneration, neuronal dysfunction, and mitochondrial damage typically over a three- to six-month period[208,209]. More recent studies have administered PFFs in other areas of the body, including muscle[210], gut[211], and olfactory bulb[212,213]. These alternative routes of administration resulted in CNS α-synuclein pathology, neuroinflammation, and, in some cases, neurodegeneration. The extent of neuronal dysfunction and loss is dependent on the site of administration of the PFFs and the species injected[214]. This approach is a useful tool to study how α-synuclein contributes to the pathogenesis of PD and is a good model to test compounds designed to prevent α-synuclein aggregation. This model has been used to study the progressive maturation of α-synuclein inclusions within individual neurons over time and the selective degeneration of these inclusion-bearing neurons[215]. The PFF models provide an elegant way of modeling late-onset PD, or similar to sPD.

A summary of the main features of α-synuclein proteostatic models of PD is described in **Table 3**.

Study of Aging in Mammalian Models of PD

Although no existing models of PD display all the cardinal features of PD, and their characterization is currently inconsistent or

incomplete, some models do display a progression of the disease with age (Tables 4–7). Some of these aging models were described in the sections above and have been characterized by several laboratories around the world. However, other aging models are not utilized by many laboratories, likely the reason their PD phenotype has not been fully characterized. We have included these additional animal models (namely Mito-PstI, a mitochondria-targeted restriction enzyme, PstI to damage mtDNA in DN; truncated FLAG-tagged human mutant Parkin [Parkin-Q311X] in DA neurons; L61 mice overexpressing WT human α-synuclein under the Thy-1 promoter; and inducible [DOX] human MAO-B expression in astrocytes) in this study, as they are important for building a picture of the current state of aging research in PD animal models. Both C57BL/6 mice and rhesus monkeys show signs of PD with natural aging[13,216]. In WT C57BL/6 mice, significant changes occur at 120 weeks of age[216], suggesting that signs may develop slowly at later ages (see Table 4). Differences in phenotypes seem to be more prominent in models where it is possible to see the slow progression of the disease and when animals are monitored for longer periods of time. As an example, Kim et al. (2019) injected PFFs at 3 months of age, and mice were assessed at 1, 3, 7, and 10 months afterward (Table 7)[217]. Mice showed a reduction in the number of TH^+ neurons only at 10 months[217]. However, when PFFs were injected at

Table 4. Summary of studies conducted with natural aging organisms.

Natural Aging/Specie	Sex	Age	Age-Related Effects Observed
C57BL6 mice[216]	N/A	60, 80, and 120 weeks	At 120 weeks
			↓ Locomotor function (rotarod, beam test)
			↓ TH + Neurons (most prominent in VTA)
			↓ DA content in the striatum
			↑ Fragmented mitochondria
Rhesus monkey[13]	F, M	9–10, 14–17, and 22–29 y	↓ TH intensity in the ventral midbrain with age
			↑ Ubiquitin-positive inclusions with age
			↓ Lysosome function with age
			↑ Neuroinflammation with age

DA, dopamine; F, female; M, male; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

Table 5. Summary of the study on the role of aging in neurotoxic models.

Toxic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
Lipopolysaccharide-induced PD[271]	C57BL/6 mouse	F	10–12 weeks and 15 months	↓ Coordination and balance starting at 10–12 weeks old
				↓ SN DA neurons starting at 10–12 weeks
				↑ Neuroinflammatory pathways (TLR2, p-NF-kB/p65, TNF-α and IL-1β) in brain of aged mice
				↑ Microglia activation
				Aging contributes to severity
Paraquat and neonatal iron exposure[272]	C57BL/6 mouse	M	2, 6, 12, and 24 months	↓ SN DA neurons, which is more pronounced at 12 and even more at 24 months of age. No change with age in saline group
Mouse $LRRK2^{R1441G}$ exposed to rotenone[273]	C57BL/6 N; homozygous knock-in mice	M	Rotenone starts at 30 weeks for further 50 weeks	↓ Locomotor activity: distance moved, movement duration, and rearing frequency with age in combined rotenone and genetic mutation mice
				↓ Striatal mitochondrial complex-I (NDUFS4) in rotenone-treated mutant with age
				No difference in the number of SN TH+ cells in all groups at 50 weeks
Chronic MPTP model (subcutaneous administration of low doses of MPTP for 3 months)[220]	C57BL/6 N mouse	M	2–3 and 12–14 months	↓ SN TH+ neurons accelerated in aged mice (higher levels after 1 month in aged animals compared to young animals treated with MPTP)
				↑ Neuroinflammation

(*Continued on next page*)

Table 5. Continued.

Toxic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
				↑ Motor deficits (accelerated in aged mice) without any sign of mortality or adverse side effects.
				No difference in α-synuclein (only assessed at in young mice)
Ercc1$^{\Delta/+}$ (one mutated Ercc1 allele) + MPTP[224]	FVB:C57BL/6 J (50:50) mouse	N/A	Starting age N/A	↓ in TH+ cells in SN more pronounced in mutant
			Mice analyzed 3 days post injection	↓ DA innervation in the striatum
Unilateral injection of 6-OHDA into the medial forebrain bundle[274]	Wistar–Han rats	F	Injection in 10 weeks and 17 months old rats. Assessment 14 weeks later	Comparing effects in young and old
				No difference in turning behavior and degree of forelimb use asymmetry
				↑ Impairments of skilled motor function (the staircase test) in old rats
				↑ TH+ cell loss in the SN in aged rats
				No difference in TH densitometry in the striatum
Rotenone (intraperitoneal injection)[275]	Sprague–Dawley rats	M	Injection at 3- or 18-month-old rats	No changes observed in young rats
				In aged rats
				↑ Behavior abnormality and striatal dopamine depletion
				No significant change in striatal serotonin level
				↑ SN malondialdehyde
				↓ Glutathione
Rotenone (subcutaneous injection)[276]	Wistar rats 2BAW	F	4–5 and 24–25 months	Both groups showed
				↑ Swollen mitochondria in the striatum
				↑ Massive lipofuscin deposits in the substantia nigra pars compacta
				↓ Mobility impairment
				↓ Dopaminergic neuron
Unilateral MPTP injection (via internal carotid artery)[13]	Rhesus monkeys	F	Injection at 8–9, 14–17, and 26.5–31 y doses of MPTP were different in the age groups	↑ Glial reactivity in all ages and all DA subregions
				↑ DA neurons degeneration
				No age-related changes in astrocyte number detected in either side of the midbrain
				This study does not allow for any age comparison as the dose of MPTP was adjusted depending on the age group.
Unilateral MPTP injection (via intracarotid MPTP)[13]	Rhesus monkeys	F	Injection at 8–9, 15–17 and 21–31 y for 3 months MPTP dose differs with age groups	↓ In striatum dopamine and homovanillic acid with age
				No difference in TH+ neurons in SN
				This study does not allow for any age comparison as the dose of MPTP was adjusted depending on the age group.

6-OHDA, 6-hydroxydopamine; DA, dopamine; F, female; M, male; MPTP, 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; SN, substantia nigra; TH, tyrosine hydroxylase.

16 months of age and the number of TH^+ cells was assessed 4 months later, no differences in TH+ cell numbers were observed[218].

Very little difference has been observed between young and old animals in some models when the disease is induced by genetic modification (e.g., L61 mice which overexpress WT α-synuclein via the Thy-1 promoter; **Table 6**)[219] or by injection of neurotoxic molecules (e.g., MPTP)[220]. This is likely because induction is very aggressive, and the disease develops over a very short period of time. Mice dosed with a low dose of MPTP for three months

Table 6. Summary of the study on the role of aging in genetic models.

Genetic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
Mito-PstI (Mitochondria-targeted restriction enzyme, PstI to damage mtDNA in DN)[277]	C57BL/6 J mouse	M	4 and >12 months	4 months
				Poor coordination by pole test (not rotarod) reversible with L-DOPA treatment
				↓ DA content
				↓ Striatal DA content, TH and DAT
				12 months
				Persistence and further aggravation of loss in striatal DA
				Too few TH+ cells to count
Truncated FLAG-tagged human mutant Parkin (Parkin-Q311X) in DA neurons[278]	FVB/NJ mouse	F, M	6, 12, 16, and 20–21 months	Most signs appear at 16 months
				↑ DA neuron degeneration in SN
				↑ Loss of DA neuron terminals in the striatum
				↑ Proteinase K-resistant endogenous α-synuclein in SN
				↓ Striatal dopamine level with Hypokinetic motor deficits
L61 (mice overexpressing wild-type human α-synuclein under the Thy-1 promoter)[219]	C57BL6/DBA2 mouse	F, M	3, 6, 9, and 12 months	Most signs already present at 3 months
				↑ α-synuclein oligomers in the brain (observed in both sexes, higher in male from early age)
				↑ Severe behavioral phenotype (in males) with hyperactivity and thigmotaxis in the open field test
				↑ Hind limb clasping and hyperactivity
Inducible (DOX) human MAO-B in astrocytes[279,280]	C57BL/6 mouse	-	6 and 14 months	Effects observed at 14 months
				↓ Behavioral tests; ambulatory function (movement, resting and stereotypy), Hindlimb clasping
				↑ TH+ neuron loss
				↓ DA content in the striatum
Homozygous mutant human A53T-α-synuclein under prion promoter[281,282]	C3H/C57BL/6 J-F1 mouse	-	2, 4, 8, and 12 months	2 and 4 months
				↓ Locomotor activity (open field test)
				No difference in grip strength, rotarod, wire hang test latency to fall
				↑ α-synuclein accumulation and aggregation in the striatum (total α-synuclein and endogenous synuclein proteins)
				↑ Anxiety-like and depressive-like behavior (thigmotaxis and aversion for elevated or open spaces)
				12 months
				↓ Wire hang test latency to fall

(*Continued on next page*)

Table 6. Continued.

Genetic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
				↓ ↓ Locomotor activity (open field test)
				↑ ↑ α-synuclein accumulation
				↓ Number of TH+ neurons in SN
Inducible (DOX) glutathione depletion in TH neurons mouse[283]	Antisense γ-glutamyl cysteine ligase—C57BL/6 mouse	-	3 and 12 months	↓ Mitochondrial complex I activity in DA neurons at 3 and 12 months
				↓ DA levels in the striatum of 12 months old mice but not at 2 months
				↓ TH+ neurons at 12 months
Expression of α-synuclein 1-103 (N103 mouse)[136]	Human α-synuclein 1-103 gene prefixed with Thy1 promoter, C57BL/6 mouse	M, F	3, 9, and 16 months	3 months
				Constipation
				α-synuclein accumulation in SN, striatum, pons
				No other sign
				9 months
				α-synuclein 103 accumulation extends to cortex
				↓ N TH+ cells
				↓ Synaptic density, DOPA, DOPAC, HVA in striatum
				↓ Locomotor function
				16 months
				Aggravation of
				α-synuclein 103 accumulation, Neurodegeneration Locomotor function
ASYN(d)/Nurr1+/− (2-hit) mouse[223]	Nurr1−/+ X human A53T α-sinuclein homozygote (prion promoter) 129SV x C57BL6/J	N/A	6, 9, 12, 15, and 22 months	↓ Spontaneous locomotor function at 6 months but not at 9 and 15 months
				↓ Stride length at 15 months
				Progressive loss of locomotor function to severe at 12–22 months
				↓ Lifespan
				↓ Number of SN TH+ cells 12 and 18 months
				↑ α-synuclein accumulation at 12 and 18 months
*VPS35 (*vacuolar protein sorting 35) D620N knock-in (KI) mouse[284]	B6(Cg) - Vps35tm1.1Mjff C57BL6/J mice	M, F	6, 9–10, and 14–16 months	Absence of constipation
				Signs of disease started at 14–16 months
				↓ Locomotor function (walking speed, total distance traveled, rotarod time to fall, grip strength)
				↓ N SN TH+ cells and DA content in the striatum
				↑ α-synuclein accumulation
				↑ Neuroinflammation (astrogliosis)
				↑ Mitochondrial fragmentation
DJ-$1^{-/-}$ (DJ1-C57)[179]	C57BL/6 J mouse	F, M	2, 4, 6, and 14–16 months	2 months
				Unilateral loss of TH+ neurons in dorsal SN

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Table 6. Continued.

Genetic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
				No motor deficits
				14–16 months
				Bilateral loss TH+ neurons in SN and LC
				Mild motor behavior deficits
DJ-1 null (9.3 kb deletion including first 5 exons)[175]	129-C57BL6/J mouse	F, M	6 and 11 months	↑ Effect on male mice in adhesive tape removal task at 6 months
				No effect in NOR and rotarod at any age
				No age-related dopamine neuron loss in SN
				No increase in α-synuclein
DJ-$1^{-/-}$ (exon 2 deletion)[285]	129-C57BL6/J mouse	N/A	3 and 12 months	No reduction in the number of SN pars compacta DA neurons at any age
				No increased in α-synuclein at any age
				Abnormalities in parameters of SN dopaminergic physiology
Mitochondrial transcription factor A (Tfam) deletion in dopamine neurons (MitoPark)[156,286]	*+/DAT-cre, Tfam^{loxP}/Tfam^{loxP}* C57BL6 mouse	F, M	6–20 weeks (do not survive beyond 45 weeks)	15 weeks
				↓ Locomotion
				↓ Exploring behavior
				↓ Number of TH+ cells
				Altered basal electrophysiological parameters
				↓ TH immunoreactivity
				↓ Cell capacitance in dopamine neurons
				↑ Age-dependent increase in input resistance
				↓ Dopamine neurotransmission
				↓ Pacemaker firing and associated ion channel currents
				20 weeks
				Progressive deterioration of locomotor behavior
				Presence of tremor, limb rigidity, twitching
Regulator of G protein signaling 6 (RGS6) knockout/ RGS6$^{-/-}$[198]	129/Sv × C57BL6 mouse	F, M	3, 9, 12, and 18 months	3 months
				↓ DA levels at 3 months with ≥ 50% ↓ within the SN but no other sign
				12 months
				↓ DA levels in SN and striatum
				↑ PD-like motor dysfunction (rotarod, open field locomotion, hind limb stride length and frequency) partially reversed by L-DOPA
				18 months
				↑ Accumulation of aberrant α-synuclein
Pink1−/− (G309D-PINK1 mutation)[287]	129/svEv mouse	F, M	4, 16, 18, and 22 months	9 months
				↓ DA content persistent at 22 months
				16 months

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Table 6. Continued.

Genetic Model	Genetic Background	Sex	Age	Age-Related Effects Observed
				↓ Spontaneous locomotor activity,
				No difference in anxiety (open field analysis)
				No difference in grip strength, coordination (rotarod).
				18 months
				No difference in hyperhidrosis assay and acoustic startle tests (early feature of sporadic PD)
				No loss of DA neurons
				↑ Dopaminergic synapse dysfunction and prominent mitochondrial dysfunction
				↓ Mitochondrial preprotein impor
Ercc1−/− deletion in dopamine neurons[224]	*DAT CRE^{loxP}* FVB: C57BL/6 J (50:50) mouse	N/A	26 and 52 weeks	↓ TH+ cells in SN progressively with age
Ercc1$^{\Delta}$/$^{+}$ (One mutated Ercc1 allele)[224]	FVB:C57BL/6 J (50:50) mouse	N/A	20 weeks	↓ DA striatal innervation in Ercc1$^{\Delta}$/$^{+}$ but not in wild type
				↑ α-synuclein (S129p) in SN
				↑ Astrocytosis in SN and striatum
				No reduction in TH + DA neurons

DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; F, female; HVA, homovanillic acid; L-DOPA, levodopa and l-3,4-dihydroxyphenylalanine; LC, locus ceruleus; M, male; NOR, novel object recognition; SN, substantia nigra; TH, tyrosine hydroxylase.

Table 7. Summary of the study on the role of aging in proteostatic models.

PD/Aging Model	Genetic Background	Sex	Age	Age-Related Effects Observed
Injection of preformed fibril in duodenum and pilorum (im)[217]	C57BL6/J mice	M, F	Injected at 3 months and follow up at 1, 3, 7, and 10 months postinjection	1 month
				Accumulation of α-synuclein (S129p) in dorsal motor nucleus of the vagus, medulla oblongata
				3 months
				Accumulation of α-synuclein (S129p) in amygdala, SN
				No difference in the number of TH+ cells in SN
				7 months
				Accumulation of α-synuclein (S129p) in hippocampus
				↓ TH+ neurons in SN
				↓ Locomotor function
				10 months
				↓↓ TH+ neurons in SN
				↓ DAT, DOPAC, HVA in the striatum
Injection of preformed fibril in duodenum (im)[218]	C57BL6/J mice	M	8–10 weeks to 16 months and observed up to 120 days from injection	8–10 weeks
				Transient Inflammatory response
				↑ α-synuclein (S129p) in enteric intestinal neurons
				No difference in α-synuclein (S129p) in SN
				No loss of locomotor function
				16 months

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Table 7. Continued.

PD/Aging Model	Genetic Background	Sex	Age	Age-Related Effects Observed
				↑ α-synuclein (S129p) in enteric intestinal neurons
				↓ GI function
				↑ α-synuclein (S129p) in Brainstem
				Persistent sensory motor deficit > 120 days post injection
				No difference in the number of TH+ cells in SN
				↓ DA content in the striatum
Artificial preformed fibrils (mouse and human injected in the upper gastrointestinal tract)[288]	Wild-type Fischer 344 rats	-	Injected at 3, 10–12, and 18 months and culled at 10 and 20 weeks postinjection	↑ Stereotypic propagation of α-synuclein pathology along the gut-brain axis in wild-type hPPF-seeded rats with age
				↑ Vagal denervation in the stomach and sympathetic cardiac denervation
				↑ Density and more proteinase K resistant phosphorylated α-synuclein
				↑ Or similar pathology in hPFFs injected old rats compared to mPFFs injected young rats, suggesting aging lowers species barrier
AAV expressing human tyrosinase (unilateral injection right SN part compacta)[289]	Sprague–Dawley rats	M	Up to 24 months postinjection	Age-dependent accumulation of neuromelanin
				↓ Number of TH+ neurons in SN with age
				↓ Striatal DA content with age
				↓ Release DA in the striatum with age following electrical stimulation
				↓ DAT striatal density
				Microgliosis with age
				↑ Marinesco bodies and LB-like with age
Mutant α-synuclein (A53T) injection into SN[290]	Rhesus monkeys	M	Injection at 2, 8, and 22 months for 8 weeks then cull	↑ Accumulation of A53T in neurites with age
				↑ Reactive astrocytes and axonal degeneration with age

AAV, adeno-associated virus; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; F, female; GI, gastrointestinal; HVA, homovanillic acid; hPFF, human preformed fibril; im, intramuscular; LB, Lewis body; M, male; mPFF, mouse preformed fribril; PD, Parkinson's disease; SN, substantia nigra; TH, tyrosine hydroxylase.

and examined one to three months post-MPTP injection, exhibited an age-dependent loss of the number of TH^+ cells, which was significant at two and three months postinjection in young mice. However, older mice were significantly more affected by chronic low-dose exposure to MPTP, which resulted in a significant loss of DA neurons even at one month postinjection[220]. In the rhesus monkey, a lower dose of MPTP was used in old animals in comparison to a dose used in young, invalidating any comparison[221,222] but the fact that the authors decided to use a smaller dose may suggest that older NHPs may be more sensitive to MPTP. Some studies suggest that a combination of factors including aging may be required. For example, in the two-hit genetic model, where transgenic mice overexpressing human A53T α-synuclein under the prion promoter were crossed with *Nurr* +/− mice (ASYN(d)/*Nurr1*$^{+/-}$) and (ASYN(d) homozygote transgenic mice), only the combination of these two factors together yielded a phenotype with age with different phenotypes manifesting at different ages (**Table 6**)[223]. Similarly, using the accelerated aging model (*Ercc1*$^{\Delta/+}$ model) of a human progeroid syndrome and a low dose of MPTP caused a loss of TH^+ DA neurons in the SN, which was not observed in vehicle-treated transgenic mice[224] (**Table 5**). It is of interest that no substantial PD phenotype is observed in *Ercc1*$^{-/\Delta}$ mice, suggesting that aging may have a more systemic influence in PD and that the very aggressive aging phenotype in the *Ercc1* mouse brain is not sufficient to produce PD. Parkin KO mice crossed with mice harboring a mutation in *Polγ* encoding the mitochondrial polymerase, which causes mitochondrial dysfunction, result in a significant loss of SN DA neurons[189]. A recent study conducted in aged mice (over 2 y of age) did show motor deficit and DA neuron loss in conjunction with mitochondrial fragmentation, indicating the importance of aging in PD pathogenesis[225].

The effect of age may be subtle and develop over a long period of time, working synergistically with other triggers. This is not

surprising and reflects what is seen in individuals with PD, where the disease develops over four to six decades with many contributing factors including genetic predisposition, exposure to environmental toxins, immune/inflammatory factors, and aging biology. The fact that there is a correlation between aging and the clinical manifestation of PD does not mean that aging is causal to the disease, but it may be a substantial risk factor. In the future, more mechanistic studies that incorporate aging in the established animal models of PD more may provide insight into the underlying causes of PD. Indeed, mice injected with PFFs at 8–10 weeks and at 16 months and analyzed 120 days postinjections, clearly showed that older animals are more severely affected[218]. This supports the hypothesis that aging contributes to the severity of the disease. Models of accelerated aging and longevity can be used to determine whether PD-like pathology can be accelerated and decelerated and further elucidate the underlying systemic biology that contributes to PD.

Barriers to the Study of PD and Aging

The study of aging biology in animal models is challenging. An obvious and major constraint to using aged animal models is the length of time required to age them (average 22–24 months for mice and 36 or more for rats; NHPs vary from 3 to >40 y)[226,227] and the specialized knowledge of the welfare of aged animals. Rodents are the preferred mammalian models as they are smaller, cheaper to maintain, and pose less ethical issues. Most knowledge available at the interface of aging and PD is from studies in mice, but even mice require a level of knowledge and infrastructure only available in labs specialized in aging research. For example, experimental design requires knowledge of attrition rates due to increased rates of death after 18 months of age, which is different in each laboratory and for each strain and may result in experiments that are underpowered. Behavioral assays require modification in aged animals to account for decreased resilience, vision, and hearing (strain-dependent) and increased variability in response. Laboratory personnel need training to ensure the use of humane endpoints appropriate for aging physiology. For example, signs of a rough hair coat are not considered as a sign of ill health in aged mice in the same way they are in young mice. Animals require weekly health checks after 18 months of age, demanding greater staff time.

The length of time it takes, the high level of monitoring and care for aging stocks, and the variability in response lead to the necessity for the use of larger cohorts of animals. This means that every experiment is a major investment in time and funding, with the risk of failure having the potential to negatively affect the output of researchers and their career progression. This discourages investigators from undertaking this type of research. Models of accelerated aging have been used to reduce the duration of experiments[228] but to date they have tended to be genetically modified in a constitutive manner, which leads to developmental defects as well as to accelerated aging. This is a problem because mechanisms driving tissue development are often different from those driving aging, making it difficult to dissect the contribution of each to various disease-related phenotypes[228]. For example, DNA repair is important at both the early developmental stages, where accumulation of DNA damage lesions can have important effects on the formation of a functional nervous system[229], and with aging leading to neurodegeneration. An understanding of which of these processes is driving which phenotype is important.

To overcome this problem, the European consortia MouseAGE brought together experts from 26 European countries and the USA to reach consensus on best practices in mouse aging studies. This consortia recommended the generation of conditionally induced models of accelerating aging[230], where the gene deletion would be induced at the end of development (e.g., approximately 4 months in mice). While this may improve the quality of the mouse models, it would bring new unknowns as to whether the models would still develop a phenotype in a short period of time and whether the use of inducers such as tamoxifen could affect processes such as DNA damage repair or produce the desired phenotype in all tissues in a similar way. In addition, even if these models were available, each accelerated model would be the result of the dysfunction of one or two mechanisms of aging (reviewed in ref. [228]). This means that the choice of model would need to be guided by the mechanisms of aging thought to be most important in driving the development of PD. The models would need to be generated and carefully characterized. As there are multiple animal models of PD, each modeling-specific mechanisms or stages of the disease, it is unknown which of these models would be most affected by aging or by a specific aging process, thus substantially escalating the number of models needed to analyze. Although such approaches would be highly informative in understanding which mechanisms of aging are most important in driving PD pathogenesis, they would require considerable upfront investment, coordination, and standardization by the research community to avoid duplication and competition. There are other ways to accelerate aging, such as the use of irradiation or a high-fat diet; however, when choosing to use these other methods, consideration needs to be given as to whether these mechanisms are associated with PD pathology. For example, obesity has not been found to be an associated risk for PD[231], perhaps making the use of a high-fat diet less desirable as an aging inducer in the context of this disease.

There would be even more barriers if one considers rats as models of aging for the study of PD. There is no availability of accelerated models of aging in this species due to the difficulties in generating genetically modified models, at least until recently. The availability of clustered regularly interspaced short palindromic repeats (CRISPR) technology has helped overcome this problem, but its implementation will require an even larger investment in both generating and characterizing these models of PD and developing better reagents and knowledge of rat aging.

The consortium for development and evaluation of late-onset Alzheimer's disease (MODEL-AD) may represent a model on how to begin to overcome these barriers. A consortium of academic and nonprofit partners, funded by the NIH, leads the program, and among its aim is the generation of animal models for AD that accurately the pathology of late-onset AD and provide predictive models for the development of therapeutics. The models are generated following consensus and under transparent and open intellectual property conditions. The models are characterized according to the standardized guidelines for rigorous preclinical testing of animal models, with deep phenotyping performed at 4, 12, 18, and 24 months of age and including transcriptomics, proteomics, and metabolomics; neuropathology; *in vivo* imaging; biomarker analysis; and behavior/cognitive tests. All data are uploaded to a web portal and openly available to all the researchers[232].

Conclusions and Recommendations

Many models have been developed and utilized in the study of PD in cells, rodents, and human primates. However, there are

relatively few studies that incorporate aging as a contributing factor. More importantly, many studies are observational, and the time of disease induction, the time of monitoring, and the tests performed to characterize the animals vary across studies, making it difficult to draw conclusions that are rigorous and reproducible.

Although there is a clear association between aging and PD, there is still some uncertainty about how important the role of aging is in driving PD pathogenesis. There is a need to systematically investigate whether aging increases the susceptibility to PD, using a combination of mammalian models, pathway analysis, measurement of the function of known PD proteins with age and standardized methodologies. As the task is complex, this is better approached through a network similar to that of MODEL-AD to ensure testing is coordinated, systematic, appropriately prioritized, and the data, resources, and knowledge gained are shared in a timely manner, including the sharing of negative results and standardized protocols. Indeed, The Michael J. Fox Foundation has recently funded a network, PD-AGE, which was launched in January 2023 and addresses the recommendations that emerged from this work. In particular, PD-AGE will:

1. Ensure that researchers on aging and PD do not work *in silos* and share their knowledge on which models of aging to use, best practices in designing experiments with aged animals, and which models of PD to prioritize.
2. Address the need for mechanistic studies where models of PD are crossed with accelerated or long-lived models of aging. In this respect, the use of mouse models of prodromal or presymptomatic disease where the disease develops slowly and not completely seems to offer an excellent starting point to determine whether mechanisms of aging may act as drivers for progressive PD. This may need to be combined with other "hits," such as infections, inflammation, or other environmental factors. As PD is a heterogeneous disease and models reproduce different aspects or stages of the disease, other mouse models and different strains should not be excluded.
3. Develop consensus on when rats offer an advantage over mice and what reagents and models need to be developed. Rats have shown characteristics of PD that are not often seen in mice, but their use has been limited due to the lack of antibodies and the ability to generate transgenic animals. With the advent of CRISPR technologies, investment in the development of rat models with access to the required reagents should be evaluated and prioritized when they are superior to mice.
4. Consider the unique value of NHP and the technological development to prioritize when they offer unique advantages.
5. Consider the value of *in vitro* aging of iPSCs or using alternative reprogramming methodologies, which have been shown to maintain some aging features, and how their use can be integrated with the use of animal models.

It is hoped that addressing the strengths and weaknesses (some of which we have outlined in this review) of existing PD models will improve our understanding of the development and progression of PD and its relationship to aging biology and ensure the generation of models that are more relevant to human PD for testing new therapeutic interventions for PD. This is particularly imperative as new approaches to treat aging biology are currently being tested clinically for safety and efficacy. Food and Drug Administration-approved drugs exist that target multiple hallmarks of aging. If the relationship between aging biology and PD is resolved, this would offer completely novel approaches to the treatment of PD.

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Author Contributions

I.B. and H.M. conceived the idea for the workshop to reach consensus on models of Parkinson's disease useful to investigate aging. S.P. and S.S. contributed to the preparation of the programme. J.K.A., E.B., M.G., T.G., W.H., W.-L.K., D.K., L.N., I.R., S.P., L.-E.T., M.S., and L.S. actively contributed during the workshop, from which the barriers and recommendations are drawn. E.R., S.J.C., M.C., I.B., and H.M. wrote this article. All authors revised and approved this article.

Conflict of Interest statement

All authors declare that they have no competing interests.

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