CHAPTER

2

Animal models for preclinical Parkinson's research: An update and critical appraisal

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Abstract

Animal models of Parkinson's disease (PD) are essential to investigate pathogenic pathways at the whole-organism level. Moreover, they are necessary for a preclinical investigation of potential new therapies. Different pathological features of PD can be induced in a variety of invertebrate and vertebrate species using toxins, drugs, or genetic perturbations. Each model has a particular utility and range of applicability. Invertebrate PD models are particularly useful for high throughput-screening applications, whereas mammalian models are needed to explore complex motor and non-motor features of the human disease. Here, we provide a comprehensive review and critical appraisal of the most commonly used mammalian models of PD, which are produced in rats and mice. A substantial loss of nigrostriatal dopamine neurons is necessary for the animal to exhibit a hypokinetic motor phenotype responsive to dopaminergic agents, thus resembling clinical PD. This level of dopaminergic neurodegeneration can be induced using specific neurotoxins, environmental toxicants, or proteasome inhibitors. Alternatively, nigrostriatal dopamine degeneration can be induced via overexpression of α-synuclein using viral vectors or transgenic techniques. In addition, protein aggregation pathology can be triggered by inoculating preformed fibrils of α-synuclein in the substantia nigra or the striatum. Thanks to the conceptual and technical progress made in the past few years a vast repertoire of well-characterized animal models are currently available to address different aspects of PD in the laboratory.

Keywords

Movement disorders, Basic science, Pathophysiology, Neurodegeneration, Neuroinflammation

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1 Introduction

Research on animal models of neurological disease is often questioned on ethical grounds (LaFollette and Shanks, 1996), conceptual grounds (Diederich et al., 2019; Drummond and Wisniewski, 2017; Gomez-Marin and Ghazanfar, 2019), or pragmatic grounds. On a pragmatic level, it is sometimes argued that studies in animal models are unable to predict the outcome of new treatments for human disease (Pound and Ritskes-Hoitinga, 2018), although it is often pointed out that clinical trial failure does not necessarily depend on the intrinsic limitations of animal models (Bespalov et al., 2016; van der Worp et al., 2010). In addition to these general considerations, the recent availability of disease models in patient-derived cells (Caiazza et al., 2020) is stimulating a debate around the necessity of animal research, at least for certain applications.

In spite of the ongoing debate, the past few years have witnessed a remarkable progress in developing and characterizing animal models of PD. This progress has been inspired by an increased understanding of the complex etiopathogenesis and multisystem pathology of the human disease. Thanks to both conceptual and technical advances, we now have unprecedented opportunities to recreate key pathological aspects of PD in laboratory animals. In this review we have sought to provide an up-to-date overview of the main rodent PD models available today, appraising both their advantages and their limitations. The wide range of PD models now available offers new opportunities, but it is at the same time a challenge for the researcher to select the most suitable model for the questions under study. Our aim is to offer both a critical reflection and an updated resource that can inform on the use of suitable models for different research applications.

2 Models in different species

Comparing results across animal species serves as a powerful approach to promote scientific rigor and to discover biological principles of universal validity (Yartsev, 2017). The possibility to model PD in a multitude of species should thus be regarded as an asset to research, ultimately leading to a better understanding of the human disease.

Using either toxins or genetic perturbations, PD-like conditions can be induced in invertebrate organisms, the most common ones being the fruit fly *Drosophila Melanogaster* (Guo, 2010) and the nematode *Caenorhabditis Elegans* (Maulik et al., 2017). These models are particularly useful for high-throughput genetic analyses (such as, experimental mutagenesis to identify genetic modifiers of α-synuclein pathology or toxicant exposure). When more advanced behavioral or functional analyses are needed, investigators usually prefer to produce PD models in vertebrate species, the most common being either small fishes (Matsui and Takahashi, 2018) or rodents. The particular strength of small fish models (such as zebrafish) is their amenability to high-throughput in vivo drug screening studies (Flinn et al., 2008),

which can be aided by new automated methods of phenotypic analysis (Palmer et al., 2017). On the other hand, rodents show a significant degree of human homology regarding the organization of cortico-basal ganglia-thalamocortical loops (Reiner et al., 1998) and their corresponding behavioral functions (Redgrave et al., 2010). Moreover, rodents can produce complex movements homologous to those in humans (Sacrey et al., 2009) and they exhibit functionally similar motor deficits after nigrostriatal dopamine (DA) lesions, as well as analogous motor responses to dopamine (DA) replacement therapy (Cenci et al., 2002). Being less expensive than non-human primates (NHPs) and ethically less problematic to use, rodents continue to provide the most widely used models in PD research, particularly for studies that require an analysis of brain functions including movement, cognition, sleep, affective behaviors. In addition, rodent models are used increasingly often in studies addressing the functionality of peripheral organs (in particular, bladder, heart, gastrointestinal tract) in the setting of experimental parkinsonism or synucleinopathy. Because of the above reasons, most of the literature review provided in this chapter is based on rodent studies.

Models in NHP, particularly those in macaque monkeys, offer the specific advantage of a striking similarity to humans regarding the phenomenology of different movement disorders (Cenci and Crossman, 2018; Johnston and Fox, 2015). This makes it possible to quantify parkinsonian and dyskinetic features in the animals using similar principles to those used in patients, streamlining the translational path from the lab to the clinic (Fox and Brotchie, 2019). Moreover, the larger brain and body size of macaque monkeys conceivably facilitates the experimental evaluation of therapeutic interventions requiring surgery, such as those needed to infuse trophic factors and implant cells or stimulation devices. The main disadvantages of NHP models are a high cost and the necessity of highly specialized housing facilities. For these reasons, NHP models are currently used only in few research centers worldwide.

3 The importance of nigrostriatal dopaminergic degeneration

Although PD is clinically and pathologically heterogeneous (Berg et al., 2014; Erro et al., 2016), a severe loss of putaminal dopaminergic innervation is a necessary prerequisite for the appearance of motor symptoms that lead to clinical diagnosis. Parkinsonian motor features become manifest when more than 50% of putaminal DA contents are lost (Fearnley and Lees, 1991), and a rapid loss of the residual putaminal DA input appears to occur during the first 5 years following clinical diagnosis (Kordower et al., 2013). Accordingly, in both rodent and macaque models of PD, motor deficits start to become manifest when striatal motor regions have lost more than 50% of their dopaminergic input (Boix et al., 2018; Decressac et al., 2012b), and a full-blown parkinsonian-like syndrome appears only after removing more than 80% of putaminal dopaminergic fibers (Francardo et al., 2011; Guigoni et al., 2005; Winkler et al., 2002). Therefore, reports of hypokinetic features in animals that

exhibit only a modest degree of DA cell loss and/or mild deficits in striatal DA contents should raise suspicion of a systemic disease or pervasive neurological intoxication depending on the model at hand (in both instances, the animal would move less). To ascertain the parkinsonian character of motor features observed in the animal model, it is recommended to evaluate the effects of L-DOPA (Cenci et al., 2002; Xu et al., 2012). Indeed, treatment with L-DOPA improves gross hypokinetic deficits (Francardo et al., 2011; Lundblad et al., 2002), although it may not improve tasks requiring a high degree of motor precision (Metz and Whishaw, 2002; Winkler et al., 2002). If the denervation of striatal motor regions exceeds 90%, the majority of animals treated with therapeutic-like doses of L-DOPA will develop abnormal involuntary movements analogous to L-DOPA-induced dyskinesia (LID) (Francardo et al., 2011; Winkler et al., 2002). A similar relationship between degree of putaminal DA denervation and incidence of LID has been reported in macaque models of PD (Schneider, 1989).

The crucial importance of striatal DA depletion to the appearance of PD-relevant motor deficits explains the continuing interest in developing experimental approaches to selectively damage dopaminergic neurons. As reviewed below, mitochondrial and oxidant toxins have been in use for many years. Additional and more recent methods involve an intracerebral delivery of proteasome inhibitors. Moreover, efficient approaches have been developed to induce α-synuclein pathology using viral vectors, inoculation of α-synuclein fibrils, or transgenic technologies. A graphic summary of these different approaches is presented in Fig. 1.

4 6-Hydroxydopamine

The first toxin-based animal model of PD consisted of rats sustaining intracerebral injections of 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1968). This chemical is a hydroxylated analog of DA that also occurs in the brain (Jellinger et al., 1995). 6-OHDA is a catecholamine-selective neurotoxin because it enters neurons via the dopamine or noradrenaline transporter. Once inside the neuron, 6-OHDA undergoes auto-oxidation and conversion to reactive oxygen species (ROS) (Rotman and Creveling, 1976). Neurons rapidly die because of oxidative damage to cellular constituents and mitochondrial dysfunction (Kupsch et al., 2014), and there is wide consensus that such mechanisms are relevant to the pathogenesis of the human disease (Grunewald et al., 2019). Moreover, the degeneration of DA cell bodies and axon terminals triggers proinflammatory glial reactions that contribute to the neurodegenerative process [reviewed in (Kuter et al., 2020)], and this mechanism is also relevant to the pathogenesis of PD.

6-OHDA does not cross the blood-brain barrier (BBB) and therefore necessitates a direct delivery to the nigrostriatal system, which leads to dopaminergic degeneration in all animal species. For the sake of producing PD models, the three most common injection targets are the substantia nigra, the medial forebrain bundle (MFB), and the striatum. Injection of 6-OHDA into the MFB is the preferred

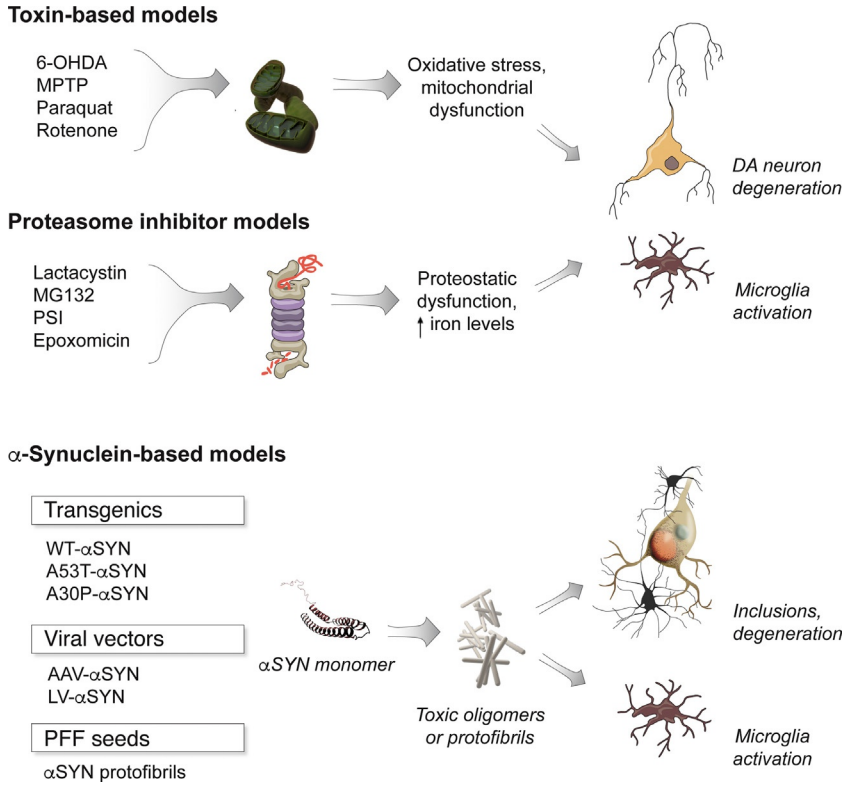

FIG. 1

Overview of the main methods currently used to obtain animal models of PD exhibiting degeneration of nigrostriatal DA neurons.

procedure to obtain a model of severe and reproducible dopaminergic degeneration with negligible tendency for animals to spontaneously compensate even at very long survival times [reviewed in (Francardo et al., 2017)]. Thanks to the predictability, stability, and severity of the DA lesion, this model is particularly useful for studies evaluating the effects of long-term pharmacological treatments or neural transplants. On the other hand, models based on intrastriatal 6-OHDA delivery afford a remarkable flexibility in modulating the severity and regional distribution of DA denervation by varying toxin dose and injection coordinates (Francardo et al., 2011; Winkler et al., 2002). Intrastriatal 6-OHDA models have proven particularly useful to study the effects of neuroprotective and neurorestorative treatments (Bjorklund et al., 1997; Francardo et al., 2017).

A lot has been learned by studying 6-OHDA lesion models of PD. Beside the elucidation of many potential treatment principles, including circuit restoration (Thompson and Bjorklund, 2012) and neuroprotection (Francardo et al., 2017),

research carried out on these models has elucidated questions of fundamental importance, such as the relationship between nigrostriatal damage and motor dysfunction (Decressac et al., 2012b; Kirik et al., 1998; Winkler et al., 2002), the postsynaptic consequences of DA denervation (Cenci and Konradi, 2010; Kostrzewa, 1995; Simola et al., 2007), and compensatory responses to dopaminergic damage (Lee et al., 2008; Zigmond, 1997). Moreover, rats with 6-OHDA lesions still provide the best validated rodent model to study L-DOPA-induced dyskinesia in the laboratory (Cenci and Crossman, 2018). Like any other approach targeting the nigrostriatal dopaminergic pathway, 6-OHDA lesions do not mimic the multisystem pathology of PD. However, it should be noted that PD-relevant pathological features are usually found also in non-dopaminergic neuronal systems, including serotonergic and noradrenergic projections and striatal neuron dendrites [reviewed in (Cenci, 2014, Fieblinger and Cenci, 2015)]. Moreover, it is technically possible to combine the injection of 6-OHDA with other genetic or chemical lesions in the same animal. Notwithstanding these possibilities, 6-OHDA-based models do not mimic two characterizing features of nigrostriatal neurodegeneration in PD, that is, the progressive time course and the formation of intracellular α-synuclein aggregates.

5 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

MPTP was discovered as a contaminant of synthetic heroin following the report of a severe parkinsonian syndrome developing in some users of illicit drugs in California (Langston et al., 1983). MPTP is a lipophilic compound that can cross the BBB. Once in the brain, it is metabolized by monoamine oxidase B (MAO-B) to the potent dopaminergic neurotoxin 1-methyl-4-phenylpyridinium ion (MPP^+) (Chiba et al., 1984), which is a structural analog of DA and can therefore be taken up by dopaminergic neurons via the DA transporter (DAT). After entering the neuron, MPP^+ becomes highly concentrated in the mitochondria and inhibits complex 1 of the electron transport chain (Ramsay et al., 1986), causing both inhibition of mitochondrial respiration and ROS accumulation. MPTP has been used to induce dopaminergic degeneration in both invertebrate and vertebrate species, the latter including nonhuman primates (Johnston and Fox, 2015), minipigs (Nielsen et al., 2016), and mice (Meredith and Rademacher, 2011). Rats are, however, resistant to MPTP toxicity (Sundstrom and Samuelsson, 1997), which is partly due to their increased capacity for vesicular sequestration of this toxin (Staal et al., 2000). Several protocols have been established to induce MPTP lesions in mice, consisting of acute, subchronic, or chronic regimens of MPTP intoxication [reviewed in (Meredith and Rademacher, 2011)]. The generally large interest in MPTP models can perhaps be attributed to the fact that the toxin is technically easy to administer (at least compared to toxins requiring intracerebral delivery), and that MPTP has been found to cause parkinsonism in humans.

Collectively, MPTP lesion models have had a remarkable scientific impact because they have been widely used to test hypotheses regarding both pathogenic

mechanisms and neuroprotective treatments for PD (Langston, 2017; Przedborski and Vila, 2003). Moreover, MPTP-lesioned monkeys have been essential to identify new symptomatic treatments based on circuit modulation [reviewed in (Wichmann et al., 2018)]. The discovery of MPTP and its dopaminergic neurotoxicity has also spurred a new wave of epidemiological research on the role of environmental toxicants in the etiopathogenesis of PD [reviewed in (Langston, 2017)].

Like 6-OHDA-based models, MPTP-lesioned animals do not reproduce the multisystem pathology of PD nor the formation of intracellular protein aggregates (Johnston and Fox, 2015). Although continuous systemic MPTP infusion with osmotic minipumps has been proposed as progressive PD model featuring α-synuclein inclusions in DA neurons (Fornai et al., 2005), these findings have been difficult to replicate (Alvarez-Fischer et al., 2008). In addition, MPTP models in mice may entail a high mortality, variability in behavioral and biochemical outcomes, and a potential for spontaneous compensation already within few months (Francardo, 2018; Meredith and Rademacher, 2011; Rousselet et al., 2003). This may explain why 6-OHDA is preferred to MPTP for the sake of producing mouse models to evaluate symptomatic and/or antidyskinetic treatments for PD. For these applications, the animal model must exhibit reproducible motor deficits that remain stable under a sufficiently long time.

6 Environmental toxicants

Several epidemiological studies have demonstrated an association between rural residence, pesticide exposure, and an increased risk of PD (Ascherio and Schwarzschild, 2016; Chade et al., 2006). Accordingly, some environmental toxicants present in rural environments have been tested for their capacity to induce nigrostriatal DA degeneration in animals [partly reviewed in (Jiang and Dickson, 2018)]. Among these toxicants, the herbicide paraquat and the pesticide rotenone have now become well-established research tools for both in vitro and in vivo applications.

6.1 Rotenone

Rotenone (a natural extract from plants) is a broad-spectrum insecticide and pesticide. Because of its hydrophobicity, rotenone can easily cross the blood-brain barrier, and once in DA neurons, it inhibits mitochondrial complex I and activates the production of ROS (Cannon and Greenamyre, 2010). Greenamyre and collaborators were the first to develop an animal model of PD based on the continuous administration of rotenone via osmotic minipumps (Betarbet et al., 2000). In part of the animals, rotenone administration induced loss of nigrostriatal DA neurons associated with formation of α-synuclein inclusions and development of hypokinetic-rigid features. Locus coeruleus noradrenergic neurons were mildly affected too (Betarbet et al., 2000). This seminal publication was followed by other studies reporting toxic effects of rotenone on multiple neuronal systems. In particular, Höglinger and

colleagues reported that chronic infusion of rotenone causes damage to striatal serotonergic fibers, striatal projection neurons and cholinergic interneurons, pedunculopontine tegmental nucleus and locus coeruleus, concluding that rotenone intoxication is more suitable to model atypical parkinsonian syndromes than PD (Hoglinger et al., 2003). In a similar vein, using different rotenone doses and administration routes in rats, other studies reported lack of correlation between loss of striatal DA innervation and motor deficits, concluding that the motor phenotype induced by rotenone intoxication may depend on some pervasive neurological effects of the toxin (Fleming et al., 2004; Lapointe et al., 2004).

Since this earlier controversy, some successful attempts have been made to increase the reproducibility and specificity of rotenone models by carefully titrating the toxicant dose and using a more lipophilic injection vehicle (Cannon et al., 2009). Moreover, unilateral infusion of rotenone into the medial forebrain bundle was reported to produce progressive dopaminergic degeneration, accompanied by increased expression and aggregation of α-synuclein, in the absence of peripheral toxicity (Ravenstijn et al., 2008).

6.2 Paraquat

The herbicide paraquat has a structure similar to MPP^+. Like MPTP, paraquat can cross the BBB, it is taken up by the DAT, and induces dopaminergic degeneration via oxidative stress and mitochondrial dysfunction (Fei et al., 2008; Powers et al., 2017). Paraquat is usually administrated orally or intraperitoneally to rats or mice, and it is most often combined with the fungicide maneb, which has been found to potentiate paraquat toxicity toward nigrostriatal DA neurons (Thiruchelvam et al., 2000). The dose and duration of the treatment have varied between studies, and so has the behavioral-histopathological phenotype of the corresponding animal models. Nevertheless, most studies have shown that paraquat can induce a dose-dependent partial degeneration of nigrostriatal DA neurons, although the effects on striatal dopaminergic fibers and DA levels have been quite variable, possibly due to compensatory mechanisms [for a recent review see (Cenci and Sgambato, 2020)]. Interestingly, low-dose chronic administration of paraquat has been reported to cause upregulation and aggregation of α-synuclein in wild-type mice (Manning-Bog et al., 2002) and to exacerbate markers of α-synuclein aggregation in the enteric nervous system in a transgenic synucleinopathy model (Naudet et al., 2017).

In summary, herbicides and pesticides are very interesting research tools because of their relevance to the environmental components of PD etiopathogenesis, although they induce only partial dopaminergic degeneration and entail a generally high risk of systemic toxicity if used at effective doses. If applied at low doses, herbicides and pesticides can provide a valuable approach to probe the vulnerability of nigrostriatal DA neurons under different conditions, such as aging (Cannon et al., 2009; Thiruchelvam et al., 2003), stress and gut dysbiosis (Dodiya et al., 2020), α-synuclein pathology and neuroinflammation (Ling et al., 2004), or any other factor that may be relevant to the etiopathogenesis of PD.

7 Proteasome inhibitors

Deficits in protein degradation are attributed a key role in the pathogenesis of PD as they are reciprocally linked with the accumulation and misfolding of α-synuclein (Xilouri et al., 2013). Since the early 2000s, there has been an increasing interest in modeling PD by administering proteasome inhibitors to a variety of species (*C. Elegans*, small fishes, mice, rats, minipigs, and non-human primates) (Bentea et al., 2017; Lillethorup et al., 2018). Proteasome inhibitors are drugs originally developed for the treatment of cancer (myeloma, in particular) based on their capacity to induce programmed cell death. Compounds of this class have been administered to rodents using systemic or intracerebral delivery methods. The most successful results have been obtained by injecting potent and irreversible proteasome inhibitors (such as lactacystin) into the substantia nigra or the MFB [reviewed in (Bentea et al., 2017)]. The corresponding rodent models exhibit a rapid, dose-dependent degeneration of nigral DA neurons (McNaught et al., 2002; Xie et al., 2010) that can be associated with L-DOPA-responsive motor deficits (Konieczny et al., 2014). The mechanisms underlying neurodegeneration in these models include apoptotic cell death, mitochondrial dysfunction, iron dysregulation, oxidative and nitrosative stress [reviewed in (Bentea et al., 2017; Le, 2014)].

The doses of lactacystin needed to obtain ≥50% loss of nigrostriatal DA neurons produce various degrees of extranigral pathology. Interestingly, in rats sustaining intranigral injections of lactacystin, substantial neurodegeneration has been detected in the ipsilateral pedunculopontine nucleus (Elson et al., 2016), causing >60% loss of cholinergic neurons and somatic hypotrophy of the remaining neurons in this region (Pienaar et al., 2015). This observation is in keeping with the reported high sensitivity of cholinergic neurons to proteasomal inhibition, as demonstrated in a dose-response study of lactacystin delivery to the basal forebrain (MacInnes et al., 2008). Furthermore, rats sustaining unilateral injections of lactacystin in the MFB have been found to exhibit a progressive pattern of brain structural changes including striatal atrophy, cortical thinning, and enlargement of the lateral ventricles, which were already significant by 3 weeks but became more severe by 5 weeks post injection (Vernon et al., 2011). The progressive extranigral pathology may depend on inflammatory mechanisms because intracerebral injections of lactacystin have been shown to produce widespread, pronounced and sustained activation of astroglia and microglia (Elson et al., 2016; Savolainen et al., 2017). These glial reactions appear to greatly exceed those reported in neurotoxin-based PD models and may stem from a direct action of proteasome inhibitors on glial cells (Ding et al., 2004).

One interesting feature of proteasome inhibitor models is the occurrence of neuronal α-synuclein accumulation within the affected regions, with the appearance of a diffusely increased cellular immunostaining for α-synuclein (Elson et al., 2016, Savolainen et al., 2017), sometimes associated with the formation of small inclusion bodies (Elson et al., 2016; MacInnes et al., 2008; McNaught et al., 2002) that may be immunopositive for Ser129-phosphorylated α-synuclein (Bentea et al., 2015). It is,

however, unclear whether α-synuclein accumulation plays a causal role in the neuronal death caused by proteasome inhibitors [reviewed in (Bentea et al., 2017)].

In conclusion, although proteasome inhibitor models may be associated with non-specific neuronal and glial cell toxicity, they provide useful tools to investigate pathways of proteostatic dysfunction and iron dyshomeostasis, and to evaluate neuroprotective treatments targeting these pathways (Bentea et al., 2017, Le, 2014). Moreover, proteasome inhibitors can be administered to animals overexpressing α-synuclein as an approach to trigger or aggravate the neurodegenerative process (Stefanova et al., 2012).

8 Alpha-synuclein models

The role of α-synuclein in PD pathogenesis goes back to the identification of a mutation in the corresponding gene (SNCA) as a cause of familiar parkinsonism (Polymeropoulos et al., 1997). Interest in the pathogenic role of this protein was further reinforced by the observation that α-synuclein is a major component of Lewy bodies and Lewy neurites (Spillantini et al., 1998), and that elevated expression of the non-mutated protein is sufficient to cause a PD-like disorder in individuals carrying SNCA duplication or triplication (Chartier-Harlin et al., 2004; Singleton et al., 2003). Attempts to replicate α-synuclein-related pathology in animals were first made using transgenic techniques, soon followed by a development of viral vectors (recombinant adeno-associated virus, AAV, or lentivirus, LV) and, most recently, intracerebral injections of α-synuclein fibrils. The tools generated in this way have added an important new dimension to the modeling of PD pathogenesis involving protein misfolding and aggregation, and they have also made it possible to study mechanisms of disease progression related to the cell-to-cell spreading of toxic α-synuclein species. The transgenic and viral vector models are complementary. The transgenic models, obtained in mice, offer opportunities to model systemic disease, but are less useful for the study of cell-type specific pathogenic processes. The viral models, on the other hand, are applicable to both mice, rats and monkeys. They offer the opportunity to study α-synuclein -related toxic processes specific to midbrain DA neurons, while also being applicable to other brain regions or neuron types.

8.1 The AAV-α-synuclein model

Like the 6-OHDA toxin, AAV mediated α-synuclein overexpression requires that the vector is injected locally in the brain using stereotactic surgery. Although this may seem a limitation, it offers distinct advantages in that the overexpression of α-synuclein (wild-type or mutated) can be selectively targeted to the midbrain region encompassing substantia nigra (SN)-ventral tegmental area (VTA) and restricted to one side of the brain, leaving the contralateral side as an internal control. A range of AAV vector serotypes have been explored for this purpose. The early studies made use of recombinant vectors of the AAV2 serotype (Kirik et al., 2002;

Yamada et al., 2004), which have later been replaced by vector serotypes with better tissue spread and transduction efficiency for midbrain DA neurons, notably AAV2/5 (Gorbatyuk et al., 2008); AAV2/6 (Decressac et al., 2012b), AAV2/7 (Van der Perren et al., 2015a), AAV2/8 (McFarland et al., 2009) and AAV2/9 (Bourdenx et al., 2015). The transduction efficiency in midbrain DA neurons achieved with LV vectors (usually not more than 50%) is clearly lower than that obtained with the more efficient AAV vectors, and the extent of DA neuron cell loss is also less pronounced (typically between 25% and 35%) (Lauwers et al., 2003, 2007; Lo Bianco et al., 2002).

AAV mediated overexpression of α-synuclein induces progressive degenerative changes in midbrain DA neurons that replicate some of the key features of the human disease, most prominently the development of α-synuclein-containing protein aggregates positive for Ser129-phosphorylated α-synuclein (p-Syn+), accompanied by prominent axonal pathology and a progressive loss of nigral DA neurons. Neuritic changes develop early and precede DA neuron cell loss. Thus, the dendritic projections of DA neurons in the SN pars reticulata are truncated with distorted morphology, and the pre-terminal axons display swollen and p-Syn+ distorted profiles. As in the human disease, these degenerative changes are associated with an early activation of microglia, an increase in pro-inflammatory cytokines, and lymphocyte infiltration preceding cell loss [for review see (Ulusoy et al., 2010, Van der Perren et al., 2015b, Volpicelli-Daley et al., 2016)].

The progressive time-course of neurodegeneration is an attractive feature of this model, making it possible to distinguish between an *early presymptomatic stage* and a later *symptomatic stage*. The *presymptomatic stage* corresponds to the first month after vector injection, and it is characterized by the development of inclusions, axonal pathology and impaired DA synthesis and release. The *symptomatic stage* develops over the subsequent months, when a significant portion (>50%) of the nigral DA neurons have degenerated and part of the still surviving neurons express p-Syn+ pathology (Decressac et al., 2012b; Lundblad et al., 2012). With this level of cell loss (50–80%) the animals show impairments in standard motor tests similar to what is typically seen following intrastriatal 6-OHDA lesions (Bourdenx et al., 2015, Decressac et al., 2012a,b, Van der Perren et al., 2015a). From a direct comparison between the two models (Decressac et al., 2012b), we have suggested that the motor impairment in the toxin-based model is well correlated with the magnitude of DA neuron loss, while the motor deficits seen in the AAV model result from the combination of DA cell death and dysfunction of the remaining nigrostriatal neurons. While both of the two models have been developed to mimic DA neuron deficiency, they differ in their temporal and neuropathological characteristics, and replicate different pathophysiological aspects of the human disease. The early developing axonal pathology and striatal DA dysfunction preceding overt nigral cell loss (Butler et al., 2015; Chung et al., 2009; Lundblad et al., 2012) is a particular feature of the AAV-α-synuclein model that mimics the disease progression seen in human PD (Burke and O'Malley, 2013; Kordower et al., 2013).

The main weakness of the AAV-α-synuclein model is the variability in the magnitude of the neurodegenerative response, which has made it difficult to obtain

consistent behavioral impairments. The magnitude of DA neuron cell loss varies considerably depending on vector types and batches. As discussed in further detail in a recent review (Volpicelli-Daley et al., 2016), this variability is due to many factors: the viral vector serotype, the promoter used, the production process, and the quality and purity of the final product. A further complicating factor is that the common measure of vector titer, genome copies/μL, does not reliably predict the in vivo transduction efficiency of the AAV vector. For this reason, it is necessary to establish the optimal working titer for each individual production round before it is used in a planned experiment. For each vector batch, however, the transduction efficiency can be expected to be consistent from animal to animal, and the variability in outcome will be the same as for 6-OHDA models (i.e., due to investigator skills in stereotactic targeting).

8.2 The PFF inoculation model

This model builds on the finding that oligomeric fibrillar α-synuclein can act as a seed to recruit the monomeric form of the protein into pathogenic aggregates. Using preformed fibrils of recombinant α-synuclein (PFFs), this property has been demonstrated in cell cultures in vitro (Lu et al., 2009; Volpicelli-Daley et al., 2011, 2014), as well as after injection into the brain (Luk et al., 2012a,b; Osterberg et al., 2015). The formation of protein aggregates and the progressive development of cellular dysfunction and cell death are not caused by the fibrils themselves, but by recruitment of endogenous α-synuclein into cellular inclusions. Thus, the injected PFFs are not pathogenic when applied to cells lacking α-synuclein (Luk et al., 2012b; Volpicelli-Daley et al., 2011, 2014). Moreover, their toxicity is increased and accelerated in the presence of elevated levels of monomeric α-synuclein (Peelaerts et al., 2015; Thakur et al., 2017), and the formation of aggregates is more efficient if PFFs and monomeric α-synuclein are from the same animal species (Luk et al., 2016; Peelaerts et al., 2015).

The robust formation of inclusions resembling Lewy bodies and Lewy neurites is a characteristic feature of the PFF model not present in AAV-α-synuclein models. This feature makes this model highly useful for studying the formation and spread of aggregated α-synuclein species. The PFF-induced inclusions share many features with those found in human PD brains: they have a filamentous structure and they are insoluble, hyperphosphorylated, ubiquitinated and morphologically similar to the spheroid inclusions seen in Lewy bodies and neurites (Volpicelli-Daley et al., 2011, 2014).

The pathogenic process is quite fast in cultured neurons, developing within 1–2 weeks (Volpicelli-Daley et al., 2011), but it progresses very slowly when PFFs are injected into the brain of normal animals not overexpressing α-synuclein. Thus, it may take up to 6 months for significant neurodegenerative changes to appear in midbrain DA neurons when the PFFs are injected into the striatum or SN (Espa et al., 2019; Luk et al., 2012a; Paumier et al., 2015; Peelaerts et al., 2015). The same

protracted time course of aggregate formation and toxicity has been observed following injections of PFFs into the cortex (Osterberg et al., 2015).

Fibril inoculation and viral vector models can have complementary applications due to differences in their associated pathogenic processes. The PFF model allows for studying the seeding process and the formation, spread and impact of toxic aggregates, while models with vector-mediated α-synuclein overexpression make it possible to study successive stages in the development of cellular and functional changes, including presymptomatic-predegenerative changes. The protracted time-course seen in PFF models is experimentally disadvantageous for evaluating the effects of neuroprotective treatments on behavioral impairments and neuronal cell loss. An additional potential concern is the limited spread of the PFFs within the tissue, which limits the number of DA neurons that can be targeted by an injection in the striatum. This may be less of an issue in the small mouse brain, but more of a problem when applying this approach to rats. In the most extensive study thus far performed in rats, Caryl Sortwell's group have studied the effects of unilateral intrastriatal PFF injections for up to 6 months (Patterson et al., 2019). At the highest PFF dose (16 μg) they observed a progressive downregulation of tyrosine hydroxylase (TH) expression in about 30% and 50% of nigral neurons at 4 and 6 months, respectively. Actual cell loss was evident only at 6 months, with a loss of approximately 30% nigral neurons (identified using the pan-neuronal marker NeuN). Notably, a reduction in nigral NeuN+ cell number was observed also on the contralateral side. The accumulation of p-Syn, which was observed in about one third of the nigral neurons, peaked at 2 months and was largely gone by 6 months. Consistent with the fairly modest degenerative changes, no or only minimal impairment in motor behavior was observed in the PFF-injected rats even at the longest time point. These data reinforce the impression that it is difficult to obtain consistent and significant behavioral impairments using intrastriatal PFF injections in rats [see (Volpicelli-Daley et al., 2016) for further discussion of this issue].

As already mentioned, the most interesting use of the PFF model consists in mechanistic studies of seeding, aggregation and spread of α-synuclein-related pathology in the brain. PFFs injected into the brain parenchyma are internalized by neurons and axons, and efficiently transported retrogradely in neurons whose axons terminate in the injected area. Thus, the initial spread of synuclein pathology is due to retrograde axonal transport of the PFFs, which will seed the formation of p-Syn+ aggregates in the parent neurons. Following injections of PFFs into the striatum, p-Syn+ inclusions will appear within 1–2 months not only in the striatum itself, but also in the principal regions projecting to the striatum, i.e., substantia nigra, cortex, thalamus, amygdala (Abdelmotilib et al., 2017; Luk et al., 2012a; Masuda-Suzukake et al., 2013; Paumier et al., 2015). Similarly, PFF injections into the hippocampus will result, at 3 months, in the appearance of pSyn+ pathology in some though not all neuronal populations projecting to the injected area (Nouraei et al., 2018). In the same study, some limited anterograde transport was observed in two of the major efferent projections from the hippocampus, entorhinal cortex and septum.

It remains unclear, however, whether any significant cell-to-cell transfer of synuclein pathology occurs in this model, and if so, how long it would take for this mechanism to become functionally relevant. Transfer of α-synuclein between cells, in monomeric or aggregated form, has been clearly demonstrated in cell culture systems, suggesting that this is likely to occur also in the brain [see (Tyson et al., 2016) for review]. The most careful investigation of this issue has been performed by Patrik Brundin's group using mice that received injections of human or mouse PFFs into the olfactory bulb (Rey et al., 2013, 2016, 2018). In this model, cellular immunoreactivity for p-Syn+ was found to spread from the olfactory bulb to anatomically connected olfactory and non-olfactory regions, a process that progressed gradually over 12 months. Over the first 9 months, the spread of synuclein inclusions appeared to occur via retrograde axonal transport as it was limited to first-order afferents to the injected area (Mason et al., 2016; Mezias et al., 2020). At longer time points, a reduction in the density of synuclein aggregates was observed in some regions, and no further spread was detected (Rey et al., 2018). Thus, in contrast to the continued disease progression seen in the advanced stages of human PD, the progression of PFF-induced pathology seems to taper off after about a year.

Taken together, these data indicate that cell-to-cell spread of PFF-induced pathology is a late event. The initial spread is clearly due to retrograde transport along afferent connections. At a later stage, only many months later, monomeric or fibrillar α-synuclein species released from the affected neurons may be taken up by adjacent cells or axons to act as seeds for further propagation of synuclein pathology to other interconnected brain regions. This slow and protracted progression limits the usefulness of the PFF model for evaluating therapies that target the spread of PD pathology (e.g., antibodies directed against α-synuclein oligomers). If the goal is to counteract or block cell-to-cell transfer, the use of assessment end-points shorter than 1 year will be irrelevant—at such short survival times the role of cell-to-cell transfer in the spread of p-Syn+ pathology is likely to be negligible. For this type of studies, the more efficient spread of pathology seen in PFF-injected transgenic α-synuclein overexpressing mice may provide a more attractive model. In a study performed by Luk et al. (2012b), human α-synuclein PFFs were injected unilaterally into the striatum in transgenic mice overexpressing human mutated (A53T) α-synuclein. The injection was given at a time point well before any pathology related to the transgene had appeared. With this approach, p-Syn+ pathology developed more rapidly and was much more widespread, than that obtained after similar injections in wild-type mice (Luk et al., 2012b). Motor deficits appeared after about 3 months, a time point when extensive p-Syn+ pathology had already emerged. In this transgenic model, p-Syn+ inclusions developed not only in structures directly connected with the injected area (including cortex, thalamus and substantia nigra), but notably also in more distant sites, such as deep cerebellar nuclei, remote brainstem areas, and spinal cord, indicating that an efficient cell-to-cell transfer had occurred in this model. Whether this represents actual trans-synaptic transmission of α-synuclein seeds, rather than passive diffusion within the extracellular space, is currently unclear and remains to be investigated (Luk and Lee, 2014).

8.3 Combined AAV-PFF α-synuclein models

A shortcoming of the AAV overexpression method is that the α-synuclein levels needed to induce sufficient DA neuron cell death (linked to significant motor impairments) are quite high, in the order of 4–5-fold above the endogenous levels (Decressac et al., 2012b; Faustini et al., 2018). This is well above what may be seen in human PD, raising the question whether the cellular toxicity associated with these high expression levels is predictive for the clinical condition. One way to circumvent this limitation is to combine viral vector-mediated α-synuclein overexpression with PFF inoculation, delivered to the SN either as two separate injections (Thakur et al., 2017) or mixed in a single injection (our yet unpublished data). As explained above, PFFs can act as seeds for the recruitment of monomeric α-synuclein into toxic fibrillar aggregates, the speed by which this happens is dependent on the level of monomeric α-synuclein (Volpicelli-Daley et al., 2011, 2014), and the seeding is most efficient if PFFs and monomeric α-synuclein are from the same species (Luk et al., 2016). In the combined AAV-PFF model, (Thakur et al., 2017) we expressed human WT α-synuclein at a low level (closer to that seen in patients with SNCA triplication) and Lewy-like pathology, neurodegeneration and DA neuron cell death were triggered by injection of human PFF seeds into the SN. Animals receiving both the AAV and the PFF exhibited an enhanced and accelerated development of pathology where the formation of p-Syn+ cellular and neuritic inclusions was evident already by 3 weeks after PFF injection. This pathology was accompanied by a prominent inflammatory response involving both activation of resident microglia and infiltration of CD4+ and CD8+ T-lymphocytes. The degeneration of nigral DA neurons was progressive, leading to a 50–60% cell loss by 24 weeks (Thakur et al., 2017). The progressive nature of the combined AAV-PFF model may offer the possibility to pre-screen the animals at an early time-point using sensitive behavioral tests (as currently done in 6-OHDA-lesioned rats and mice) in order to identify those animals that will become fully symptomatic at a later time point. In this way, it would become possible to evaluate potential neuroprotective treatments in well-matched groups of animals that are on the way to developing a significant disease phenotype.

This combined approach is applicable also for the induction of cortical Lewy-like pathology analogous to that seen in human Lewy body disease. In a recent study (Espa et al., 2019), we induced overexpression of human wild-type α-synuclein bilaterally in rat medial prefrontal cortex using an AAV2/6 synuclein vector, followed 3 weeks later by an injection of human PFFs. The PFF injection targeted the rostromedial striatum based on the expectation that PFFs would be efficiently transported from this region to the AAV-targeted cortical areas via retrograde axonal transport (which proved to be the case). While neither the α-synuclein overexpression nor the PFFs induced any behavioral phenotype if given alone, their combined application induced significant impairments in tests of working memory, attention and inhibitory control, accompanied by the development of prominent proteinase K-resistant, p-Syn+ inclusions, swollen and distorted cortical dendrites, and cortical neuronal

loss by 24 weeks (Espa et al., 2019). These results further support the notion that inoculating fibril seeds into a brain region expressing high levels of monomeric α-synuclein leads to an accelerated and amplified development of pathology (which was found to also involve frontocortical afferent regions). Thanks to this approach, it became possible to experimentally reproduce cognitive and pathological features relevant to Lewy body disease using the rat frontocortical circuits as a model system.

In the studies by Thakur et al. (2017) and Espa et al. (2019), vector and PFFs were administered with a 3–4-week interval. In a recent follow-up study (Hoban et al., under revision) we have now proceeded to combine the two preparations in a single, mixed injection, which is experimentally more convenient. Following injection into the rat SN, the same, accelerated and enhanced p-Syn+ pathology, inflammatory response, and progressive DA neuron cell loss are obtained also in this version of the AAV-PFF model. Significant motor impairments, as assessed in tests of forelimb use (cylinder and stepping tests), are observed already by 3–4 weeks after injection, at a time when most of the affected neurons still survive but in a down-regulated state characterized by impaired striatal DA release and reduced expression of TH and vesicular monoamine transporter 2 (VMAT2), as well as downregulation of nuclear receptor related-1 (Nurr1) transcription factor (which controls the expression of DA phenotype genes). At 3–4 months, when DA neurodegeneration is complete, marked impairments in forelimb use is seen in at least half of the injected animals, those with $>50\%$ nigral cell loss.

8.4 Transgenic α-synuclein overexpressing mice

Considerable efforts have been made to generate transgenic models of PD based on overexpression of human α-synuclein in its wild-type or mutated forms. A database assembled by the Joint Programme for Neurodegenerative diseases (JPND) lists a total of 24 transgenic α-synuclein models published and characterized to date [list available through (Joint Programme for Neurodegenerative Diseases, 2019)]. Only few of them develop dopaminergic dysfunction and DA cell death of a magnitude justifying their use as models of PD [see (Jiang and Dickson, 2018, Magen and Chesselet, 2010) for review]. The first transgenic models in this category were generated by Eliezer Masliah's group and expressed wild-type human α-synuclein under either the platelet-derived growth factor beta (PDGF-beta) promoter (Masliah et al., 2000) or the Thy-1 promoter (Chesselet et al., 2012; Rockenstein et al., 2002). In these mice, expression of the α-synuclein transgene is widespread, and cytoplasmic and nuclear inclusions containing human α-synuclein develop in several brain areas, including cortex, hippocampus, olfactory bulb, and to some extent also in the SN.

In the PDGF-beta transgenic model, α-synuclein-positive inclusions at the level of the SN were limited to only a few scattered cells. Nevertheless, the mice with the highest level of transgene expression (Line D) were impaired in motor performance, as assessed at 12 months in the rotarod test. This was accompanied by a 50%

reduction in striatal TH immunostaining and TH protein levels, but no DA neuron cell loss. The extent of nigral pathology was more prominent in the Thy-1 mice, but the impact on the integrity of the nigrostriatal system remained quite modest: a 40% reduction in striatal DA and 17% reduction in striatal TH, seen at 14 months, without any measurable nigral cell loss (Chesselet et al., 2012). The changes in motor behavior were bi-phasic: an early phase of locomotor hyperactivity at 4–5 months of age was followed by a slow development of sensorimotor impairments that became evident only at 14 months. The time-dependent changes seen in these two transgenic strains seem compatible with a slowly developing axonopathy in nigrostriatal DA neurons accompanied by a reduction in TH expression in the surviving neurons, although the presence of neurodegenerative changes in several brain regions makes it difficult to attribute the observed deficits exclusively to the pathology of nigrostriatal DA neurons. Indeed, these mice also developed gut dysfunction and changes in circadian rhythm that preceded the motor impairments, suggesting an early impact of the α-synuclein transgene in areas outside the nigrostriatal system, akin to the prodromal phase of human PD.

In a similar way, a constipation-like phenotype preceding motor impairments has been observed in a bacterial artificial chromosome (BAC) transgenic mouse line expressing wild-type α-synuclein from the complete SNCA locus at disease-relevant levels (Janezic et al., 2013). These mice showed a 30% loss of nigral DA neurons at 18 months, preceded by a 30% reduction in striatal DA release, without any observable α-synuclein pathology. These results suggest that the functional impairments seen in these mice were caused by their elevated levels of monomeric α-synuclein rather than a formation of toxic aggregates.

A more interesting PD-like phenotype has been obtained in transgenic mice expressing an aggregation-prone, truncated version of human α-synuclein (1–120 α-synuclein) driven by the TH promoter (Tofaris et al., 2006; Wegrzynowicz et al., 2019). In these mice the transgene expression is confined to DA neurons in the SN and the olfactory bulb (though also occurring in locus coeruleus noradrenaline neurons), having a pronounced impact on the integrity and function of the nigrostriatal pathway. In a first version of this mouse model, the α-syn120 line (Tofaris et al., 2006), dense α-synuclein cytoplasmic inclusions were observed in atrophic TH-positive nigral neurons at 12–14 months, accompanied by a 30% reduction in striatal DA levels, while the number of TH cell bodies remained unchanged. At the longest time-point analyzed, 18 months, the mice exhibited reduced spontaneous locomotor activity.

The Spillantini lab has recently published an improved version of this TH promoter-driven 1–120 synuclein mouse, referred to as the MI2 line (Wegrzynowicz et al., 2019). These mice show more prominent, progressive aggregation of α-synuclein in nigral DA neurons and striatal DA terminals. The first protein aggregates appear at 1.5 month of age in the form of small puncta, and then develop into larger Lewy body-like aggregates at 6–12 months, some of which are ubiquitin-positive and proteinase K-resistant. In these mice there was a significant

loss of nigral TH neurons starting at 9–12 months and amounting to about 50% at 20 months of age. The first signs of motor impairment (affecting gait pattern) developed at 9 months of age, at a time when there was a marked reduction in striatal DA release measured with microdialysis, but preceding the appearance of any significant nigral cell loss. A more overt behavioral phenotype was observed only later (at 20 months) when 50% of nigral TH-positive neurons were lost. This sequence of events—axon terminal dysfunction followed by cellular pathology and DA cell death—is reminiscent of the histopathological progression seen in human PD, and supports the view that α-synuclein-induced degenerative changes start at the level of the axon terminals.

A large number of transgenic mouse lines have been generated carrying mutated versions of α-synuclein (A53T, A30P or E46K). Many of these develop motor impairments linked to a widespread synucleinopathy with intraneuronal α-synuclein inclusions, but no overt damage to nigrostriatal DA neurons [see (Jiang and Dickson, 2018, Magen and Chesselet, 2010) for review]. One notable exception is a conditional transgenic model obtained using a tetracyclin-dependent inducible system to overexpress human A53T α-synuclein selectively in midbrain DA neurons (Lin et al., 2012). In these mice there is a 2–4-fold increase in α-synuclein protein and mRNA in the midbrain, with a development of granular α-synuclein deposits in nigral TH neurons and α-synuclein aggregates in striatal axons and terminals by 12–18 months of age. This is accompanied by a gradual loss of nigral DA neurons, amounting to 15% at 1 month and 40% at 12–20 months of age. In this mouse model, a motor impairment in open field and rotarod tests was reported to develop already at 1–2 months, before any major cell loss had occurred. At this early time point, Lin et al. (2012) observed a marked downregulation of both TH, DAT, VMAT2 and Nurr1 in the α-synuclein-overexpressing nigral neurons, similar to what has been found in human PD (Chu et al., 2006) and in the AAV-α-synuclein model at a similar early time point (Decressac et al., 2012a). In line with these findings, Lin et al. detected a 70–80% reduction in baseline and evoked striatal DA release at 3–4 months. The protracted time course of SN degeneration in this model is consistent with observations made in other transgenic or viral α-synuclein models (see above) and indicates that the earliest impact of elevated levels of wild-type or mutated α-synuclein occurs at the level of axons and presynaptic terminals (reflected in a downregulation of the DA synthesis machinery and a reduction in striatal DA release). These changes precede cell death and may be sufficient to cause motor deficits. Nevertheless, caution should be exerted when attributing motor deficits to nigrostriatal DA pathology if a transgenic model also exhibits non-PD-specific phenotypic features. For example, in the above-mentioned study (Lin et al., 2012), the transgenic human α-synuclein protein was expressed not only in the midbrain but also, at very high levels, in cerebellum and hippocampus. Moreover, compared to their wild-type controls, the α-synuclein transgenic mice exhibited a significant and progressive body weight reduction starting at young adult age, whose implications remain unclear.

9 Other genetic models of PD

As already mentioned, the discovery of gene mutations associated with autosomal recessive and autosomal dominant forms of PD has prompted the development of a large number of transgenic rodent lines expressing PD-causing mutations [for review see (Blesa and Przedborski, 2014, Creed and Goldberg, 2018, Konnova and Swanberg, 2018, Xu et al., 2012)]. In this section, we have chosen to focus on four well-established genetic PD models exhibiting nigrostriatal DA deficits, the *Engrailed1, Nurr1, Pitx3-Aphakia*, and *mitochondria-deficient "MitoPark"* mouse models. Engrailed1, Nurr1 and Pitx3 are transcription factors involved in the development and survival of midbrain DA neurons during development. They are, however, expressed not only during development but also in adulthood, playing a role in the maintenance and survival of mature DA neurons. The MitoPark mouse, by contrast, carries a genetic defect that will impair mitochondrial function and replication selectively within midbrain DA neurons. These models are interesting and valuable as they can replicate a PD-like progressive dopaminergic neurodegeneration; at the same time, their usefulness to mimic the disease process is limited by the lack of α-synuclein-related pathology.

In the *heterozygous Engrailed1 (En1$^{+/-}$) knock-out mouse,* ablating one of the two Engrailed1 genes induces a progressive degenerative process in nigrostriatal DA neurons that starts at the level of axon terminals at about 4 weeks of age and progresses over the subsequent months, resulting in a significant loss of DA neurons at 3–5 months of age (Nordstroma et al., 2015; Sonnier et al., 2007). The magnitude of DA neuron loss is, however, too small to induce any significant motor phenotype. In this model, the early axonopathy/axon terminal loss followed by nigral DA neuron degeneration is reminiscent of the disease process seen in human PD (Burke and O'Malley, 2013; Kordower et al., 2013). Similar to the human disease, the En1$^{+/-}$ mice exhibit decreased mitochondrial complex-I activity and signs of impaired autophagy, although they lack α-synuclein aggregate formation, a hallmark of human PD. In a recent extension of the model by the Brundin group, PFFs were injected into the striatum to induce synuclein pathology in En1$^{+/-}$ mice (Chatterjee et al., 2019). In the absence of one Engrailed allele, the induction of pathological α-synuclein aggregates was found to be more pronounced than in wild-type mice, suggesting that the mitochondrial and autophagic deficits seen in En1$^{+/-}$ mice may help to accelerate the seeding and aggregation process. This combined approach thus brings the Engrailed model one step closer to mimicking PD-like pathology.

In the *Nurr1 model*, one or both of the Nurr1 genes are ablated. Because homozygous Nurr1 knockout (KO) mice do not survive beyond birth, Nurr1 hypomorphic models are obtained by using either heterozygous mice lacking one allele through life (Jiang et al., 2005) or conditional KO mice where one or both alleles are selectively removed in mature DA neurons (Kadkhodaei et al., 2009, 2013). There is evidence to suggest that Nurr1 is involved in the pathophysiology of PD. Indeed, the expression of Nurr1 is reduced in DA neurons affected by synuclein pathology

(Chu et al., 2006; Decressac et al., 2012a) and polymorphisms in the Nurr1 gene point to reduced Nurr1 expression being a risk factor for the development of PD (Grimes et al., 2006; Xu et al., 2002; Zheng et al., 2003). In line with these findings, reduced Nurr1 expression, as seen in heterozygous Nurr1$^{+/-}$ mice, is associated with a slowly developing nigrostriatal dysfunction including DA neuron loss and reduced striatal DA levels, which becomes evident at 15–24 months of age (Jiang et al., 2005), as well as an increased sensitivity to the DA neurotoxin MPTP (Le et al., 1999). These changes are even more pronounced in conditional knock-out mice where the Nurr1 gene is ablated by administering tamoxifen treatment at 5 weeks of age. In this model, the Nurr1 target genes TH, VMAT2, and DAT are markedly down-regulated by 4 months, and tissue DA levels are reduced by over 80% in both striatum and nucleus accumbens at 11 months, accompanied by motor impairments in the open field and vertical pole tests developing gradually over time (Kadkhodaei et al., 2013). No loss of cell bodies was detected for up to 11 months after tamoxifen administration, while TH-positive axons and dendrites developed clear signs of pathology, exhibiting a swollen and fragmented morphology. This pattern of pathological changes—axon terminal degeneration and reductions in striatal DA preceding DA neuron cell loss—suggests similarity with early stage PD. However, similarly to the En1$^{+/-}$ model, Nurr1 deficient mice do not develop any signs of α-synuclein pathology.

The Pitx3-Aphakia mouse is the best characterized and most commonly used of the three transcription factor-related models. The expression of Pitx3 (paired-like homeodomain transcription factor 1) is restricted to the developing eye and midbrain DA neurons from embryonic day 11 throughout adult life (Smidt et al., 1997). Aphakia mice carry a spontaneous deletion at the *Pitx3* locus causing microphthalmia and aphakia (i.e., absence of the lens of the eye). Mice homozygous for this mutation (termed ak/ak mice) exhibit an almost complete loss of DA neurons in the pars compacta of the SN that is present already at birth, while DA neurons in the VTA are relatively spared up to about 6 weeks of age. At later time points (100 days), about 50% of the VTA neurons are lost (Hwang et al., 2003; Nunes et al., 2003; van den Munckhof et al., 2003). As a result of the marked DA cell depletion in the SN, the motor part of the striatum is severely denervated and exhibits an over 90% reduction in DA levels. This is associated with supersensitivity of DA receptor-mediated signaling in striatal neurons (Hwang et al., 2005), which enables the induction of dyskinesia by repeated L-DOPA administration (Ding et al., 2007; Suarez et al., 2018). Differently from the dorsolateral (motor) striatum, nucleus accumbens and ventral striatal areas exhibit only approximately 70% DA loss in adult mice (3 months old) (van den Munckhof et al., 2003). Ak/ak mice do not show any gross alterations in motor behavior, but display clear L-DOPA-reversible defects in sensitive measures of nigrostriatal motor function, such as longer latency and shorter steps in the beam walking test and impaired performance in the vertical pole test (Hwang et al., 2005). Moreover, these mice show impairments in striatum-dependent cognitive tests including rotarod learning, T-maze and inhibitory avoidance tasks (Ardayfio et al., 2008). The Pitx3-Aphakia mouse has been proposed as valid model

for PD because of its conspicuous loss of nigral DA neurons with relative sparing of the VTA, resembling the pattern of dopaminergic degeneration in the human disease. Nevertheless, this model has limited utility for studying PD pathogenesis and treatments thereof, since the loss of SN DA neurons results from a developmental defect as opposed to an adult degenerative process. The applications benefiting the most from this mouse model are pathophysiological-behavioral studies addressing the effects of severe nigrostriatal DA depletion and the associated compensatory mechanisms. Due to its bilateral phenotype, the Pitx3-Aphakia mouse offers an interesting complement to the common, unilateral 6-OHDA lesion models for this type of studies.

The MitoPark mouse is a conditional knock-out mouse where the gene for mitochondrial transcription factor A (Tfam) is disrupted selectively in DA neurons. The TFAM protein is a regulator of mitochondrial replication and decreased levels of this protein result in a reduction of mitochondrial DNA copy number (Ekstrand et al., 2004), similar to what has been observed in nigral DA neurons in human PD (Grunewald et al., 2019). In the affected DA neurons, Tfam disruption induces a respiratory chain deficiency, which in turn causes a progressive degenerative phenotype. The dopaminergic deficiency yields a L-DOPA-responsive motor impairment that is first observed at around 12–15 weeks of age, accompanied by a gradual loss of DA neurons in both SN and VTA, which reaches about 80–90% at 10 months of age (Ekstrand et al., 2007). Prior to the onset of cell loss these mice show a presymptomatic impairment in striatal DA release (Good et al., 2011) and also changes in somatodendritic morphology in DA neurons (Lynch et al., 2018). The protracted time-course of degenerative changes, leading to a near-complete loss of midbrain DA neurons within a reasonable time span, is an attractive feature of the MitoPark mouse. Mitochondrial dysfunction is a characteristic feature of human PD and the MitoPark mouse provides a highly useful model for this aspect of the disease.

10 Concluding remarks

During the past decades, an increased understanding of pathological features, genetic and environmental factors underlying PD has prompted the development of a vast and diversified repertoire of animal models. Today we have unprecedented opportunities to recreate and study virtually all critical aspects of PD pathogenesis in laboratory animals. This is a very active research field in continuous communication with other research disciplines, in particular, molecular genetics, protein biochemistry, pharmacology, physiology, and comparative anatomy. In spite of the criticism often raised on the validity of animal models, there is no question that disease models in adult living organisms will continue to be indispensable for many fundamental applications. Although no single animal model replicates all pathogenic and clinical features of PD, the range of rodent models available today offers opportunities to reproduce specific disease features within a tightly controlled in vivo system. If carefully chosen and correctly applied, animal models are requisite

for scientific progress and invaluable for the exploration and development of novel therapeutic ideas. To this end, selecting the most suitable model for the questions under study is essential, and a continuous, bidirectional dialogue between experimentalists and clinical researchers is of vital importance.

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