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The non-motor complications in Parkinson's disease — what can we learn from animal models?

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Abstract: Sporadic Parkinson's disease is a widespread human disease that has never been reported in non-human vertebrates. The etiopathogenesis of the non-motor symptoms in the disease is not well understood and it is difficult to interpret the roles of affected neurotransmitters in currently available animal models. Most of the non-motor symptoms do not correlate with the stage of motor deficits and precede the development of motor symptoms by many years, before the permanent loss of dopaminergic neurons in the basal ganglia. The aim of this review is to briefly summarize the advantages and limitations of the well-recognized mammalian animal models with special regard to the non-motor complications of the prodromal and early stage Parkinson's disease.

Key words: early Parkinson's disease, non-motor symptoms, animal models.

It has been almost two centuries since James Parkinson published his monograph entitled "An essay on the Shaking Palsy" and still, there is no cure for the disease [1]. These days we are at least aware of the very complex nature of the sporadic Parkinson's disease (PD). It affects all races equally, with slight male predomination. In Europe, the prevalence is 1.6/100 inhabitants, with a wide variability between different countries [2]. Its complete clinical manifestations consist of the more obvious motor symptoms such as resting tremor, hypokinesia, rigidity, postural instability [3] as well as less obvious, yet burdensome, non-motor symptoms including mainly olfactory, gastrointestinal, thermoregulatory and genitourinary dysfunction, sleep disturbances, anxiety, fatigue or even dental abnormalities [4–7]. From the neuropathological point of view, it is defined by the continuous formation of immunoreactive inclusion bodies, which develop only in susceptible neuronal types within the central and peripheral (including enteric part) nervous systems. The

inclusions occur in the form of spherical, granular Lewy bodies in cell somata as well as elongated, spindle-shaped or thread-like Lewy neurites in axons and dendrites [8–10]. These lesions, so called "Lewy pathology", tend to play a key role in the pathogenesis of PD [8–13]. Only neurons with long, thin and weakly myelinated or unmyelinated axons become involved. Interneurons and projection cells with short axons or those with densely myelinated axons are not vulnerable [14–16]. It should also be mentioned that Lewy pathology is not limited to dopaminergic neurons of the substantia nigra but also occurs in glutamatergic [17, 18], noradrenergic, serotonergic [19], histaminergic, and cholinergic neurons. Thus, during the past decade, PD has come to be recognized as a multi-systemic disorder rather than a mono-systemic disease with selective dysfunction and loss of nigral neurons [8, 20–23].

For this review, relevant articles (full texts written in English language) were primarily identified by electronic search of the PubMed, Cambridge Journals Online and EBSCO databases ("non-motor symptoms" AND "Parkinson's disease" AND "animal model") as well as an examination of the reference lists of the identified studies and other distinguished reviews in the field. The majority of papers were published during the past decade.

Animal models of Parkinson's disease

Sporadic Parkinson's disease is a widespread human disease that has never been reported in non-human vertebrates [24]. This might partially explain the minimal translation of findings from the animal studies into the new effective treatments, with the exception of the deep brain stimulation which was tested in the MPTP-treated monkeys [25]. Still, animal models are a very important tool to improve our understanding of the pathogenesis and pathophysiology of the disease as well as for the testing of new therapeutic interventions [26]. The pathological process underlying PD consists of an early non-symptomatic period, followed by a prodromal phase, often characterized by olfactory dysfunction [27, 28], autonomic dysregulation [29], as well as sleep [30–33] and mood disturbances [34]. The non-motor features of PD [35] that can appear years, sometimes decades, before the onset of the motor symptoms, do not meaningfully respond to dopaminergic medication and are a challenge to the clinical management of PD [36–38]. The prodromal phase transitions into a phase that is accompanied by the classical somatomotor symptoms and subsequently, impaired cognitive functioning. The duration of each phase varies among patients. Braak's grading with six neuropathological stages incorporates all of them [8, 39] Thus, the "perfect" PD model should reproduce: the main pathological features such as cell loos in the substantia nigra and cytoplasmatic inclusions (in case of animals — Lewy-like pathology) possibly spreading from cell to cell manner, the progressive evolution of the neuronal damage, characteristic motor and non-motor symptoms, and finally be responsive to the therapeutics currently available in the market [26].

Up-to-date, such a model does not exist. Following a classical categorization strategy, existing animal models can be grouped into "fidelity models" and "discrimination models". A fidelity model reproduces the maximum number of characteristics of the original, whereas a discrimination model reproduces only one particular characteristic of the original.

A high fidelity model might seem superior to a discrimination model, but the latter can help by teasing out the irrelevant factors from the important. Regarding PD modeling, **the reserpine model** sets an excellent example. This toxin works by inhibiting vesicular monoamine transporter 2, leading to a reversible depletion of monoamines. This model mimics the akinetic symptomatology of PD without being associated with the nigral dopaminergic cell degeneration. The model made it possible to demonstrate that dopamine was involved in the movement process so that L-DOPA was developed [40].

So far, epidemiological studies have suggested an association between environmental factors, including drinking well water, rural living, farming, exposure to agricultural chemicals, and PD. Elevated levels of organochlorines have been found in the brains of PD patients. Pesticides inhibiting the mitochondrial Complex I and increasing oxidative stress are more prone to induce PD upon exposure. Parkinson's disease has also been linked to the exposure to different metals (manganese, lead, copper, iron, zinc, aluminium or amalgam) and industrial compounds. Higher incidence of PD has also been reported in manganese miners. Manganese, a component of various pesticides, reproduces Parkinsonian symptoms after long and chronic exposures (between 6 months and 16 years). Moreover, environmental toxins can induce the release of pro-inflammatory signals [41]. Hence, it is quite obvious that **toxic models** of PD remains the most popular. In animal models, the most widely used chemicals are MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) [42], 6-OHDA (6-hydroxydopamine) [43], rotenone [24, 44] and paraquat [45]. These models have been extensively reviewed in the literature [26, 40–50].

MPTP has been used to understand the effect of mitochondrial inhibition, to test different neuroprotective strategies or to observe the effect of dopamine absence in different brain functions and areas. MPTP produces an irreversible and severe parkinsonism in humans, characterized by all of the cardinal features of PD, including tremor, rigidity, slowness, postural instability and freezing, but it has two main drawbacks: firstly, induces only an acute or subacute neurodegeneration and secondly, neither Lewy bodies formation (except Lewy-like pathology in the locus coeruleus of older squirrel monkeys) nor the characteristic pathology progression has been observed so far [26, 40, 41]. There are also several methodological limitations [49].

6-OHDA has to be injected directly in the central nervous system, as it is not able to cross the brain-blood barrier and when administered systemically, it destroys sympathetic neuron nerve terminals in the peripheral nervous system. Similarly, this model does not produce the characteristic Lewy-like pathology nor does it show the pathology progression. In fact, in case of 6-OHDA, it is more accurate to talk about at least two separate models: the medial forebrain bundle (more suitable for studying the consequences of dopaminergic neuronal death and to test therapeutic strategies to treat motor symptoms) and the striatal (more relevant to unmask the mechanisms of cell death involved in PD) [26, 40–43].

Rotenone is a naturally occurring pesticide derived from the roots of plants within the Lonchocarpus and Derris genera and acts through mitochondrial Complex I inhibition [24]. The rotenone model largely gained its attention following the seminal paper by the Greenamyre group, which demonstrated for the first time that rotenone administered systemically to rats can reproduce the hallmarks of PD [51]. Fore mostly, chronic rotenone

treatment seems to generate proteinaceous inclusions in some of the surviving dopaminergic neurons that cannot be found in the standard 6-OHDA and MPTP models. These inclusions were first described in experiments with the osmotic pump administration and it remains to be determined if the daily injection regimen triggers the formation of the same kind of inclusions. Interestingly, the low dose prevents rotenone from reaching the brain but spreads the synucleinopathy from the enteric to the central nervous system [44–50]. Oral administration appears to cause little neurotoxicity. Thus, the chronic systemic administration using osmotic pumps has been the most common delivery regimen, especially in the Lewis rat, which may be more sensitive to rotenone than other strains of rats [52].

Paraquat toxicity is mediated by the induction of redox cycling with a cellular diaphorase such as nitric oxide synthase, which gives rise to the subsequent production of reactive oxygen species. As its chemical name indicates, paraquat is a pyridinium that shares structural similarities with another herbicide used in the past called cyperquat, which is an active ion of MPTP (MPP^+). This fact suggested that both chemicals may share some mechanisms of neurotoxicity, though the exact details are still uncertain. Rotenone and paraquat models are not yet thoroughly understood (in the contrary to MPTP and 6-OHDA) and have to be fully validated, especially in terms of doses, routes of administration and experimental periods [26, 40, 41].

Less common model substances include **lipopolysaccharide** (acute and chronic administration), **tetrahydroisoquinoline derivatives** (such as salsolinol, for example) or another pesticide, **maneb** (especially in non-human primates) [26, 40, 48–50, 53, 54].

Regarding the molecular mechanisms of cell death, the toxin-based models have emphasized apoptosis, oxidative stress, mitochondrial dysfunction and microglial activation as key players in PD-related neurodegeneration. At the same time, they are not useful for screening of potential neuroprotectants, probably because of the acute nature of cell death in comparison with the natural progression of the disease [49].

Transgenic models are mostly associated with the familial cases of PD, however with some exceptions, for instance, the GBA1 gene encoding for lysosomal enzymes **glucocerebrosidase** (GCase). Heterozygous mutations in the GBA1 gene are the most important risk factor for sporadic PD, leading to the 20 to 30-fold increased risk. Between 5 and 10% of Parkinsonian patients carry such mutations. The loss of glucocerebrosidase activity causes lysosomal dysfunction and α-synuclein aggregation [55]. Currently, in vivo models replicating such impairment are being developed, since mice with GCase deficiency show mitochondrial impairment [56], enhanced vulnerability to PD-inducing mitochondrial toxins (MPTP, MPP+) [57], gial activation and α-synuclein accumulation (in the cortex, hippocampus, substantia nigra and striatum) as well as dopaminergic synapse alterations including reduced striatal dopamine release and reduced post-synaptic density size [58].

Indeed, Parkinson's disease seems to be heterogeneous in its etiology, and both, environmental and genetic factors should be taken into consideration. Perhaps the most promising models are those that could effectively combine genetic models with exposure to toxins.

Animal models of the non-motor symptoms in Parkinson's disease

The non-motor symptoms in Parkinson's disease appear throughout the course of the disease while some appear before motor symptoms are even fully recognized. In fact, the non-motor features remain under-recognized, under-researched, and under-treated [59, 60]. The early non-motor signs comprise impaired olfactory function, mood and personality disturbances, subtle neurocognitive dysfunctions and visuomotor control abnormalities. These signs and symptoms are neither specific nor sensitive for PD. However, in combination, they are highly suggestive for the disease [4, 6, 29]. The complete clinical manifestations, mainly due to the autonomic failure, include orthostatic hypotension, dry mouth, oropharyngeal dysphagia, upper gastrointestinal dysmotility, constipation, defecatory dysfunction, urinary urgency and urine incontinence, hyperhidrosis, and sexual dysfunction [29]. One should also bear in mind that besides those symptoms particularly attributable to pathologic changes in the nervous system many of them represent adverse effects of the drug therapy, especially L-DOPA treatment. For instance, drug effects on the mesolimbic pathways can lead to impulse control disorders such as pathologic gambling and shopping, pending, binge eating and hypersexuality [61, 62]. Their effects on the autonomic system can cause the known non-motor side effects such as cardiovascular autonomic dysfunction (including orthostatic hypotension, syncope, impaired baroreflex, reduced heart rate variability), constipation, nausea and urological disturbances [63, 64]. Impulsive–compulsive behaviors constitute a serious clinical problem in PD because they interfere with a successful management of the motor symptoms. However, these type of the non-motor complications exceed the scope of this review [61].

The usefulness of toxic models for modeling the non-motor complications of the prodromal and early stage PD is limited by the fact that many of these symptoms are at least partially independent of dopamine. The etiopathogenesis of the non-motor symptoms is not well understood and it is difficult to interpret the roles of each altered neurotransmitter systems in currently available models. Most of the non-motor symptoms do not correlate with the stage of the motor deficits and precede the development of the motor symptoms by many years [6, 7, 65, 30] before the severe and permanent loss of dopaminergic neurons in the basal ganglia [66, 67].

6-OHDA models have surely great value in studying the motor aspects of the PD and the efficacy of classical anti-parkinsonian drugs. However, the analysis of the non-motor symptoms seems to be rather limited. And it is also believed that the non-motor changes found in animals with complete 6-OHDA-induced lesions are closely linked to changes in dopaminergic function [68, 69]. According to literature, some of the neuropsychiatric non-motor symptoms of PD can be replicated in the 6-OHDA-induced rat model, suggesting the indirect involvement of various neurotransmitter systems. What is more, this model might also be used to mimic pain [70], circadian deficits [71] and gastrointestinal dysfunction [72, 73]. Zheng *et al.* have reported a reduce of dopaminergic neurons in the substantia nigra following the bilateral microinjection of 6-OHDA. 6-OHDA rats exhibited impaired gastric motility and delayed gastric emptying, accompanied by increased dopamine content and the over-expression of D2 receptors in the stomach. Subdiaphragmatic vagotomy prevented the increase in the gastric dopamine content and the dopamine D2 receptor expression and improved gastric dysmotility in 6-OHDA rats [74].

A few studies have analyzed the **MPTP-induced parkinsonism** in non-human primates in search for deficient cognition. Similar phenomenon was found in rats, which displayed an impairment of memory acquisition and retention processes that was worsened with L-DOPA treatments [67]. It is likely that L-DOPA-induced increase in dopamine release in extrastriatal circuits can impair cognition in some PD patients while improving motor functioning [75]. At the same time, neither acute nor chronic exposure to MPTP in non-human primates was able to produce the sympathetic denervation of the heart [76] or induce gastrointestinal dysfunction despite a significant decrease of neurons immunoreactive for tyrosine hydroxylase in the enteric nervous system [77].

MPTP-intoxicated monkeys exhibited deficits in maintenance of a response set and difficulties in shifting attention sets. Impaired ability to sustain spatial attention or to focus attention, deficits in motor readiness and planning and impaired time estimation was also observed in these animals. As in PD patients, these cognitive alterations are not reversed by L-DOPA. Instead, monkeys could also experience psychotic-like behaviors that may be related to the neuropsychiatric symptoms that PD patients often suffer from after chronic treatment with L-DOPA [40].

In an MPTP mouse model reported by Anderson *et al.*, the toxin exposure caused a 57% decrease in neurons immunoreactive for tyrosine hydroxylase in the substantia nigra pars compacta, a 52% decrease in the striatum and a 40% reduction in the enteric nervous system 10 days after the injections. However, there were no changes in gastric emptying and most notably, the behavioral phenotype of gastrointestinal dysfunction was limited to a temporary increase in colonic motility (the opposite of what is observed in patients) [78]. Olfactory deficits are yet another complication difficult to replicate in the MPTP mouse model. It was shown a number of years ago that unlike Parkinsonian patients, individuals with MPTP-induced parkinsonism (usually due to parenteral self-administration) did not show any olfactory dysfunction [79]. Multiple studies also report lack of anxiety after MPTP exposure, suggestive of extranigral involvement in this frequent symptom in PD [80].

In general, the model of MPTP-induced parkinsonism has been extremely valuable in testing efficacy of dopaminergic therapies [81]. The toxin does not accurately replicate the global and progressive degeneration seen in the disease but its specificity can be used to help elucidate the role of dopamine in the development of some non-motor symptoms in PD. One should also remember that despite some similarities between the physiology of rodents and humans, it is clear that the significant differences do exist. For example, rats and mice are relatively resistant to MPTP, whereas humans are quite sensitive to this toxin. The sensitivity of humans to MPTP became apparent in 1983 when several drug addicts unfortunately injected themselves with MPTP thinking it was synthetic heroin. In rodents in order to produce some of the pathophysiological and behavioral symptoms seen in humans it is more effective to administer MPTP with probenecid (which blocks the rapid clearance of MPTP and its metabolites from the kidney) [49].

Rotenone administration in rats has been shown to lead to lesions of the nigrostriatal pathway and α-synuclein accumulation, yet the lack of specificity of the toxin and the variability of its effects in earlier experiments have limited its use for evaluating neuroprotective strategies. According to a growing body of literature, rotenone could mimic at least some

of the non-motor complications seen in PD patients [24, 26, 50, 67]. Rats that underwent bilateral infusion of rotenone into the substantia nigra displayed depressive behavior as well as changes to hippocampal levels of serotonin and noradrenaline metabolites [82]. Chronic rotenone administration also altered cognition in rats [83]. What is more, two groups have recently presented evidence that rotenone can induce gastrointestinal dysfunction as determined by α-synuclein aggregation in the enteric nervous system, loss of the myenteric neurons of the small intestine [84], a delay in gastric emptying and abnormal functioning of inhibitory neurons in the enteric nervous system [85]. Intragastric administration of rotenone in mice resulted in a progressive deposition of α-synuclein in both the enteric and the central neurons (the dorsal motor nucleus of vagus and the substantia nigra) [86]. One major advantage of the rodent rotenone model is its ability to induce the formation of a-synuclein-positive cytoplasmic inclusions in nigral neurons, replicating the neuropathological hallmark of Lewy bodies seen in PD. The major limitations of the rodent rotenone model include lack of reproducibility in the amount of animals that develop dopaminergic nigrostriatal lesions. However, despite the high variability of rotenone effects, the progressive and non-dopaminergic specificity of the toxin expands its experimental opportunities [24]. It also remains unknown whether the mechanisms of the non-motor complications observed in rotenone-treated animals are etiopathogenetically similar to those observed in PD patients [67].

Recently, Ngwa *et al.* have also demonstrated that $\mathbf{V_2O_5}$ induces dopaminergic neurotoxicity via protein kinase C delta (PKCδ)-dependent oxidative signaling mechanisms in dopaminergic neuronal cells. Their results revealed a significant decrease in olfactory bulb weights, tyrosine hydroxylase levels, levels of dopamine and its metabolite, 3, 4-dihydroxyphenylacetic acid as well as increases in astroglia of the glomerular layer of the olfactory bulb in the treatment groups relative to vehicle controls. Neurochemical changes were accompanied by impaired olfaction and locomotion [87].

Unfortunately, few animal models recapitulate the non-motor symptoms in PD. The relationship between Lewy pathology, neurodegeneration and the corresponding clinical deficits awaits further elucidation [88].

Alpha-synuclein

Accumulating evidence suggests that sporadic Parkinson's disease has a long prodromal period during which various non-motor features start to develop. Early sites of Lewy pathology are the olfactory bulb and the enteric plexus [8, 11, 12]. Those eosinophilic intracytoplasmic neuronal inclusions are abnormal aggregates of proteins such as α-synuclein and ubiquitin, stored in the cytoplasm as non-degraded by-products of the degenerative process [14–16, 20, 89]. The main component is α-synuclein which is abnormally phosphorylated, nitrated and oxidized, has an abnormal crystallographic structure and abnormal solubility, and is prone to the formation of aggregates and insoluble fibrils [90]. According to Braak *et al.*, environmental or neurotropic pathogens might adhere to the mucous membrane of the upper gastrointestinal tract and/or nose and penetrate into local neurons, where they might trigger a formation of those pathological inclusions, which might be transported along unmyelinated axons from the enteric nervous system to the dorsal motor nucleus of the vagus

nerve [8, 14, 15, 91]. Indeed, experimental models have made it possible to detect seeding mechanisms [92] and spreading of α-synuclein aggregates from the periphery via the vagus nerve to the central nervous system following intragastric, intraduodenal and peripheral vagal nerve inoculations [8, 93].

It is believed that the non-motor symptoms in PD are directly linked to widespread distribution of α-synuclein pathology not restricted to the dopaminergic nigrostriatal system. In addition to the non-nigral brainstem nuclei, α-synuclein pathology involves sympathetic and parasympathetic, enteric, cardiac and pelvic plexuses, and many other organs indicating a topographical and chronological spread [88, 94–96]. For example, Lewy bodies that contain α-synuclein are observed in central and peripheral regions associated with cardiovascular autonomic function such as the medulla, locus coeruleus, cardiac plexus and stellate ganglia. Furthermore, patients with a familial form of PD associated with duplication or triplication of the α-synuclein locus show impaired parasympathetic function in the Valsalva maneuver and reduced cardiac sympathetic innervation suggesting an important role for α-synuclein in the pathogenesis of sympathetic and parasympathetic dysfunction in PD [64].

Mutations of α-synuclein gene used to be mostly linked to monogenic forms of familial cases of Parkinson's disease. The discovery of various mutations associated with those familial forms including α-synuclein, Parkin, DJ-1, ubiquitin C-terminal hydrolase L1 T (UCHL1), PTEN-induced putative kinase 1 (Pink1), and Leucine-rich repeat kinase (LRRK2) has led to the generation of genetic mouse models of parkinsonism [52, 97–99]. Genome-wide association studies also suggested that sporadic PD is at least associated with the genetic variations in SNCA gene. The mouse model, among many others, has strong advantages for studying complex genetic disorders including PD: inbred strains of the same genetic background, sophisticated yet accessible methods of genetic manipulation, and a broad spectrum of phenotypical manifestations to cover many of PD symptoms. There is still a tremendous need for genetic PD mouse models that can recapitulate at least the degeneration of dopaminergic neurons in a manageable time window because the ultimate goal in PD research would be the prevention or halting of the degenerative processes of these neurons and others [100]. However, in comparison with toxin models, the genetic models are at the early stages of behavioral and pharmacological characterization, and the phenotypical characterization of the non-motor symptoms in genetic mouse models constitutes an emerging area of research [97].

The enteric nervous system abnormalities preceding the central nervous system changes and α-synuclein-positive aggregates in the enteric ganglia have been reported in mice transgenic for artificial chromosomes containing PD-associated α-synuclein (A53T or A30P of the human SNCA gene) mutations [101]. Lee *et al.* have shown that injection of the total brain extract from Dementia with Lewy bodies patients into the gastric walls of α-synuclein A53T transgenic mice resulted in an accumulation of α-synuclein aggregates in the enteric neurons. In contrast, injection of control brain extract failed to produce the aggregates. The results suggest that once α-synuclein aggregates are formed in some neurons in the gastrointestinal tract, they can spread to a larger neuronal population in the enteric nervous system, thereby increasing the chance to propagate the aggregates to the central nervous system [102].

Mice that overexpress wild-type α-synuclein under the Thy1 promoter has also been examined for the presence of the non-motor complications [103]. These animals exhibited

a progressive development of sensorimotor deficits beginning at two months of age, and α-synuclein accumulation in multiple brain regions such as the substantia nigra, however except the spinal cord. By three months of age the mice displayed olfactory deficits together with progressive alterations in circadian rhythms (lower night-time activity) and signs of autonomic dysfunction (increased heart rate variability). Beginning around four to six months of age cognitive changes became evident as well. These mice also showed decreases in classical tests of anxiety [67, 104]. Another study revealed gastrointestinal dysfunction exhibited by abnormal colonic motility in 11-month-old mice [105]. Noreen *et al.* have found that gastrointestinal expression of human α-synuclein in this transgenic line was limited to efferent fibers projecting from the dorsal motor nucleus of the vagus nerve to the enteric nervous system in an α-synuclein A53T transgenic mice model expressed under control of the prion promoter (AS mice). Older transgenic mice had a lower density of human α-synuclein expression in the gastrointestinal tract, suggesting an age-related disruption of efferent vagal fibers in this model. At the same time, mice developed age-related declines in stool frequency and gastric emptying consistent with those seen in human PD [106].

Zhang *et al.* have also found that α-synuclein A53T transgenic mice at 6 months or older displayed a deficit in odor discrimination and odor detection without any motor deficit and nigral dopaminergic neuron loss. In 10 months old α-synuclein A53T transgenic mice compared to wildtype littermates, dopaminergic neurons were found increased in glomerular layer, accompanied with an increase in tyrosine hydroxylase activity as well as a marked decrease in cholinergic neurons in mitral cell layer and a decrease in acetylcholinesterase activity, which might resemble the observation that olfactory dysfunction usually precedes motor deficits in PD patients [107]. At the early stages of the disease in α-synuclein over-expressing mice, α-synuclein depositions are more widespread in the olfactory nucleus, olfactory tubercle, piriform cortex and lateral entorhinal cortex than in the substantia nigra, indicating that pathways involved in these earlier symptoms are the most vulnerable to pathophysiological changes [108].

However, there is no obvious dopaminergic neuronal loss in most α-synuclein transgenic mice, mechanistic studies of dopaminergic neuronal death by α-synuclein have been aided through the study of the impact of α-synuclein expression following MPTP intoxication. Mitochondrial dysfunction appears to be sufficient to induce α-synuclein aggregation and downstream toxicity for dopaminergic neuronal loss in MPTP mouse models. As expected, the dopaminergic neurons of α-synuclein transgenic mice show more sensitivity for mit chondrial toxins [102].

Unfortunately, rodent models of PD are difficult to mimic the non-motor symptoms of PD, such as depression, anxiety and emotional abnormalities. Brain anatomy and neuronal circuitry are significantly different between primates and rodents. Non-human primates would serve as good animal models to uncover the non-motor behavioral changes that may mimic early clinical symptoms in PD and would also be valuable for identifying biomarkers [109]. Niu *et al.* have established transgenic PD rhesus monkeys that express mutant α-synuclein (A53T). The A53T monkey model allowed to identify cognitive defects and anxiety-like behaviors, without detectable sleeping disorders, as early phenotypes [110].

Compared with toxin-induced PD animal models, transgenic PD models should show more stable and replicable pathological changes and phenotypes, which are critical for developing effective treatments [110].

Studies of pathological mechanisms that underline the conformational change and aggregation of α-synuclein, as well as those involving the formation of pathogens that can be transmitted trans-synaptically to interconnected nerve cells, cannot be performed on formalin-fixed autopsy tissue [8]. It is also crucial to differentiate physiologic and pathological forms of α-synuclein with appropriate histological stains. Traditional staining shows Lewy bodies to be eosinophilic, but α-synuclein immunohistochemistry (antibodies to the amino- and carboxyl-terminal sequences) is now the standard method of localizing Lewy bodies and Lewy neurites in tissue specimens [111]. Alpha-synuclein is a normal constituent of neurons and is expected to be present in a soluble form. This must be distinguished from the phosphorylated pathological α-synuclein that forms β-rich sheets and can be detected with either appropriate antibodies or stains for Thioflavin-S [112].

A wide range of models, even evolutionarily remote, have been used to study Parkinson's disease together with its various complications. Yeast, worms, fruit flies or fish can be useful for studying cellular processes involved in the pathogenesis of neurodegeneration and Parkinson's disease, such as apoptosis, autophagy, oxidative stress, protein misfolding and degradation or vesicle-mediated transport, however cannot replicate the loss of neurons in the brain. Rodents and non-human primates are another important scientific resource but the limitations of these models must be kept in mind when interpreting results, since none of them completely reproduces the clinical symptoms and pathology seen in PD patients so far. Non-human primate models are anatomically, physiologically, and behaviorally more similar to humans, but they are rarely used because of cost and ethical concerns [49].

Because of these limitations, some studies are still best done in the clinic. A search for noninvasive biomarkers of PD sets a good example of this type to study. If one is to identify blood biomarkers of PD, the investigation could be done directly and therefore the results obtained from the study would be directly applicable to the patients [49]. The identification of specific biomarkers and validation of some candidate proteins is needed [113, 114], since the diagnosis of Parkinson's disease is currently assessed by the clinical evaluation of extrapyramidal signs [115, 116].

The connectivity between the enteric and the central nervous system via the vagus nerve probably play a critical role in the progression of PD so the best but challenging scenario would be to design a human-based enteric nervous system model [8], which would provide more credible insight into the mechanism of α-synuclein aggregation and transmission among Parkinson's disease patients.

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Conflict of interest

None declared.

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