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EXPERIMENTAL MODELS OF PRECLINICAL PARKINSON'S DISEASE

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ABSTRACT
Parkinson's disease (PD) is the second most common progressive neurodegenerative disease after Alzheimer's disease. Animal models are the best tools to study the pathogenesis of PD and are highly effective in the discovery of novel treatments for motor symptoms of PD. Models specifically based on pathogenic mechanisms may lead to the development of neuroprotective agents that slow disease progression. In this review, we try to provide summary of models for PD. These models include those produced by 6-hydroxydopamine (6-OHDA), 1-methyl-1,2,3,6-tetrahydropiridine (MPTP), paraquat, maneb and rotenone. Furthermore, now that we know that 10% of all PD cases are genetic in nature, we discuss some of the more common genetic rodent models of PD, like those related to alpha-synuclein, PINK1, Parkin and LRRK2 alterations. We also discuss some other models of PD.

KEYWORDS: Parkinson's disease; Alzheimer's disease; 6-hydroxydopamine; 1-methyl-1,2,3,6-tetrahydropiridine; paraquat; maneb and rotenone.

INTRODUCTION
Parkinson's disease [PD] is a progressive disease of the nervous system marked by tremor, muscular rigidity, and slow, imprecise movement, chiefly affecting middle-aged and elderly people. It is associated with degeneration of the basal ganglia of the brain and a deficiency of the neurotransmitter dopamine.[1] The term Parkinsonism is used for a motor syndrome whose main symptoms are tremor at rest, stiffness, slowing of movement and postural instability. Parkinsonian syndromes can be divided into four subtypes according to their origin.[2]

1. Primary or Idiopathic
2. Secondary[acquired] or Drug Induced
3. Hereditary parkinsonism, and
4. Parkinson plus syndromes or multiple system degeneration.

Animal models are an essential tool to study human diseases, not only to enable a thorough investigation into the mechanisms involved in the pathogenesis of a disease but also to help in the development of therapeutic strategies. It was through the use of an animal model that striatal dopamine deficiency was first associated with symptoms of Parkinson's disease (PD) and levodopa was first used to compensate for striatal dopamine loss. However, the mechanisms involved in PD pathogenesis and therefore its cure remain elusive to this day. It is therefore important to develop animal model(s) to understand the pathogenesis of PD and to develop therapeutic strategies to treat it. In the present chapter we will describe genetic as well as pharmacological manipulations used to develop animal models that mimic PD and discuss the advantages and disadvantages of the various models.[3]

A wide array of uncommon strategies and organisms has been used to produce animal PD models. However, due to space limitations here, we focus on rodents and non-human primates, and on approaches that have been widely used and reproduced. Basically, animal models of PD can be classified into three main categories: those based on neurotoxins or neuropharmacological agents targeting catecholaminergic neurons, those based on genetic manipulations relevant to PD (sometimes these two approaches are combined), and others (table 1).[4]

Locomotor activity[5]
Object: To study the locomotor activity of mice using actophotometer (activity cage).

Principle: Most of the central nervous system acting drugs influence the locomotor activities in man and animals. Most of the CNS depressant drugs such as

barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamines increase the activity. In other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity.

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats and mice may be used for testing in this equipment.

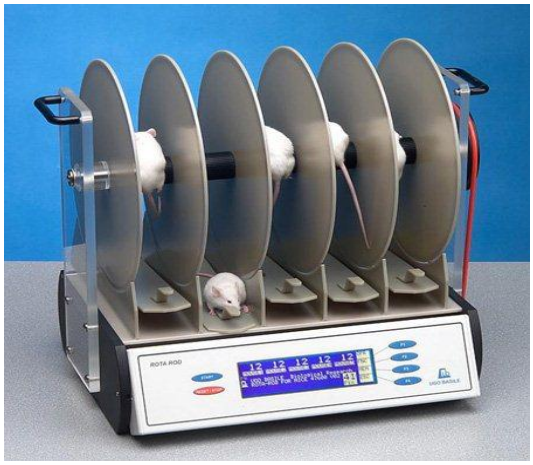
Requirements
Animal: Mice (20-25 g)
Drugs: CNS depressant drug (e.g. Chlorpromazine hydrochloride)
Equipment: Actophotometer.

Procedure
1. Weigh the animals and number them.
2. Turn on the equipment (check and make sure that all the photocells are working for accurate recording) and place individually each mouse in the activity cage for 10 min. Note the basal activity score of all the animals.
3. Inject drug, and after 30 min re-test each mouse for activity scores for 10 min. Note the difference in the activity, before and after injecting drug.
4. Calculate percent decrease in motor activity.

Inference: Reduction in the motor activity indicates CNS depressant property of the drug.

Motor Co-ordination test[5]
Object: To study the muscle grip strength in mice using Rota-rod apparatus.

Principle: One of the important pharmacological actions of antianxiety agents of benzodiazepine class of drugs is muscle relaxing property. The skeletal muscle relaxation together with taming or calming effect, these agents reduce anxiety and tension. The loss of muscle-grip is an indication of muscle relaxation. This effect can be easily studied in animals using rotating rods.



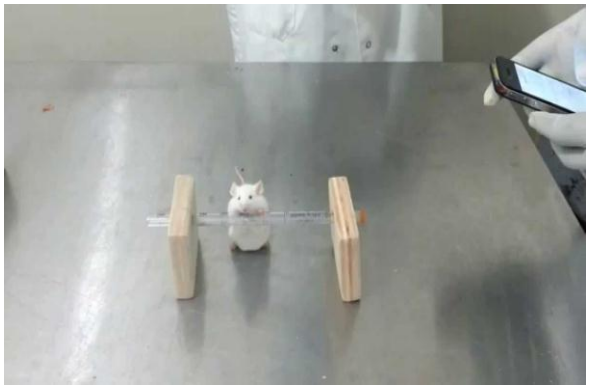
The difference in the fall off time from the rotating rod between the control and drug treated animal is taken as an index of muscle relaxation. The rate of rotation of the rod should be adjusted such that a normal mouse can stay on the rod for an appreciable period (3-5 min) of time.

Requirements
Animal: Mice (20-25 g).
Drugs: Muscle relaxant (e.g. Diazepam).
Equipment: Rota-rod.

Procedure
1. Weigh the animals and number them.
2. Turn on the Rota-rod. Select an appropriate speed (20-25 rpm is ideal).
3. Place the animal one by one on the rotating rod. (If the rod is divided into several compartments, one can place more than one mouse at a time). Note down the 'fall off time' when the mouse falls from the rotating rod. A normal (untreated) mouse generally falls off within 3-5 minutes.
4. Inject drug to all the animals. After 30 min. repeat the experiment as done in step 3. Note the fall off time.
5. Compare the fall off time of animals before and after drug treatment.

Catalepsy test[5]
Object: To study the antiparkinsonian effect using bar test.

Principle: Antipsychotic type of drugs are known to produce extrapyramidal side-effects in man. These effects, such as akinesia, rigidity and tremors, are called Parkinson's-like because in Parkinson's disease the major clinical symptoms include difficulty to move and change posture (akinesia and rigidity) and tremors. These effects of antipsychotic drugs are due to excessive blockade of dopamine receptors in the extrapyramidal motor system.



Requirements
Animal: Mice (20-25 g).
Drugs: Antipsychotic drug (e.g. Haloperidol).
Equipment: Two wooden bars, one being 3 cm high and other 7 cm high.

Procedure
1. Weigh and number animals.
2. Inject drug and observe severity of catatonic response as follows.

Stage I- Animal moves normally when placed on the table, score=0.
Stage II- Animal moves only when touched or pushed, score=0.5.
Stage III- Animal placed on the table with front paws set alternately on a 3 cm high bar fails to correct the posture in 10 seconds, score=0.5 for each paw with a total of 1 for this stage.
Stage IV- Animal fails to remove its front paws when placed alternately on a 7 cm bar, score=1 for each paw with a total score of 2 for this stage.

3. Observe the severity of catatonia at 5, 15, 30, 45, 60, 90 and 120 min after drug treatment.

1. The neurotoxin models
Several toxic animal models are currently in use in primates and rodents and, interestingly, with the exception of 6-hydroxydopamine (6-OHDA) and MPTP, these models are actually pesticide based. There are some drawbacks to the use of these models, but this fact does not negate the value of these animal models to the study of PD. They have opened crucial doors to increase our knowledge base of the events that may lead to the PD neurodegenerative process. Below are some of the positives and the negatives of these toxin-based PD models.

- **6-Hydroxydopamine (the classic PD model)**

6-Hydroxydopamine (6-OHDA) is the classic animal model of PD. The structure of 6-OHDA is similar to that of dopamine, but the presence of an additional hydroxyl group makes it toxic to dopaminergic neurons. This compound does not cross the blood–brain barrier which necessitates its direct injection into the substantia nigra pars compacta (SNpc) or the striatum. Injection of 6-OHDA into the SNpc, as first demonstrated by Ungerstedt in 1968, knocks out about 60% of the tyrosine hydroxylase (TH)-containing neurons in this area of the rat/mouse brain, with the subsequent loss of the TH-positive terminals in the striatum. 6-OHDA uses the DA transporter to gain access to the cytosol where it can auto-oxidize, hence generating an intracellular oxidative stress.[6]

The neurotoxicity of 6-OHDA occurs through a two-step mechanism involving accumulation of the molecule into catecholaminergic neurons by the DA and noradrenaline membrane transporters, followed by its inhibitory action on mitochondrial complex I. Injection of 6-OHDA in the SNc or in the MFB produces a massive (> 90%) and rapid (few days) degeneration of DAergic neurons.[7]

The use of 6-OHDA remains widespread today for both in vitro and in vivo investigations. Mice, cats, dogs, and monkeys are all sensitive to 6-OHDA; however, it is used much more frequently in rats. The lesions formed by this compound are directly proportional to the amount injected. The amount of injected drug corresponds to the severity of the PD like symptoms. In small amount it is useful to produce partial lesions to create “early stage PD”, and as the concentration increases it can be utilized to induce “severe PD”.[8]

6-OHDA is best used as a unilateral model as the bilateral injection of this compound into the striatum produces not only severe adipsia, aphagia and seizures but also death more often than not. Although 6-OHDA does not produce or induce proteinaceous aggregates or Lewy-like inclusions like those seen in PD.[9]

The 6-OHDA model has also been used successfully to study the action mechanisms and the effects of classical antiparkinsonian therapies, such as L-DOPA, deep brain stimulation or both, as well as novel approaches such as, for instance, targeting metabotropic glutamate receptors, all of which can improve (at least partially) motor behaviour, possibly by normalizing synaptic transmission in the striatum and the BG. Finally, the concomitant depletion of the DAergic neurons by 6-OHDA injection in the MFB, combined with noradrenaline and/or serotonin depletions, significantly induced anxiety-like states, anhedonia and ‘‘depressive-like’’ behaviour.[10]

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

MPTP is the gold standard model for PD. Ithas repeatedly demonstrated this ranking among PD researchers by replicating almost all of the hallmarks of PD in monkeys and other higher mammals and a significant number of these hallmarks in mice, but not in rats, which were found to be resistant to this toxin.[11]

MPTP is a highly lipophilic mitochondrial complex I inhibitor that easily crosses the BBB and thus it can be administrated systemically to obtain experimental PD models. It is not toxic per se, but it becomes toxic

through its transformation to 1-methyl-4- phenyl-2,3-dihydropyridinium (MPDP+) by monoamine oxidase B (MAO-B) in glial cells and serotoninergic neurons and its subsequent spontaneous oxidation into 1- methyl-4-phenylpyridinium (MPP+), which is the ‘‘real’’ toxic agent.[12]

The DA transporter (DAT) then carries MPP+ into DAergic neurons, where it accumulates in the cytoplasm and into synaptic vesicles by the vesicular monoamine transporter (VMAT). The driving force of mitochondrial membrane potential (DCm) lets MPP+ enter these organelles, where it blocks complex I. MPTP is administered in mice following several protocols, for example a single high dose or repeated intraperitoneal (i.p.) injections of low doses leading to significant loss of DAergic neurons of the SNpc (and in some cases also of the VTA), while rats are immune to this toxin. Generally, MPTP-treated mice develop motor impairments that can be detected by behavioural tests sensitive to subtle changes in DA levels. Protocols for monkeys usually consist in multiple low dose injections, generally producing consistent DAergic neuron loss accompanied by akinesia, ‘‘freezing’’, bradykinesia, muscle rigidity, abnormal posture, stereotypy and, sometimes, tremor. Also in this model, the degeneration of DAergic neurons is not accompanied by LB and general loss of catecholaminergic cells, at least in non-chronic models. However, some papers report that chronic MPTP intoxication in mice can generate LB-like neuronal inclusions containing ubiquitin and a-synuclein.[4]

- **Paraquat and Maneb Model**

Paraquat (N, N -dimethyl- 4–4–4 -bipiridinium) is an herbicide (weed killer) that exhibits a structural resemblance to MPP+ and because of this structural similarity, it was reasoned that paraquat should behave like MPP+. However, unlike MPP+, paraquat exerts its deleterious effects through an oxidative stress mediated by redox cycling, which generates reactive oxygen species, particularly the superoxide radical, hydrogen peroxide and the hydroxyl radical that lead to the damage of lipids, proteins, DNA and RNA.Recent evidence on the effects of paraquat in the nigrostriatal DA system is somewhat confusing in that, on the one hand, reports note that, following the systemic application of this herbicide to mice, the animals exhibit reduced motor activity and a dose-dependent loss of striatal TH-positive striatal fibres and SNpc neurons. Paraquat does cross the blood–brain barrier, although slowly, and to a limited extent. When injected systemically into mice it caused a dose-dependent decrease in dopaminergic nigral neurons and striatal dopaminergic innervation, followed by reduced ambulatory movement.[13]

Maneb (Manganese ethylene-bis-dithiocarbamate) has been shown to decrease locomotor activity and potentiate MPTP effects, the major active fungicidal component of maneb, manganese ethylene-bis-dithiocarbamate (Mn-EBDC), when administered directly into the lateral ventricles in rats, was selectively toxic to the dopaminergic system, induced extensive striatal dopamine efflux and preferentially inhibited mitochondrial complex III.[14]

Combined paraquat and maneb exposures produced greater effects on the dopaminergic system then either of the chemicals alone including reduced motor activity and increased degeneration of striatal dopaminergic terminals and nigral cell bodies. The evidence that combined exposure to paraquat and maneb targets the nigrostriatal dopamine systems and induces motor impairment gives credence to the theory that environmental toxins and pesticides may have a role in PD pathogenesis. Further investigations using these pesticide models will help to determine the involvement of environmental exposures in the pathological, biochemical and clinical symptoms of PD.

- **Rotenone model**

Unlike paraquat, which is a pure herbicide, rotenone is both an herbicide and an insecticide. Its half-life is 3–5 days depending on exposure to sunlight. And, like MPTP, it is highly lipophilic and readily crosses the blood–brain barrier. Rotenone seems to replicate almost all of the hallmarks of PD, including complex I blockade, behavioural alterations, inflammation, synuclein aggregation, Lewy-like body formation, oxidative stress and gastrointestinal problems. It is the most potent member of the rotenoid family of neurotoxins found naturally in tropical plants. The half-life of rotenone is 3–5 days depending on its exposure to sunlight, and it is rapidly broken down in soil and in water.

Chronic exposure to low doses of rotenone results in inhibition of the mitochondrial electron transport chain in the rat brain. In animals, rotenone has been administered by different routes. Oral administration appears to cause little neurotoxicity. 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. Intraperitoneal injections have been reported to elicit behavioural and neurochemical deficits, although mortality is very high. Intravenous administration is able to cause damage to nigrostriatal DA neurons that is accompanied by α-synuclein aggregation, Lewy-like body formation, oxidative stress, and gastrointestinal problems.[15]

However, Rotenone causes high mortality in laboratory animals, and DAergic damages that are variable and difficult to replicate. Finally, an interesting outcome has emerged from intragastric administration of this toxin, showing a-synuclein aggregates the enteric nervous system, the dorsal motor nucleus of the vagus, the intermediolateral nucleus of the spinal cord and the SN, supporting the hypothesis of a trans-synaptic spreading of synucleopathy in PD originating in the guts.[16]

- Other Model: Amphetamine-like psychostimulants

2. Genetic Models

- **a-Synuclein**

a-Synuclein, a presynaptic protein, is heterogeneously expressed throughout the brain. a-Synuclein's association with PD followed the identification of mutations in a-synuclein gene in some rare cases of familial PD as well as its presence in LBs. Point mutations (A30P, A53T and E46K) or multiplications (duplication and triplication) of the SNCA gene (a-synuclein, PARK1 and 4) have been identified as the cause of autosomal dominant forms of familial PD. However, the role of a-synuclein in neurodegeneration and PD pathogenesis is not clearly understood. Therefore, animal models have been developed to investigate the role of a-synuclein in the etiology of PD. These model systems have focused on the use of transgenic mice, or Drosophilia or fruit flies and Caenorhabditis elegans overexpressing wild-type or mutated a-synuclein.[17]

- **Parkin**

Parkin (coded by PRKN gene, PARK2) is a ubiquitin E3 ligase, and the loss of this enzymatic activity is thought to play a role in both familial and sporadic PD. Autosomal Recessive Juvenile Parkinsonism (AR-JP) was first identified in Japanese PD patients due to mutation of PARKIN gene., and they account for autosomal recessive and early-onset PD cases, generally consisting in deletions in exons 2, 3 and 8, duplications in exons 2–4 and 9, and P437L substitution.[18]

Mouse models are typically produced by full deletion, deletions in exons 2, 3 or 7, and in some cases parkin deletion combined with the expression of mutated a-synuclein. Exons 2 and 3 mutated mice show no overt DAergic loss and little or no behavioral deficits, while exon 3-null also display increased striatal glutamate levels, mild mitochondrial dysfunction, reduced antioxidant capacity, and increased oxidative damage. Exon 7-null mice lack significant loss of SNcDAergic neurons and exhibit several changes in the brain catecholaminergic systems, but no behavioural deficits. Thus, overall, these parkin PD models are not considered very useful.[19]

- **PINK1**

PINK1 protein (PTEN-induced putative kinase 1, PARK6) interacts with parkin to induce autophagy of depolarized mitochondria (mitophagy), thus is thought to protect cells from mitochondrial dysfunction.[115] Mutations (missense or nonsense) in PINK1 gene were first identified in Italian families with autosomal recessive and early-onset PD cases. PINK1/ mice show mild mitochondrial deficits and increased vulnerability to oxidative stress and ROS and a progressive reduction of weight and of locomotor activity at old age accompanied by a mild reduction of brain DA levels.[20]

In general, PINK1/ mice show neither changes at the level of the striatonigralDAergic system nor LB-like aggregates. Although one paper has reported that overexpression of a-synuclein in the substantia nigra of PINK1/ mice resulted in enhanced DAergic neurodegeneration. Overall, PINK1 models do not produce functional disruption of the nigrostriatal DAergic neurons or other PD-related defects, thus their usefulness is questionable.[21]

- **DJ-1**

The protein coded by DJ-1 gene (PARK7) has various functions, including transcriptional regulation, oxidative stress sensor, protease and mitochondrial regulation. Missense DJ-1 mutations are linked to autosomal recessive and early-onset PD, initially found in Dutch and Italian families. DJ-1/ mice have no loss of SNcDAergic neurons, but they show reduced striatal DA release and responsiveness to D2 autoreceptor stimulation, decreased locomotor activity and altered corticostriatal synaptic plasticity, as well as an increased vulnerability to MPTP.[22]

In another model in which DJ-1 loss was obtained by ''gene trap'' technique, mice showed upregulation of mitochondrial respiratory activity, less DAergic neurons in the VTA but no decreased DAergic terminals in the striatum, paralleled by changes in object recognition, suggesting the implication of this protein in early phase non-motor symptoms of PD and the existence of compensatory mechanisms, while, using DJ-1- null mice backcrossed with C57BL/6, reports early-onset, unilateral nigrostriatal degeneration, which progresses and becomes bilateral with age leading to mild motor deficits.[23]

- **LRRK2**

The large LRRK2 protein (leucine-rich repeat kinase 2, also known as dardarin, PARK8) has multiple domains and functions, among which a kinase domain, a RAS domain and a GTPase domain, and it interacts with parkin. Missense and point mutations in LRRK2 gene have been found in late-onset and autosomal dominant cases of PD and they are particularly enriched (20% and 40%, respectively) in PD patients. The most common mutations of LRRK2 gene mainly involve the kinase (G2019S) or the GTPase (R1441C/G) domain, and both have been reproduced in mouse models, where mild DAergic degeneration is sometimes observed, as well as alterations in DA release and uptake, while LRRK2 KO mice do not show DAergic deficits or other neuropathological features.[24]

BAC transgenic mice expressing mutated LRKK2 have also been developed, showing age-dependent and progressive motor impairment and slight reduction of DA release in the striatum, but no nigrostriatal degeneration. Overall, LRRK2 mouse models produce only mild DAergic deficits or other pathological processes relevant to PD, therefore it is unlikely that they

will be valuable for testing therapeutic agents or studying PD pathophysiology. On the other hand, a rat LRRK2 model with neuron-specific, adenoviral-mediated expression of LRRK2G2019S in the nigrostriatal system has been produced, which develops a progressive degeneration of nigral DAergic neurons.[25]

3. Other models

- **SHH, Nurr1, Pitx3 and EN1 models**

The conditional ablation of Shh from DAergic neurons resulted in progressive, adult-onset degeneration of DAergic, cholinergic, and fast spiking GABAergic neurons of the mesostriatal circuit, imbalance of cholinergic and DAergic neurotransmission, and motor deficits. Ablation of Nurr1 in midbrain DAergic neurons of young mice resulted in rapid loss of striatal DA, DAergic neuron markers and striatonigral degeneration without LB-like aggregates, while a more slowly progressing process was observed after ablation in adults. Interestingly, increased Nurr1 expression is found in midbrains of PD patients (particularly in neurons containing LB), and several variants of Nurr1 have been reported in association with PD, suggesting that this transcription factor is not only essential in the development and maintenance of mensencephalic DAergic neurons, but it may also play a role in PD pathogenesis. The Pitx3 KO mouse (also called ‘‘aphakia mouse’’) shows a complete loss of nigrostriatal DAergic neurons at early postnatal age and motor deficits corrected by L-DOPA. Interestingly, Pitx3 polymorphisms have been associated with an increased risk for PD. Finally, En1+/ mice have been shown to develop a progressive retrograde degeneration of nigrostriatal DAergic neurons.[26,27,28]

- **The ‘‘MitoPark’’ model**

The mitochondrial transcription factor A (TFAM) is required for the transcription of mtDNA coding for essential subunits of the mitochondrial respiratory chain. The conditional deletion of TFAM gene in DAergic neurons in mice resulted in the MitoPark model, characterized by adult-onset and slowly progressive motor impairment, degeneration of nigrostriatal DAergic neurons and intraneuronal inclusion not containing a-synuclein. Due to such strong outcome in terms of phenotype, pathology and progressivity, and to the involvement of mitochondrial deficits in PD, the MitoPark model may represent a valuable tool for testing neuroprotective strategies as well as further characterize PD pathogenesis.[29,30]

- **The PDC model**

This model is based on the rationale that oxidative stress due to GSH depletion and excitotoxicity have been implicated in DAergic neuron vulnerability in PD, and on the knowledge, that the EAAT substrate inhibitor PDC can cause both these insults. A single, unilateral injection of PDC in the SN of rats triggers a progressive loss of SNcDAergic neurons, without affecting non-DAergic neurons of the substantia nigra pars reticulata (SNr). More precisely, DAergic neuron loss was significant (21%) at 4 days postinjection (dpi) in the ipsilateral SNc, and it progressed to 31, 41, 47 and 57% at, respectively, 15, 30, 60 and 120 dpi. Surprisingly, neuronal death also reached the contralateral SNc with a 19 and 36% loss at 60 and 120 dpi, when motor deficits were detected, and the neurodegeneration followed a caudo to rostral pattern at both sides. Interestingly, non-DAergic neurons of the SNr were spared, and slice electrophysiological recordings showed that PDC application depolarized these cells, via glutamate signaling, much less than SNcDAergic ones, suggesting that glutamate-mediated mechanisms might underlie such selective vulnerability. Moreover, PDC injection provoked a significant decrease in GSH content and an increase in lipid peroxidation. Accordingly, NMDA receptor antagonists (ifenprodil and memantine) as well as the antioxidant N-acetylcysteine could reduce SNcDAergic neuron loss. Such novel model with progressive, self-propelling nigrostriatal neurodegeneration, and recapitulating several main pathophysiological processes of PD, might thus be useful for testing neuroprotective and disease-modifying strategies.[31,32]

Table 1-Summary of the main models of Parkinson's disease. The outcome of each model is rated with a 4-level scale: (-) absent; (+) weak; (++) moderate; (+++) strong.

Category	Model	Main pathogenic mechanisms	Usual administration of induction mode	Nigrostriatal degeneration	Progressivity	LB-like inclusions	Motor symptoms
Neurotoxic models	6-OHDA	Complex-I inhibition	Injection in SNc or MFB	+++	-	-	+++
			Injection in striatum	++	+		++
	MPTP	Complex-I inhibition	Systemic injection (single or repeated)	++	+	-	++/+++
	Paraquat*	Complex-I inhibition, ROS production	Systemic injection (single or repeated)	++	+	+	+
	Maneb*	Impairment of glutamate and DA uptake	Systemic injection	+	+	-	+
			Associated with Paraquat	++	+	+	++
	Rotenone	Complex-I inhibition, ROS production, etc.	Systemic (injection, oral, etc.) or brain injection	++	+	+	+
Genetic models	a-synuclein (PARK1,4)	LB-like aggregate toxicity?	Overexpression, mutation, injection of aggregates	+	+	++	+
	Parkin (PARK2)	Loss of ubiquitin E3 ligase activity?	Mutations	+/-	+/-	+/-	+/-
	Pink1 (PARK6)	Mitochondrial insult?	KO	+/-	+/-	+/-	+/-
	DJ-1 (PARK7)	Oxidative stress, mitochondrial dysfunction?	KO	+/-	+/-	+/-	+/-
	LRRK2 (PARK8)	Loss of enzymatic activity?	Mutations, KO	+/-	+/-	+/-	+/-
Others	SHH, Nurr1, Pitx3, EN1	Impaired protein synthesis in DAergic neurons	KO	++	+/-	-	+/-
	MitoPark	Mitochondrial deficit	KO	++	++	+/-	+
	PDC	Acute EAATs blockade, excitotoxicity, oxidative stress	Injection in SNc	++	+++	-	+

6-OHDA: 6-hydroxydopamine; DA: dopamine; DAergic: dopaminergic; EAATs: excitatory amino acid transporters; EN1: engrailed 1; LB: lewy body; MFB: medial forebrain bundle; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Nurr1: nuclear receptor related protein-1; PDC: L-trans-pyrrolidine-2,4-dicarboxylate; PINK1: PTEN-induced putative kinase 1; Pitx3: pituitary homeobox 3; ROS: reactive oxygen species; SHH: sonic hedgehog; SNc: substantia nigra pars compacta.

CONCLUSION

Although animal models of PD do not reproduce the human condition, they have however contributed to clarify some hypotheses about its pathophysiology, to test the efficacy of novel antiparkinsonian treatments and to study the action mechanisms of ''classic'' treatments such as L-DOPA and deep brain stimulation. Given the strengths and weaknesses of PD animal models, the main ''take home message'' of this review would be that each of them can be appropriate for addressing specific questions, such as PD pathophysiology (genetic models), neuroprotection (genetic and other models) or symptomatic treatments (toxin models). Possibly, in the future, a combination of two or more of these models that would, for instance, mimic the contribution of genetic and environmental factors, thus providing novel models more relevant to the complexity of PD etiology, pathology and symptomatology. Chronic treatment (e.g. MPTP, rotenone) appears to simulate PD pathology more accurately. Furthermore, transgenic models are useful for evaluating the role of genetics in PD pathogenesis. The choice of model to be used depends upon the goals of the particular experimental paradigm and the questions being asked.

REFERENCES

1. http://www.oxforddictionaries.com/definition/english/Parkinson's-disease.
2. Jankovic J "Parkinson's disease: clinical features and diagnosis". Journal of Neurology, Neurosurgery, and Psychiatry, 2008; 79(4): 368–376.
3. Carlsson A, Lindqvist M, Magnusson T (1957). 3, 4-dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature, 180: 1200.
4. Gubellini P, Kachidian P. Animal models of Parkinson's disease: An updated overview. Revue neurologique (2015).
5. Kulkarni SK. Handbook of experimental pharmacology. New Delhi: Vallabh Prakashan, 2002; 131, 136, 158.
6. Lee CS, Sauer H, Bjorklund A. Dopaminergic neuronal degeneration and motor impairments following axon terminal lesion by instrastriatal 6-hydroxydopamine in the rat. Neuroscience, 1996; 72: 641–53.
7. Blum D, Torch S, Lambeng N, Nissou M, Benabid AL, Sadoul R, et al. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog Neurobiol, 2001; 65(2): 135–72.
8. Amalric M, Koob GF. Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reactiontime performance in rats. J Neurosci, 1987; 7(7): 2129–34.
9. Alves da Costa C, Dunys J, Brau F, Wilk S, Cappai R, Checler F. 6-Hydroxydopamine but not 1-methyl-4-phenylpyridinium abolishes alpha-synuclein anti-apoptotic phenotype by inhibiting its proteasomal degradation and by promoting its aggregation. J Biol Chem, 2006; 281: 9824–31.
10. Oueslati A, Sgambato-Faure V, Melon C, Kachidian P, Gubellini P, Amri M, et al. High-frequency stimulation of the subthalamic nucleus potentiates L-DOPA-induced neurochemical changes in the striatum in a rat model of Parkinson's disease. J Neurosci, 2007; 27(9): 2377–86.
11. Chiueh CC, Markey SP, Burns RS, Johannessen JN, Jacobowitz DM, Kopin IJ. Neurochemical and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig, and monkey. Psychopharmacol Bull, 1984; 20: 548–53.
12. Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. Trends Pharmacol Sci, 2010; 31: 141–2; author reply: 142–3.
13. Brooks AI, Chadwick CA, Gelbard HA et al. (1999). Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res, 823: 1–10.
14. Chung KK, Zhang Y, Lim KL et al. (2001). Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. Nat Med, 7: 1144–1150.
15. Greenamyre JT, Cannon JR, Drolet R, Mastroberardino PG. Lessons from the rotenone model of Parkinson's disease. Trends Pharmacol Sci, 2010; 31(4):141–2.
16. Hawkes CH, Del TK, Braak H. Parkinson's disease: the dual hit theory revisited. Ann N Y Acad Sci, 2009; 1170: 615–622.
17. RANJITA BETARBET, J. TIMOTHY GREENAMYRE "Parkinson's disease: animal models" Handbook of Clinical Neurology, Vol. 83 (3rd series) Parkinson's disease and related disorders, Part I, 2007 Elsevier
18. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature, 1998; 392(6676): 605–8.
19. Perez FA, Palmiter RD. Parkin-deficient mice are not a robust model of parkinsonism. Proc Natl Acad Sci U S A, 2005; 102(6): 2174–9.
20. Gispert S, Ricciardi F, Kurz A, Azizov M, Hoepken HH, Becker D, et al. Parkinson phenotype in aged PINK1- deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. PLoS One, 2009; 4(6): e5777.
21. Oliveras-Salva M, Macchi F, Coessens V, Deleersnijder A, Gerard M, Van der PA, et al. Alpha-synuclein-induced neurodegeneration is exacerbated in PINK1 knockout mice. Neurobiol Aging, 2014; 35(11): 2625–36.
22. Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, et al. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc Natl Acad Sci U S A, 2005; 102(14): 5215–20.[125]
23. Pham TT, Giesert F, Rothig A, Floss T, Kallnik M, Weindl K, et al. DJ-1-deficient mice show less TH-positive neurons in the ventral tegmental area and exhibit non-motoric behavioural impairments. Genes Brain Behav, 2010; 9(3): 305–17.
24. Hinkle KM, Yue M, Behrouz B, Dachsel JC, Lincoln SJ, Bowles EE, et al. LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. Mol Neurodegener, 2012; 7: 25.
25. Dusonchet J, Kochubey O, Stafa K, Young Jr SM, Zufferey R, Moore DJ, et al. A rat model of progressive nigral neurodegeneration induced by the Parkinson's diseaseassociated G2019S mutation in LRRK2. J Neurosci, 2011; 31(3): 907–12.
26. Jankovic J, Chen S, Le WD. The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. Prog Neurobiol, 2005; 77(1–2): 128–38.
27. Nordstrom U, Beauvais G, Ghosh A, PulikkaparambilSasidharan BC, Lundblad M, Fuchs J, et al. Progressive nigrostriatal terminal dysfunction and degeneration in the engrailed1 heterozygous mouse model of Parkinson's disease. Neurobiol Dis, 2015; 73: 70–82.
28. Le PG, Sonnier L, Hartmann A, Bizot JC, Trovero F, Krebs MO, et al. Progressive loss of

dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1: a new genetic model for Parkinson's disease? Parkinsonism Relat Disord, 2008; 14(Suppl. 2): S107–11.

29. Falkenberg M, Larsson NG, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem, 2007; 76: 679–99.
30. Ekstrand MI, Galter D. The MitoPark Mouse – an animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. Parkinsonism Relat Disord, 2009; 15(Suppl. 3): S185–8.
31. Nafia I, Re DB, Masmejean F, Melon C, Kachidian P, Kerkerian-Le GL, et al. Preferential vulnerability of mesencephalic dopamine neurons to glutamate transporter dysfunction. J Neurochem, 2008; 105(2): 484–496.
32. Assous M, Had-Aissouni L, Gubellini P, Melon C, Nafia I, Salin P, et al. Progressive Parkinsonism by acute dysfunction of excitatory amino acid transporters in the rat substantia nigra. Neurobiol Dis, 2014; 65: 69–81.