Animal Models of Parkinson’s Disease

O. von Bohlen und Halbach
Interdisciplinary Center for Neurosciences (IZN), University of Heidelberg, Heidelberg, Germany

Key Words
Mouse · Monkey · α-Synuclein · DJ-1 · Substantia nigra · Striatum

Abstract
Parkinson’s disease (PD) is one of the major neurodegenerative disorders. The etiology of this disease is likely due to combinations of environmental and genetic factors. Symptomatic hallmarks of PD are tremor, bradykinesia, rigidity and postural instability. On the morphological and anatomical level, PD is characterized by massive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to a severe loss of striatal dopaminergic fibers and to a massive reduction of dopamine levels in the striatum. In addition, PD is characterized by the appearance of Lewy bodies within the surviving dopaminergic neurons. Animal models of PD allow getting insight into the mechanisms of several symptoms of PD thereby providing indispensable tools for basic and applied research. The biochemical and cellular changes that occur following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rodents or monkeys are remarkably similar to those seen in idiopathic PD. In this review, the main characteristics of experimental models of PD induced by the neurotoxic compound MPTP are reviewed.

Copyright © 2005 S. Karger AG, Basel

Introduction
Parkinson’s disease (PD) is the second most common neurodegenerative disease, primarily affecting people of ages over 55 years, although young adults and even children can also be affected. PD, first reported by James Parkinson in 1817, is a neurological disorder that is characterized by resting tremors, rigidity, bradykinesia and postural instability. Although the etiology of this disease is still unknown, it is thought to be multifactorial, with a predominance of environmental factors that act on genetically predisposed individuals with aging [1]. It is widely accepted that the main behavioral disturbances in PD are the consequence of a substantial loss of dopaminergic nigral neurons and a depletion of dopamine in the striatum. However, the clinical symptoms do not fully develop until there is a loss of about 50–60% of the dopaminergic neurons within the substantia nigra pars compacta and a loss of about 70% of the striatal dopamine [2]. A further histopathological hallmark of the disease is the presence of intracytoplasmic inclusions called Lewy bodies within the surviving dopaminergic neurons. The major compound of these eosinophilic Lewy bodies are aggregated forms of the normally presynaptically located protein α-synuclein [3]. This abnormally aggregated α-synuclein in the Lewy bodies is associated with synphilin-1 [4]. Although the mechanisms underlying the gradual transition of soluble α-synuclein into vir-
tually insoluble Lewy bodies or Lewy neurites are still unknown [5], this feature serves as a marker for the visualization of PD-related lesions [6]. In this context, it should also be mentioned that specific cases of PD exist that do not show the classic Lewy body pathology, e.g. a somatic recessive juvenile form of PD that is due to a mutation in a gene coding for parkin [7]. Patients with parkin mutations exhibit all classic symptoms of PD, but lack Lewy bodies [8]. Moreover, in many instances, the parkinsonism seen in association with toxins is not that of typical Lewy body PD [9].

Neurological disorders in humans can be modeled in animals using standardized procedures that recreate specific pathogenic events and their behavioral outcomes. In addition to providing an indispensable tool for basic research, animal models of human disorders allow to investigate therapeutic strategies as a prerequisite to their testing in patients [10]. Since the common pharmacological therapies are limited to a treatment of the symptomatology of PD without arresting the course of the disease, it is extremely important to develop experimental animal models that replicate several aspects of this human disease. Availability of suitable animal models could further allow analyzing the mechanisms that underlie the etiology of this disease and may facilitate the development of novel neuroprotective agents or therapeutic strategies. Such animal models should reproduce the main characteristics of the human disease, such as: (1) selective lesion of dopaminergic neurons that evolves over time; (2) depletion of dopamine from the striatum, and (3) presence of Lewy bodies in the remaining dopaminergic neurons.

In addition, from the behavioral point of view, a parkinsonian syndrome should be observed, ideally with akinesia, rigidity and resting tremor.

However, the relationship between genetic and environmental factors in PD is poorly understood, and most models of PD focus on a single gene or a single toxin. The recent genetic discoveries have led to the identification of several genes and proteins that are involved in PD, e.g. α-synuclein, synphilin-1, parkin, DJ-1, UCH-L1 and dardarin (LRRK2). All these genes and proteins seem to be linked to the ubiquitin-proteasomal pathway [11, 12], and gene-based animal models will help to examine the biochemical pathways. Specific knockout mice, or mice overexpressing one of these genes as well as mice carrying a missense mutation in one of these genes are indispensable for getting insight into these mechanisms. Indeed, several genetic mouse models are available; however, the use of specific neurotoxins are available; however, the use of specific neurotoxins reproducing specific features of PD is currently more common.

Agents that selectively disrupt or destroy the catecholaminergic system, such as 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), are widely used as animal models of PD [13]. Moreover, it has been reported that agricultural chemicals, such as rotenone, paraquat or maneb, when administered systemically, can induce specific features of PD [14]. These above-mentioned substances were also used to develop animal models of PD. A common feature of these chemical compounds is that they all act on mitochondria of nigral dopaminergic neurons, either by inhibiting mitochondrial complex I or by inhibiting complex III (fig. 1).

**Fig. 1.** MPTP is converted by monoamine oxidase B (MAO B) to MPP⁺. MPP⁺ is taken up by dopamine transporters (DAT) and can then be accumulated by mitochondria, leading to complex I inhibition. MPP⁺ can also be taken up by VMAT and transported into synaptic vesicles, which reduces the toxicity of MPP⁺.
This review focuses on one of the most widely used toxin-induced PD model: the MPTP model.

**Biochemistry**

MPTP is a side-product of the chemical synthesis of a meperidine analog with potent heroin-like properties. Drug addicts who took MPTP accidentally developed a syndrome that clinically and pathologically resembled PD [15]. Seven patients developed chronic and severe parkinsonism, which was L-dopa responsive, after repeatedly injecting MPTP intravenously. Four of the 7 patients with moderate to severe MPTP-induced parkinsonism exhibited a tremor indistinguishable from the characteristic rest tremor of PD [16]. Several years later, neuropathological examination revealed moderate to severe depletion of pigmented nerve cells in the substantia nigra in each case, but an absence of Lewy bodies [17].

It soon became clear that MPTP apparently acted as a neurotoxin, which preferentially affected dopaminergic cells in the substantia nigra pars compacta. MPTP itself is highly lipophilic and readily crosses the blood-brain barrier. MPTP-mediated toxicity is induced through conversion to the 1-methyl-4-phenyl-2,3-dihydropyridine ion (MPP+) in astrocytes by monoamine oxidase B [18]. To exert its toxicity, MPP+ must be transported into dopaminergic neurons by neurotransmitter transporters. Once inside the neuron, MPP+ accumulates within mitochondria. Within mitochondria, MPP+ acts by inhibiting the electron transport system of the mitochondrial complex I (NADPH-ubiquinone oxidoreductase I; fig. 1). This leads to an impairment in ATP production, to an elevation of the intracellular calcium concentration and to the generation of free radicals, resulting in cellular energy failure [19] and in the formation of superoxide anions [20]. The selective toxicity of MPP+ to dopaminergic neurons derives, at least in part, from its high affinity for the dopamine transporter [21]. In line with this, mice lacking this transporter are protected from MPTP toxicity [22]. MPP+ is also sequestered into synaptic vesicles by the vesicular monoamine transporter (VMAT), and sequestration into vesicles decreases MPP+ toxicity by preventing its interaction with mitochondria [23, 24]. Thus, mice with a 50% depletion of VMAT2 show increased vulnerability to MPTP [25].

**MPTP Animal Models**

MPTP currently represents the most important and most frequently used parkinsonian toxin applied in animal models [13, 26, 27] and has several advantages over other neurotoxic PD models, since, in humans, it induces symptoms virtually identical to PD and directly elicits a specific intoxication of dopaminergic structures [28]. Most of the current evidence concerning mechanisms of cell death in PD originates from studies using the MPTP model [2], indicating that dopamine agonists, iron chelators, nitric oxide synthase inhibitors, radical scavengers, and certain calcium channel antagonists provide neuroprotection if administered prior to the intoxication by MPTP [29].

MPTP is mainly used in nonhuman primates and in mice but also in several other species such as dog, cat, rat, and goldfish [13]. With regard to the species used, several distinct routes of MPTP administration have been established. In principle, MPTP can be given by a variety of regimens, such as gavages or stereotactical injections, but the most common and reproducible form of application is the systemic (e.g. intramuscular, intraperitoneal, intravenous or subcutaneous) administration [27]. At present, the mouse MPTP model provides the most useful animal model of PD to study neuropathological and neurochemical changes [30], whereas for behavioral tests, the monkey MPTP model appears much more suitable [13].

In contrast to mice, rats are relatively insensitive to MPTP [31]. Thus, rats injected with doses of MPTP comparable to those in mice do not exhibit any significant dopaminergic neurodegeneration for unknown reasons. Only injections of high doses of MPTP cause dopaminergic neurodegeneration in rats. However, rats have to be pretreated with guanethidine to prevent peripheral catecholamine release and extensive mortality [32].

**The Mouse MPTP Model**

In mice, systemic or intracranial application of MPTP can lead to severe damage of the nigrostriatal dopaminergic system, including symptoms of motor control disturbances resembling those of human PD, such as akinesia, rigidity, tremor, gait and posture abnormalities [33]. In MPTP-injected mice, a dramatic loss of dopaminergic neurons has been detected leading to impaired dopaminergic neurotransmission, which is accompanied by a loss of dopaminergic nerve terminals in the striatum and a dramatic reduction in striatal dopamine levels (fig. 2). The cell loss in the substantia nigra pars compacta is ac-
companied by an increase in the number of α-synuclein-immunoreactive neurons located in this brain area [34, 35] and an increase in α-synuclein mRNA [36]. However, when MPTP-treated animals developed α-synuclein inclusion bodies, these aggregates usually failed to reproduce the structure of Lewy bodies [37]. Thus, the presence of true Lewy bodies in the remaining neurons of the murine substantia nigra has not convincingly been demonstrated. Likewise, in humans, MPTP intoxication induces a depletion of pigmented nerve cells in the substantia nigra, but Lewy bodies have not been detected [17].

The mode of MPTP administration in mice has been shown to determine the mode of neuronal cell death occurring in the substantia nigra. Chronic regimens cause apoptotic cell death of dopaminergic neurons [38], whereas acute regimens of MPTP cause necrotic cell death [39]. Acute regimens of MPTP did not only result in death of dopaminergic neurons but also, at least during the first 4 days after injection, in a loss of the dopaminergic phenotype without necessarily destroying these neurons [39].

The mouse MPTP model can also mimic several further characteristics of human PD. In addition to classic motor signs and symptoms, PD is characterized by neuropsychological and emotional deficits, including a blunted emotional response. One of the brain structures critically involved in the processing of emotions is the amygdala. It has been shown that in humans suffering from PD, the amygdala is also affected [40]. PD patients, in contrast to normal healthy subjects, display only a minor bilateral amygdala response during a hypodopaminergic state in a paradigm that involves perceptual processing of fearful stimuli [41]. These results provide evidence for a role of dopamine in modulating the response of the amygdala to sensory information in humans. Moreover, it has been demonstrated that there is a decrease in corticotropin-releasing factor concentration in patients with PD [42]. Chronic MPTP treatment also decreases the number of corticotropin-releasing-factor-immunoreactive neurons, e.g. in the central nucleus of the amygdala [43], indicating that the amygdala might also be disturbed in this mouse model of PD. This raises the question whether the dopaminergic innervation of the amygdala might also be affected in the mouse MPTP model. In line with this, it has recently been shown that the numbers of fibers immunoreactive for tyrosine hydroxylase (the rate-limiting enzyme in catecholamine biosynthesis) are decreased in the amygdala of MPTP-treated mice [44].

However, the mouse MPTP model has several drawbacks. Like all other mouse models of PD that are based on pharmacological treatments, the slow time course of the progression of the human disease cannot be replicated in these models, and, as mentioned before, the mouse MPTP model normally failed to induce the generation of true Lewy bodies (see above).

It should also be mentioned that mouse strains differ in their sensitivity to MPTP. Especially the C57/Bl6 strain was found to be more sensitive to systemic injection of MPTP and highly selective in terms of targeting the nigrostriatal dopaminergic neurons compared to other mouse strains [30]. At least in some mouse strains MPTP intoxication does not only induce neurodegeneration within the substantia nigra, but also in other brain areas. CD-1 mice, for example, that received a single 50 mg/kg dose of MPTP intraperitoneally display signs of neuronal degeneration not only in the substantia nigra pars compacta, but also in the ventral tegmental area and retrorubral field as well as in several thalamic nuclei [45].

**Genetic Mouse Models and MPTP**

Several gene polymorphisms have been identified that contribute to the development of PD in humans. Among them are e.g. different mutations in the α-synuclein gene [46–48].

Mice with a targeted deletion of the α-synuclein gene have been generated; these mice are viable and develop normally [49]. Their brain architecture is not affected and they show a normal complement of dopaminergic neurons. Even so, they display a reduction in the striatal dopaminergic content and an attenuated dopamine-dependent locomotor response following amphetamine treatment.
The reserve pool of neurotransmitter vesicles in the hippocampus is reduced [50]. Several different lines of α-synuclein knockout mice have been generated that either display a strong [51] or partial resistance [52, 53] towards neuronal changes induced by MPTP intoxication.

In addition, several transgenic mouse lines have been created that express mutated forms of α-synuclein. Mice overexpressing the mutant human α-synuclein A30P display an increased vulnerability of dopaminergic neurons to MPTP [54]. In contrast, overexpression of the mutant human α-synuclein A53T in the substantia nigra of normal and MPTP-treated mice by recombinant adeno-associated virus-mediated gene transfer does not increase the susceptibility of dopaminergic neurons to MPTP [55]. A further mouse mutant that has been developed overexpresses human wild-type α-synuclein under the Thy1 promoter. When these mice are treated with MPTP, they display extensive mitochondrial alterations, increased mitochondrial size, filamentous neuritic aggregations, axonal degeneration, and formation of electron-dense perinuclear cytoplasmic inclusions in the substantia nigra [56].

A further gene polymorphism has been described that contributes to the development of PD. In 2003, it was discovered that mutations in the DJ-1 gene can cause early-onset autosomal recessive PD [57]. DJ-1 mRNA [58] and protein [59] are expressed in the murine brain. DJ-1-deficient mice have recently been generated; these mice are viable, fertile, and show no gross anatomical or neuronal abnormalities [60]. However, DJ-1-deficient mice reveal increased striatal denervation and dopaminergic neuron loss induced by MPTP [60]. Interestingly, wild-type mice that received an adenoviral delivery of DJ-1 resisted MPTP-induced striatal damage, and neurons overexpressing DJ-1 were protected from oxidative stress in vitro [60].

Although the phenotypes of the α-synuclein knockout and transgenic mice as well as of the DJ-1 knockout mice are not dramatically altered, MPTP treatment of these mice revealed a higher sensitivity towards a challenge with MPTP (for a summary, see table 1). Since the etiology of PD is likely due to combinations of environmental and genetic factors, the use of mice with known genetic mutations associated with PD in combination with an MPTP treatment will be important for understanding the etiology and progression of PD.

### The Monkey MPTP Model

Aside from the mouse MPTP model, the monkey MPTP model has emerged as an important tool to investigate MPTP-induced neurodegeneration and associated behavioral disturbances. In both, humans and monkeys, MPTP causes damage to the nigrostriatal dopaminergic pathway comparable to damage seen in PD. Moreover, it is well established that MPTP produces, in both humans and monkeys, an irreversible and severe parkinsonian syndrome, featuring all of the cardinal symptoms of PD, including tremor, rigidity, slowness of movement, postural instability, and even freezing [61]. A further advantage of the monkey MPTP model is that the anatomical structures involved in the pathology of PD are not as different in humans as they are in mice. These differences include, for example, the fact that in monkeys and humans the striatum (nucleus caudatus and putamen) comprises discrete structures, whereas in rodents it is a single structure. Since the striatum is the target of many developing treatments for PD, this disparity in structural arrangement between the species is an important issue [62].

Like in humans, MPTP does not induce the formation of Lewy bodies in adult monkeys. However, in old MPTP-injected monkeys, intraneuronal proteinaceous inclusions reminiscent of Lewy bodies have been observed.
Like in mice or in humans, MPTP-treated baboons develop fine granular α-synuclein accumulations and some larger deposits, but these inclusions do not seem to represent fully developed Lewy bodies [64]. These α-synuclein aggregates might be indicative of early-onset Lewy body formation according to the stages proposed by Wakabayashi et al. [65]. Therefore, these MPTP-induced inclusions differed somewhat from ‘true’ Lewy bodies, and thus may represent immature Lewy bodies [66].

The monkey MPTP model represents an important tool for testing new therapeutic and neuroprotective strategies [27]. For example, electrophysiological studies in MPTP monkeys have shown that hyperactivity of the subthalamic nucleus is a key factor in the development of bradykinesia and rigidity [67, 68]. These findings were the basis for reconsidering the thalamus as a target for stereotactic thalamotomy and/or high-frequency stimulation [69, 70]. Indeed, electric stimulation of the subthalamic nucleus can be used as an effective neurosurgical treatment of PD [71, 72].

Nevertheless, because of ethical and economical constraints that are related to the experimental use of monkeys, this animal model of PD is exploited in relatively few laboratories worldwide [10, 13]. Moreover, there are some limitations in the MPTP model in general. In many cases, acute MPTP treatments were performed, which do not mimic the progressive degeneration of nigrostriatal dopaminergic neurons in PD. This can be overcome by a model of chronic MPTP regimens. However, long-term treatment with low doses of MPTP has resulted in recovery of motor deficits once the treatment is stopped [14]. In addition, it should always be taken into account that MPTP intoxication mimics several aspects of PD. Thus, in contrast to the normal time course of PD, the neurodegeneration seen in the substantia nigra after MPTP intoxication develops fast. Moreover, there is no major development of authentic Lewy bodies in case of MPTP intoxication, neither in rodents, monkeys nor even humans.

Currently, none of the genetic models and none of the models using neurotoxins fully recapitulate all key features, which clinically and pathologically characterize PD. However, each of the available animal models recapitulates specific features of PD. Nevertheless, aside from these aspects, the animal MPTP model is one of the most important animal models to study certain aspects of PD. Especially in combination with mutant animals, the MPTP model might allow to get further insights into the mechanisms and processes that contribute to the development of PD.

**Acknowledgement**

The work for this review was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 636).

---

**References**


318 Neurodegenerative Dis 2005;2:313–320

von Bohlen und Halbach
Animal Models of Parkinson’s Disease

Neurodegenerative Dis 2005;2:313–320


23 Reinhard JF Jr, Dilberio EJ Jr, Viversoh OH, Daniels AF: Subcellular compartmentalization of 1-methyl-4-phenylpyridinium with catecholamines in adrenal medullary chromaffin vesicles may explain the lack of toxicity to adrenal chromaffin cells. Proc Natl Acad Sci USA 1987;84:8160–8164.


