# REVIEW

# NEUROTOXIN-BASED MODELS OF PARKINSON'S DISEASE

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**Abstract**—Animal experimentation in the Parkinson's disease (PD) field is a classic example of how the use of animal models to study diseases can have a significant impact on human health. Among the different neurotoxin-based animal models of PD that are presently available, the 6-hydroxydopamine (6-OHDA) and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models have been established and validated as useful models for the development of therapeutic strategies aimed to treat motor symptoms and to study alterations of the basal ganglia that occur in this disease. The 6-OHDA rat model and the MPTP primate model have contributed enormously to translate animal experimentation into clinical practice, including pharmacological treatments and deep brain stimulation of the subthalamic nucleus. These models, along with the MPTP mouse model, are helping to elucidate the pathogenic mechanism of neurodegeneration in PD. The roles of oxidative stress, apoptosis, mitochondrial dysfunction, inflammation, and impairment of the protein degradation pathways have also come under careful consideration thanks to these models. The more recently developed paraquat and rotenone rodent models are also contributing to our understanding of neuronal cell death. However, none of the neuroprotective strategies that have worked in the pre-clinical stage have thus far been successfully translated to a clinical setting to treat PD patients. At the same time, the lack of any effective neuroprotective strategy for PD is preventing the validation of any one particular model as a screening tool for such neuroprotective strategies. Therefore, it seems that we are trapped in a vicious circle that casts doubt on the suitability of the neurotoxin-based models for this purpose. Here, we discuss how epidemiological data may help to validate a specific model with data linking a lower risk of developing PD with nutritional/consumption habits or with a specific chronic drug therapy.

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## Contents

The use of toxin-induced animal models has been crucial to elucidation of the pathophysiology underlying Parkinson's disease (PD) and to the development of therapeutic strategies aimed to treat its motor symptoms. Moreover, these models are being employed to shed light on the pathogenic mechanisms involved in PD-associated neuronal cell death with the ultimate objective that neuroprotectants can be developed to halt the progression of the disease. PD is a chronic, progressive neurodegenerative movement disorder that affects more than six million people worldwide (www.epda.eu.com). The average age at onset is 60, with age being an irrefutable risk factor for this idiopathic disease (de Rijk et al., 1997). Historical references to PD-like motor symptoms can be found throughout the ages, from 12th century B.C. Egyptian papyrus to the notebooks of the renaissance genius Leonardo da Vinci. However, it was not until James Parkinson published "An Essay on The Shaking Palsy" in 1987 and Jean-Martin Charcot added more symptoms to the former's original description, that PD gained recognition as a distinctive clinical entity. In this regard, PD is characterized by resting tremor, slowness of voluntary movements, rigidity, and postural instability. Importantly, these symptoms can be accompanied by non-motor symptoms that were initially eclipsed by the obvious movement impairment. Some of

these non-motor symptoms occur early in PD and may even precede the diagnosis that is based on motor signs (Tolosa et al., 2009). These non-motor symptoms include neuropsychiatric and sleep disorders, olfactory deficits, constipation, and male erectile dysfunction among others (Chaudhuri et al., 2006).

The anatomical substrate of PD remained unknown until 1919, when Konstantin Tretiakoff (Tretiakoff, 1919) presented his thesis linking for the first time the loss of neuromelanin-containing neurons of the substantia nigra pars compacta (SNpc) with PD. Nevertheless, the underlying pathophysiology of the condition was only revealed several decades later thanks to animal experimentation with drug-induced PD models. Arvid Carlsson and collaborators laid the foundation for the use of L-dopa as a PD treatment through their discovery that dopamine was a neurotransmitter involved in movement control (Carlsson et al., 1958). They demonstrated that a reserpine-induced akinetic state was reversed by the administration of the dopamine precursor L-dopa (Carlsson et al., 1957) and subsequently, Ehringer and Hornykiewicz (Ehringer and Hornykiewicz, 1960) demonstrated that PD patients exhibit a deficit in striatal dopamine that can be attributed to the loss of neurons in the SNpc. Later on, George Cotzias started treating PD patients with L-dopa, which to this day remains the gold standard treatment (Cotzias et al., 1968). Indeed, most of the PD motor symptoms can be explained by the impairment of the nigrostriatal pathway, although histological abnormalities are also found in many other dopaminergic and non-dopaminergic neurons, which may explain the sum total symptomatology of the disease. For instance, there is a dramatic loss of noradrenergic neurons in the locus coeruleus that accompanies the loss of the dopaminergic neurons in the SNpc. Both neuronal cell populations contain the pigment neuromelanin and both can develop intraneuronal eosinophilic inclusions called Lewy bodies, which are the histological hallmark of PD, also present in other neuronal populations (Fahn, 2003).

Following a classical categorization strategy, animal models can be grouped into "fidelity models" and "discrimination models" (Russell and Burch, 1959). On the one hand, a fidelity model reproduces the maximum number of characteristics of the original, whereas on the other a discrimination model reproduces only one particular characteristic of the original. One might think that a high fidelity model is always superior to a discrimination model; however, the latter can help by teasing out the irrelevant factors from the important. Regarding PD modeling, the case of the reserpine model is quite instructive. This toxin works by inhibiting vesicular monoamine transporter 2 (VMAT2), leading to a reversible depletion of monoamines. This model mimics the akinetic symptomatology of PD without being associated with nigral dopaminergic cell degeneration. However, the model made it possible to demonstrate that dopamine was involved in movement and thereby for L-dopa to be developed. As such, a PD model can be useful without having to recapitulate all the characteristics of the disease.

One of the characteristics of PD that all the toxin-induced animal models mimic is the neurodegeneration of the dopaminergic neurons of the SNpc. Each model nonetheless has its own particularities depending upon the species involved and the toxin used. In other words, dopaminergic cell death is the cornerstone of these animal models but the mechanism of action and the behavioral impairments induced differ among them. PD can be modeled at the histological, molecular, and behavioral levels. If the aim of the research is to study the consequences of dopaminergic cell death and to test dopaminomimetics or other strategies to treat the symptoms, the mechanism of action of the neurotoxin is irrelevant. However, this is not the case if the aim is to study the pathways involved in neuronal cell death and to develop neuroprotective strategies. For both circumstances, validation of the animal model used is based on the possibility to translate the results obtained with the model into a clinical application to treat PD patients. Regarding the first case, these models made it possible for L-dopa and other drugs targeting the symptoms to be developed, for the physiology of basal ganglia to be more clearly understood, and consequently for subthalamic deep brain stimulation to be developed as a treatment option. In the second case, the models helped to shed light on some aspects of the PD pathogenesis puzzle such as, for example, the roles of oxidative stress, apoptosis, mitochondrial dysfunction, inflammation, impairment of the protein and organelle degradation pathways, and $\alpha$-synuclein accumulation among others.

The mammalian models of PD produced by 6-hydroxy-dopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) administration are the best characterized and most widely used. Over the last decade, two new neurotoxin-based models, the rotenone and the paraquat rodent models, have emerged and evolved to reach the status of "model," though not without controversy. The amount of data generated with the 6-OHDA and the MPTP models is much greater than that obtained with the newer models, and as such our approach to review them will be different. With the 6-OHDA and the MPTP models, we will discuss in more detail some practical and conceptual issues within the different sections of this paper, whereas for the rotenone and paraquat models, we will summarize their evolution and the latest data generated. All the data discussed will be related to *in vivo* results unless otherwise specified. Moreover, data generated with non-mammal animals has been intentionally excluded. With the exception of MPTP, which is used to model PD both in monkeys and mice, the other models discussed are with respect to rodent models.

## THE 6-OHDA RODENT MODEL: BEYOND TURNING BEHAVIOR

6-OHDA is the neurotoxin *par excellence* to model PD in rats and is also being increasingly used with genetically modified mice. When administered systemically, 6-OHDA destroys sympathetic neuron nerve terminals in the peripheral nervous system (Porter et al., 1963). Indeed, it exhibits
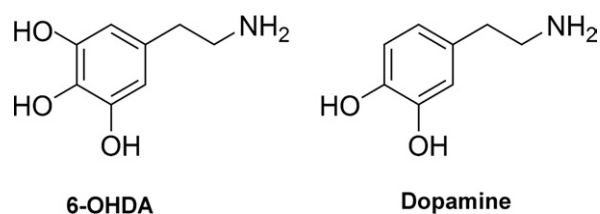
**Fig. 1.** Comparison of the chemical structures of 6-hydroxydopamine (6-OHDA) and dopamine.

high affinity for the catecholamine transporters due to its chemical similarity with the cathecolamines (Fig. 1) and is thus able to damage the cathecolaminergic neurons (Luthman et al., 1989). However, since 6-OHDA only sparingly crosses the blood–brain barrier, it has to be injected directly into the brain to trigger neuronal cell death in the CNS, as demonstrated by Ungerstedt more than 40 years ago (Ungerstedt, 1968). Solutions containing 6-OHDA should be protected from light and must contain ascorbic acid to avoid oxidation of the 6-OHDA because this compound is known to be particularly labile. In addition, 6-OHDA is often used in conjunction with a selective noradrenaline reuptake inhibitor such as desipramine to spare the noradrenergic neurons from damage and to selectively kill dopaminergic neurons.

**Stereotaxic administration: the medial forebrain bundle (MFB) and striatal models**

Even though 6-OHDA can induce a bilateral lesion when injected into both hemispheres or into the ventricles, the most popular protocol involves a unilateral lesion. Depending upon the site of injection and the dose employed, different profiles of cell death can be elicited. If the compound is injected into the SNpc or the MFB, the dopaminergic neurons begin to degenerate within 24 h and exhibit a non-apoptotic morphology (Faull and Laverty, 1969; Jeon et al., 1995). However, if injected into the striatum, 6-OHDA produces a protracted retrograde degeneration that can take 1–3 weeks to complete (Przedborski et al., 1995; Sauer and Oertel, 1994), and the neurons exhibit a heterogeneous morphology, including an apoptotic-like morphology (Marti et al., 1997, 2002). Based on these differences, it is more accurate to talk about at least two 6-OHDA differentiated models: the MFB model and the striatal model. In the MFB model, the maximum reduction of striatal dopamine levels is reached within 3–4 days after the lesion (Faull and Laverty, 1969), and in the majority of studies, the residual dopamine content is less than 20% of control. After an injection of 6-OHDA into the MFB, a complete loss of dopamine content is achieved in the striatum within 3 weeks. The number of dopaminergic neurons in the SNpc decreases gradually, with a near-complete lesion (>90%) obtained only after about 5 weeks, indicating that neurodegeneration is still ongoing when total dopamine denervation of the striatum is stable (Sarre et al., 2004).

In the striatal model, a single injection of 6-OHDA produces a dose-dependent decrease in striatal dopamine levels and in the number of dopaminergic neurons in the

SNpc, accompanied by a significant long-lasting atrophy of the remaining dopaminergic neurons (Lee et al., 1996). This dose-dependent effect enables the dose to be tuned to generate a partial lesion that resembles the initial stages of PD when about 50% of the neurons are still intact (Fearnley and Lees, 1991). Moreover, rats can receive from one to four injections into the striatum, with a subsequent attainment of dopaminergic cell loss ranging from 30% to 75% (Kirik et al., 1998).

Mortality in the MFB and the striatal models (i.e. involving unilateral lesion) is almost non-existent compared with the bilateral model where a higher proportion of animals die as a result of aphagia, adipsia, and seizures (Ungerstedt, 1971a; Bourn et al., 1972).

**Mechanism of action and pathways of neuronal death**

There is consensus that the deleterious effect of 6-OHDA is due to the oxidative stress triggered by the production of reactive oxygen species (ROS) after entering the neuron via the dopamine transporter (Cohen, 1984; Soto-Otero et al., 2000) (Fig. 5). It seems that auto-oxidation may account for ROS generation, since under physiological conditions 6-OHDA is subjected to a rapid non-enzymatic auto-oxidation (Heikkila and Cohen, 1972; Seitz et al., 2000; Soto-Otero et al., 2000) that generates toxic species such as quinones, hydrogen peroxide, superoxide radicals, and hydroxyl radicals (Fig. 5) (Saner and Thoenen, 1971; Cohen and Heikkila, 1974). However, it has also been suggested that iron via the Fenton reaction may contribute to this process as iron chelators prevent the deleterious effect of 6-OHDA (Ben-Shachar et al., 1991; Borisenko et al., 2000). Supporting the relevance of oxidative stress in this model, it has been shown that mice overexpressing superoxide dismutase and glutathione peroxidase are protected against 6-OHDA toxicity (Asanuma et al., 1998; Bensadoun et al., 1998), as are rats treated with the antioxidant vitamin E (Cadet et al., 1989).

In postmortem human PD brains, dopaminergic neurons display characteristic markers of different types of programmed apoptotic and non-apoptotic cell death (Perier et al., in press). The molecular pathways involved in 6-OHDA-induced cell death have been studied mainly in cell cultures and to a lesser extent *in vivo*. Even though it is believed that dopaminergic neurons die by necrosis after MFB injection of 6-OHDA, much less is known about the mechanism of progressive dopaminergic neuronal death induced by the striatal injection of 6-OHDA. Apoptotic profiles have been observed *in vivo* in the SNpc of adult rats and mice after a striatal lesion (Cutillas et al., 1999; Marti et al., 2002; Oiwa et al., 2003; Mladenović et al., 2004; Ries et al., 2008) based on morphological assessment and TUNEL labeling, but it is still a matter of debate if caspase activation is involved in the programmed cell death that occurs in the striatal model. The fact that the general caspase inhibitor Z-VAD-FMK has been reported to be neuroprotective (Cutillas et al., 1999) and that two studies found caspase-3 activation in adult (Hanrott et al., 2008) and juvenile (Jeon et al., 1999) rats, contrasts with three

studies on adult rats or mice that reported no caspase activation in the striatal model (Crocker et al., 2001b; Ebert et al., 2008; Ries et al., 2008). Regardless of this controversy, the c-jun N-terminal kinase (JNK) pathway has been shown to be involved in dopaminergic cell death in both the MFB and the striatal models, since pharmacological inhibition of JNK in rats (Crocker et al., 2011) or homozygous jnk2/3 double null mutations in mice (Ries et al., 2008) entails neuroprotection. In addition, it has been demonstrated that the downstream molecule c-jun activated by JNK mediates dopaminergic cell death since expression of a dominant negative form of c-jun inhibits its phosphorylation and neuron death in the SNpc (Crocker et al., 2001a). More recently, it has been demonstrated that apoptosis signal-regulating kinase 1 (ASK1) activates the JNK/c-jun pathway in 6-OHDA-induced cell death (Hu et al., 2011). ASK1 can be activated in response to a variety of stressors that include ROS and can be inhibited by the antioxidant enzyme peroxiredoxin 2, which is increased in PD brains. Indeed, overexpression of peroxiredoxin 2 preserves both neuron cell bodies and their axon projections (Hu et al., 2011), although the molecular pathways involved in axon and cell body degeneration may be different (Cheng et al., 2011).

Microglial activation contributes to degeneration of dopaminergic neurons, which is in contrast to the beneficial effects of astroglial activation (Saura et al., 2003). In this regard, exogenous administration of fractalkine (CX3CL1), a neuron-derived suppressor of microglial activation, protects dopaminergic neurons against 6-OHDA-induced toxicity (Pabon et al., 2011). Interestingly, it has been demonstrated that microglial activation in the SNpc precedes the actual cell loss both in the striatal (Cicchetti et al., 2002; Depino et al., 2003) and the MFB models (Marinova-Mutafchieva et al., 2009) and may also account for the delayed phase of neurodegeneration (Harms et al., 2011).

Following the demonstration that mutations or triplications in the $\alpha$-synuclein gene induce familial Parkinsonism, the role of $\alpha$-synuclein in the pathogenesis of PD has been extensively explored. For instance, it has been demonstrated that $\alpha$-synuclein knockout mice are protected in the 6-OHDA striatal model (Alvarez-Fischer et al., 2008b) as was shown several years earlier with the MPTP mouse model (Dauer et al., 2002). Indeed, the 6-OHDA model often follows behind the MPTP model in terms of the study of molecular pathways involved in neurodegeneration.

**Behavioral alterations in the 6-OHDA model**

The 6-OHDA model is well-recognized by the characteristic rotational or circling behavior of affected rodents in response to the administration of dopaminomimetics. This rotational behavior is related to the degree of the nigrostriatal lesion (Ungerstedt and Arbuthnott, 1970; Hefti et al., 1980; Przedborski et al., 1995), although this is not an accurate indicator of the dopaminergic cell loss (Kirik et al., 1998). Several compensatory mechanisms, such as the increase of dopamine D2 postsynaptic receptor density for example (Ungerstedt, 1971b), are activated in response to the lesion to compensate for the decrease of dopamine.

This dopamine hypersensitivity on the lesioned side implies an imbalance between the two striata and an asymmetrical response to systemically administered dopaminergic drugs. When dopaminergic agonists such as apomorphine or L-dopa are administered, the animal exhibits a contralateral rotation (away from the lesion), but with drugs that stimulate the release of dopamine, such as amphetamines, the rodent turns in the direction of the lesion. This is due to the fact that the non-lesioned side is able to release more dopamine than the lesioned side. The rotational behavior can be quantified with automatic rotomoters and has been used to test the antiparkinsonian effects of new drugs (Ghosh et al., 2010), to asses the efficacy of transplantation and gene therapies (Kirik et al., 2002; Bjorklund et al., 2002), and to establish a model of L-dopa motor fluctuations after chronic treatment (Papa et al., 1994; Bové et al., 2002). The amount of time that a rat engages in rotational behavior is significantly reduced after a 3-week chronic and intermittent treatment with L-dopa, a phenomenon that has been linked to the wearing-off that PD patients experience after the initial period of high therapeutic success. Moreover, some doses of L-dopa fail to have any effect on rotational behavior, thus resembling the on–off fluctuations that some patients experience after years of treatment with L-dopa. The increase in the number of rotations that is seen over time with this model has been linked to L-dopa-induced dyskinesia; however, a more accurate abnormal involuntary movements rating scale has been developed to assess dyskinetic-like features (Cenci et al., 1998). As in the case of the motor fluctuations, the abnormal involuntary movements are clearly evident in the MFB model but not in the striatal model (Winkler et al., 2002; Cenci and Ohlin, 2009).

The fact that rotational behavior is the fingerprint of the 6-OHDA model should not undermine the use of other behavioral tests, which in some cases are more versatile than this one (Deumens et al., 2002). These tests might not only have a better correlation with the degree of cell loss, but they are also useful for evaluating the long-lasting effect of dopaminomimetics. The stepping test (Olsson et al., 1995), the paw reaching test (Montoya et al., 1990, 1991), and the cylinder test (Vandeputte et al., 2010) are three techniques that have been used to correlate behavioral impairments with 6-OHDA-induced lesions. The stepping test evaluates the slowness of movement of the limbs, whereas the paw reaching test evaluates skilled use of the paw. The threshold of lesion needed to detect alterations is lower with the stepping test than with the paw reaching test (Kirik et al., 1998). Alterations in the paw reaching test are patent with the MFB model and with four injections in the striatum, but not with fewer injections in the striatum as is the case for the stepping test. The stepping test makes evident a limb bradykinesia that is relieved with dopaminomimetics and which resembles the slowness of movement seen in PD patients. In this test the rat is held by the limbs that are not being monitored, and the animal is moved along a 90 cm table surface over 5 s; the free limb being allowed to touch the table surface. The number of adjusting steps done with the paws controlled by the dam-

aged hemisphere is clearly reduced, with the most striking effect seen with the MFB model (Chang et al., 1999; Kirik et al., 1998). The cylinder test, also called the limb use asymmetry test, reveals the defective use of the contralateral forelimb according to the preference of the animal to use its non-lesioned paw to contact the wall while rearing. The number of contacts that an animal makes using the impaired forelimb when placed in a glass cylinder of diameter 20 cm and height 30 cm is expressed as a percentage of total forelimb contacts (Vandeputte et al., 2010).

The behavioral impairments exhibited by 6-OHDA-lesioned mice have their own particularities and have been characterized by several groups. With the MFB model, the amphetamine-induced rotation test and the rotarod test have been reported to be most sensitive and reliable for detecting tyrosine hydroxylase-immunoreactive cell loss in the substantia nigra (Iancu et al., 2005). In addition, Grealish and collaborators (Grealish et al., 2010) established the corridor task as a useful behavioral test to predict the severity of the lesion obtained with intranigral 6-OHDA injections. The corridor task was first developed for rats (Dowd et al., 2005) and is based on the fact that rodents with unilateral nigrostriatal damage fail to orient to tactile, visual, or olfactory stimuli presented on the contralateral side of the body, neglecting food placed on that side. The corridor test has been adapted for mice, with a long narrow plastic corridor (60 cm long, 4 cm wide, and 15 cm high) used that has 10 pairs of adjacent pots containing four to five sugar pellets that are placed at 5-cm intervals along the length of the corridor. The number of ipsilateral and contralateral retrievals made by each mouse is counted until the mouse makes a total of 20 retrievals, or a maximum time of 5 min has elapsed.

**Usefulness of the 6-OHDA model**

The MFB model and the striatal model have different applications. The MFB model is more suitable for studying the consequences of dopaminergic neuronal death and to test therapeutic strategies to treat motor symptoms. On the other hand, the striatal model may be more relevant to elucidate the mechanisms of cell death involved in PD. The MFB 6-OHDA model has been used for pre-clinical testing of new symptomatic therapies including strategies to prevent L-dopa-induced motor fluctuations and dyskinesias (Ghosh et al., 2010; Bové et al., 2002, 2006a; Rylander et al., 2010) and transplantation approaches to replace dopamine levels (Jungnickel et al., 2011; Roy et al., 2006). Moreover, the MFB model has contributed enormously to our understanding of the basal ganglia circuitry (Gerfen et al., 1985, 1987a,b, 1990; Blandini et al., 2008) and to describe the consequences of the dopaminergic denervation in the striatum that occurs in PD and leads to hyperactivity of the subthalamic nucleus (Hassani et al., 1996). It has also been used to demonstrate the importance of dopamine stimulation for the proliferation of precursor cells in both the subependymal and subgranular zones in the adult rat brain (Hoglinger et al., 2004). The MFB model also has been used to test neuroprotective strategies targeting neuroinflammation (Broom et al., 2011).

The striatal model is mainly being used to unmask the pathogenesis of PD and to test neuroprotective strategies (Hu et al., 2010, 2011). For instance, it has been demonstrated that intrastriatal transplantation of undifferentiated human mesenchymal stem cells protects against 6-OHDA (Blandini et al., 2010), that activated AKT/protein kinase B has a neurotrophic and anti-apoptotic profile (Ries et al., 2006), and that deep brain stimulation of the subthalamic nucleus may have a neuroprotective effect (Spieles-Engemann et al., 2010).

It is worth emphasizing that 6-OHDA has been found in the human caudate nucleus (Curtius et al., 1974) and in the urine of L-dopa-treated PD patients (Andrew et al., 1993). Therefore, we can speculate that 6-OHDA may play a role in the pathogenesis of PD as an endogenous hydroxylated metabolite of dopamine. Moreover, several indirect pieces of evidence demonstrate that the dopaminergic neurons of PD patients are exposed to heightened oxidative stress. Diminished glutathione peroxidase (Kish et al., 1985) and catalase (Ambani et al., 1975) activity have been reported, along with lower concentrations of reduced glutathione (Sofic et al., 1992) in the SNpc of PD brains, as well as lipid peroxidation (Dexter et al., 1989) and DNA oxidative damage (Sanchez-Ramos et al., 1994) in the dopaminergic system of PD patients. As an inducer of oxidative stress, 6-OHDA may recapitulate some of the features observed during the course of the disease.

## MPTP: MODELING COMPLEX I INHIBITION

In the last quarter of the 20th century an analogue of the synthetic opioid meperidine with the chemical name 1-methyl-4-phenyl-4-propionpiperidine (MPPP) and with an effect comparable to that induced by heroin was introduced as a recreational drug in the United States. A 23-year-old graduate student set up a home laboratory to synthesize MPPP and after one injection of a new batch of MPPP, he experienced severe bradykinesia that only improved with L-dopa (Fahn, 1996). Investigations were held to clarify what was the etiology of his condition; along with MPPP they found in the glassware that was used to prepare the drug the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Fig. 2) as a byproduct of the reaction. MPTP was injected into rats but only transient Parkinsonian-like symptoms were induced since rats are exceptionally resistant to MPTP as was later learned (Boyce et al., 1984; Chiueh et al., 1984). This would have been the end of MPTP as a neurotoxin to model PD if it had not been injected into monkeys several years later in a new investigation carried out to understand what happened to several drug users with the same parkinsonian symptoms after the i.v. injection of MPPP (Langston et al., 1983). Indeed, this batch of MPPP was also contaminated with
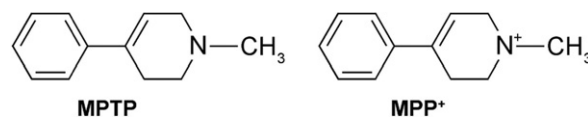


**Fig. 2.** Chemical structures of MPTP and MPP$^+$.

MPTP. MPTP not only induced a permanent parkinsonian syndrome in the monkeys but also neurodegeneration of the dopaminergic neurons of the ventral midbrain. It is now well established that MPTP produces an irreversible and severe parkinsonian syndrome in humans, characterized by all of the cardinal features of PD, including tremor, rigidity, slowness, postural instability, and even freezing (Langston and Irwin, 1986). However, the neurodegenerative process in PD is thought to be progressive over a course of years, whereas in the human intoxicated with MPTP, the most active phase of neurodegeneration takes place within a few days of the injection and is followed by a silent progression over several decades (Langston et al., 1999; Vingerhoets et al., 1994). MPTP has been used to model PD in a variety of species ranging from non-human primates to invertebrates such as worms (Kopin, 1987; Kitamura et al., 1998), though the most used species are the mouse and monkey. As reviewed elsewhere (Jackson-Lewis and Przedborski, 2007; Przedborski et al., 2001), very strict safety measures have to be taken when MPTP experiments are carried out.

**The mouse MPTP model**

The mouse MPTP model is the most commonly used model to elucidate the mechanisms of cell death involved in PD. Rats have been generally excluded from the modeling of PD with systemic MPTP administration since the dose required to induce a dopaminergic degeneration comparable with that induced in mice implies a high mortality rate (Giovanni et al., 1994). However, stereotaxic injection of the toxic metabolite MPP$^+$ has been used to model PD in rats (Staal and Sonsalla, 2000). More recently it has been demonstrated that intranasal administration of MPTP triggers neurodegeneration in the SNpc as well as motor impairments in rats (Prediger et al., 2006, 2009) and mice (Rojo et al., 2006). This emerging model may open the door to a new approach to model PD with MPTP in rodents.

It is important to bear in mind that there are striking variations in the sensitivity to MPTP among some strains of mice (Smeyne et al., 2005; Muthane et al., 1994); the election of the strain cannot therefore be done randomly. Along with the strain there are several parameters such as the gender, age, and body weight that influence the reproducibility and sensitivity of the lesion in mice. Female mice exhibit higher mortality rates than males, so when possible it is better to exclude them from the experiment. Male mice used should weigh at least 22 g and be at least 8 weeks of age (Jackson-Lewis and Przedborski, 2007). Another important issue related to strain differences is the variation in rates of mortality due to peripheral toxicity and hypothermia. It is thus advisable to perform preliminary injections to test the regimen of intoxication with the particular mouse strain that will be used.

*Regimens of intoxication in the MPTP mouse model.* The magnitude of the lesion and the mode of cell death induced by MPTP depend on the regimen of administration (Przedborski and Vila, 2003). The most common routes of administration are the i.p. and the s.c. injection sites. At the same dose, s.c. administration elicits a more severe lesion of the dopaminergic system than i.p. administration due to avoidance of the first hepatic pass (Chiba et al., 1988). Along with the route of administration, the dose and the time between injections are the parameters that will determine the degree of dopaminergic cell death. Even though a lot of different regimens of intoxication can be found in the literature, the best characterized regimens are the widely referred as acute and sub-acute regimens. The acute regimen was developed by Jackson-Lewis and Przedborski and involves a total of four injections at a dose of 20 mg/kg with 2 h intervals between injections (Jackson-Lewis et al., 1995). This regimen leads to 90% striatal dopamine depletion and about 70% loss of dopaminergic neurons in the SNpc; these properties are stable within 7 days after the MPTP administration. With the acute model the dopaminergic neurodegeneration occurs morphologically by a non-apoptotic type of cell death. This contrasts with the sub-acute regimen established by Tatton and Kish that yields a more progressive neurodegeneration with apoptotic cell death (Tatton and Kish, 1997). The regimen implies one i.p. injection of 30 mg/kg MPTP daily for five consecutive days, and causes a 40–50% depletion of striatal dopamine and a 30–40% cell loss (Perier et al., 2007). The dopaminergic lesion stabilizes within 21 days after MPTP administration. The fact that these regimens are the most popular does not mean that they are superior to others. Other regimens that try to more optimally mimic the progressive nature of PD need to be considered (Bezard et al., 1997), as well as those developed to study the compensatory mechanisms elicited in response to subtoxic doses of MPTP (Aubin et al., 1998). Attempts have been made to develop a more progressive model with a continuous infusion of low doses of MPTP with the use of osmotic pumps (Fornai et al., 2005). However, several groups have not been able to reproduce these results (Alvarez-Fischer et al., 2008a). Another strategy that has been used to obtain a more chronic model is the co-administration of MPTP and probenecid that reduces MPTP metabolism and reduces urinary and neuronal clearance (Petroske et al., 2001).

*Mechanism of action and pathways of neuronal death.* MPTP is a protoxin with complex toxicokinetics that give rise to the actual toxic metabolite 1-methyl-4-phenylpyridinium (MPP$^+$) (Heikkila et al., 1984) (Fig. 2). It is widely accepted that MPP$^+$ impairs mitochondrial respiration by inhibiting the multi-subunit enzyme complex I (NADH-ubiquinone oxireductase) of the mitochondrial electron chain (Fig. 5) (Nicklas et al., 1985). After systemic administration, MPTP crosses the blood–brain barrier in a matter of seconds (Markey et al., 1984) due to its high lipophilicity (Riachi et al., 1989). Once in the brain, it is rapidly converted into MPP$^+$ by a two-step process. First, MPTP undergoes a two-electron oxidation catalyzed by monoamine oxidase B (MAO-B), producing the intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP$^+$) (Chiba et al., 1984) probably in the glial and serotoninergic cells.

$MPDP^+$ is an unstable molecule that readily undergoes spontaneous dismutation to $MPP^+$ and MPTP (Chiba et al., 1985; Peterson et al., 1985). The $MPP^+$ is released into the extracellular space and enters the dopaminergic neurons via the plasma membrane dopamine transporter (DAT) for which it has a high affinity (Bezard et al., 1999; Javitch et al., 1985). The fact that pharmacological inhibition of the dopamine transporter (Javitch et al., 1985) or its genetic ablation (Bezard et al., 1999) completely prevents MPTP neurotoxicity demonstrates the importance of this step.

Once inside neurons, $MPP^+$ can be accumulated in the synaptic vesicles (Liu et al., 1992) and by means of passive transport it accumulates in the mitochondrial matrix (Davey et al., 1992; Hoppel et al., 1987). Once in the mitochondria, $MPP^+$ inhibits complex I that triggers a transient reduction in midbrain ATP levels of about 20% *in vivo* (Chan et al., 1991), and an increase of ROS production (Rossetti et al., 1988; Hasegawa et al., 1990). ROS contribution to MPTP-mediated cell death *in vivo* has been demonstrated indirectly by the neuroprotection elicited by the modulation of ROS scavengers like superoxide dismutase (Andreassen et al., 2001; Klivenyi et al., 1998; Przedborski et al., 1992) and by the detection of an indirect hydroxyl radical marker (Ferger et al., 2000). This ROS production is triggered not only by complex I inhibition but also by the auto-oxidation of dopamine resulting from the massive release of vesicular dopamine induced by $MPP^+$ (Lotharius and O'Malley, 2000).

The data generated with the MPTP mouse model relating to molecular mechanisms of cell death are much more abundant than those generated with the 6-OHDA model; these data have been extensively reviewed (Przedborski and Vila, 2001; Perier et al., in press). In addition, some of the pathways described to be implicated in the 6-OHDA model, such as the ASK1/JNK/c-jun pathway (Hunot et al., 2004; Karunakaran et al., 2007), were described first with the MPTP model.

The time course of the deleterious events involved in MPTP-induced cell death can differ among the different regimens of intoxication. However, an overlap of many of the molecular pathways implicated may occur for the different regimens. The acute and the sub-acute regimens discussed previously have been used for two distinct purposes. The acute regimen has been used mainly to study the role of inflammation associated with neurodegeneration, whereas the sub-acute regimen has been used primarily to study the molecular pathways of cell death (Przedborski and Vila, 2001, 2003; Perier et al., in press). Postmortem studies in three individuals who survived 3–16 years after exposure to MPTP (Langston et al., 1999) and in six monkeys who survived 5–14 years after exposure to MPTP (McGeer et al., 2003) showed activated microglia, which is indicative of an ongoing degenerative process with neuroinflammation. This finding suggests that a single acute MPTP insult can set in motion a cascade of cellular and molecular events with long-lasting deleterious effects. In the acute mouse model, astrocytosis parallels dopaminergic-cell death (Liberatore et al., 1999; Członkowska et al., 1996; Kohutnicka et al., 1998), suggesting that astrocytic reaction is secondary to the neuronal death. In contrast, the activation of microglial cells (Członkowska et al., 1996; Kohutnicka et al., 1998; Liberatore et al., 1999; Dehmer et al., 2000) occurs much earlier than that of astrocytes and reaches a maximum before the peak of dopaminergic neurodegeneration, indicating that microgliosis could be involved in neuronal death. Finally, cells of the peripheral immune system are also likely to play a part in neurodegeneration as the infiltration of $CD8^+$ T-cytotoxic and $CD4^+$ T-helper lymphocytes into the nigrostriatal system have been observed after MPTP injection (Brochard et al., 2009; Kurkowska-Jastrzebska et al., 1999).

As previously reviewed extensively by us (Perier et al., in press), dopaminergic neurodegeneration after sub-acute MPTP intoxication occurs, at least in part, via the activation of mitochondria-dependent apoptotic molecular pathways (Perier et al., 2005, 2007; Vila et al., 2001; Vila and Przedborski, 2003). A time-dependent and region-specific mitochondrial release of cytochrome c occurs in association with caspase-9 and caspase-3 activation (Perier et al., 2005). All of these molecular alterations, including SNpc dopaminergic cell death, are regulated by the pro-apoptotic protein Bax, as they coincide with Bax up-regulation and translocation to the mitochondria and are prevented by genetic ablation of Bax (Perier et al., 2005; Sriram et al., 2002). However, contrary to initial views, the deleterious effects of programmed cell death pathways in PD may not be limited to mitochondria-mediated caspase-dependent apoptosis but may also involve caspase-independent non-apoptotic cell death, including programmed necrosis (for review, see Perier et al., in press). We recently demonstrated that, along with the permeabilization of the mitochondrial outer membrane, a permeabilization of the lysosomal membrane takes place that contributes to the neurodegenerative process (Vila et al., 2011; Dehay et al., 2010). This contribution is two-fold; on the one hand there is an ectopic release of lysosomal proteases, whereas on the other there is an impairment of autophagy.

*Motor impairments in the mouse MPTP model.* Three important aspects have to be taken into account when carrying out behavioral tests with MPTP-lesioned mice. The first is that there are large behavioral differences among various inbred strains, and often also among subtypes of one particular strain (Wahlsten et al., 2003; Krackow et al., 2010). For instance, based on our experience, tail climbing is clearly impaired in MPTP-lesioned C57BL/6J mice, whereas naïve C57BL/6Ncrl mice (both Charles River, Paris) are unable to climb up their tails when suspended. Actually, a wide variability in the propensity for tail climbing has been confirmed for several strains (Mayorga and Lucki, 2001). These basal behavioral differences may account in part for the apparent lack of reproducibility of some results with the MPTP mouse model. It is therefore advisable to specify with the standard nomenclature the mouse strain used in order to facilitate comparison

of the data generated. The second important aspect is that, after recovering from the injections, mice look normal, meaning that highly challenging behavioral test are required to unravel some of the deficits they may display. Indeed, MPTP-intoxicated mice exhibit a remarkable capacity for functional recovery, for which reason some motor impairments are only transient in nature (Willis and Donnan, 1987; Sedelis et al., 2001). Finally, as motor impairment is correlated with the lesion magnitude, it is therefore more difficult to conduct behavioral tests in the case of moderate lesions of the dopaminergic system. In this regard, only retired-breeder mice elicit motor impairments that are persistent over time following a moderate dose of MPTP. This can be evidenced by measuring the forepaw stride length during walking, and the forepaw distance, wall time, and forepaw faults in the grid (Tillerson et al., 2002). Along with the grip coordination tests, several tests have been used to analyze the motor impairments induced by MPTP administration in mice such as the tail suspension test (Mori et al., 2005), the rotarod test, the pole test, and the nest-building test (Sedelis et al., 2001). In the rotarod test, the latency to fall from a rotating rod is measured as an indicator of motor skills (Rozas et al., 1998). Hutter-Saunders and collaborators reported better results when normalizing individual performances with pre-treatment test performance to decrease the variation, and using a rat-size rod instead of a mouse-size rod (Hutter-Saunders et al., in press). Using a variation of the acute regimen that triggers a cell loss of 52% and a 66% decrease in dopaminergic terminals in the striatum, they reported a decrease of the latency to fall with a constant rotation speed of 10 rpm. Interestingly, an adaptation of the stepping test has been reported to be useful to evaluate akinesia in mice (Blume et al., 2009). This motor impairment arises with a 65% cell loss and is reversed with L-dopa. Indeed, to validate that a particular motor impairment is due to the dopaminergic lesion, the impairment has to be able to undergo a total or partial reversal by a dopaminergic agonist.

*Usefulness of the mouse MPTP model.* As indicated previously, the MPTP mouse model is the classic model used to study the molecular pathways involved in PD neuronal cell death and to test the effectiveness of neuro-protectants. The relevance of this model is based on the fact that there is a mitochondrial dysfunction in PD (Vila et al., 2008) and that MPTP intoxication mimics the mitochondrial dysfunction that occurs in PD. In this regard, it has been demonstrated that complex I activity is reduced in the brain, platelets, and skeletal muscle of patients with PD (Parker et al., 1989; Schapira et al., 1990). In addition, cell lines engineered to contain mitochondria derived from the platelets of PD patients (cybrids) exhibited reduced complex I activity (Swerdlow et al., 1996), indicating that PD-derived mitochondrial DNA (mtDNA) encodes pathogenic information. More recently, it has been shown that mitochondria in PD brains undergo substantial misassembly of complexes I–V that form the functional unit of electron transport and ATP synthesis, providing additional support

to the view that mitochondrial dysfunction is an important component in the PD pathological process (Arthur et al., 2009).

One of the strengths of the MPTP mouse model is the possibility to work with genetically modified mice. For instance, the deleterious effects of $\alpha$-synuclein and Bax in the MPTP model have been demonstrated with $\alpha$-synuclein and Bax KO mice (Dauer et al., 2002; Vila et al., 2001), whereas the relevance of the microglial glucocorticoid receptor (GR) in neuroinflammation was assessed in MPTP-treated mice in which the GR gene had been selectively inactivated (Ros-Bernal et al., 2011).

**Non-human primate MPTP model**

A wide variety of species has been used to develop the non-human primate PD model. The species used so far are the rhesus macaque (Burns et al., 1983), cynomologus (Mitchell et al., 1985), common marmoset (Jenner et al., 1984), squirrel monkey (Langston et al., 1984), African green monkey (Taylor et al., 1997), and the baboon (Todd et al., 1996). The strength of this model comes from the phylogenetic proximity between humans and non-human primates. Non-human primates have behavioral correspondence with humans, neuroanatomical similarities like the separation of the caudate nucleus and the putamen, and age-related impairment of the dopaminergic system as in humans (Collier et al., 2007, 2011; Eriksen et al., 2009). Neuropathological data in both humans and monkeys (Davis et al., 1979; Forno et al., 1993) indicate that MPTP causes damage to the nigrostriatal dopaminergic pathway that is identical to that seen in PD (Agid et al., 1987). As in PD, MPTP causes a greater loss of dopaminergic neurons in the substantia nigra than in the ventral tegmental area (Seniuk et al., 1990; Muthane et al., 1994). Moreover, in monkeys intoxicated with low doses of MPTP, a greater degeneration of dopaminergic nerve terminals is seen in the putamen than in the caudate nucleus (Moratalla et al., 1992; Snow et al., 2000). Indeed, it has been demonstrated that the dramatic reductions in spine density and dopamine levels are greater in the sensorimotor post-commisural putamen (Villalba et al., 2009). Regarding the presence of Lewy bodies, thus far they have not been convincingly observed in the ventral midbrain of MPTP-induced parkinsonism monkeys (Forno et al., 1993), even 10 years after the injections (Halliday et al., 2009). However, in older MPTP-injected squirrel monkeys, intraneuronal proteinaceous inclusions reminiscent of Lewy bodies have been found in the locus coeruleus accompanying cell loss. Other studies have shown that the serotoninergic system can also be affected in the MPTP primate model (Pérez-Otaño et al., 1991; Russ et al., 1991). More recently, it has been demonstrated that cholinergic neurons in the pedunculopontine nucleus (PPN) degenerate in aged monkeys but not in young monkeys intoxicated with MPTP. Cell loss in this neuronal population correlates with balance and postural deficits that are not present in young monkeys. Interestingly, elderly PD patients with balance deficits and falls also exhibit cell loss in the PPN (Karachi et al., 2010). Unlike the degeneration of the monoaminer-

gic neurons, it seems that the cholinergic cell loss is a consequence of the dramatic reduction of the dopaminergic innervations of the PPN that occurs with MPTP intoxication (Rolland et al., 2009).

*Intoxication regimens.* MPTP can be injected systemically to induce a bilateral parkinsonism, or by intracarotid infusion to induce hemiparkinsonism (Bankiewicz et al., 1986). A bilateral intracarotid model has also been developed, with a period of several months between the two administrations (Smith et al., 1993). Nonetheless, the most broad-based regimens of intoxication involve repeated i.p., s.c., or i.v. administrations of MPTP (1–2 mg/kg) over several days to months (Burns et al., 1983; Fox and Brotchie, 2010). The dopaminergic cell loss depends on the regimen followed, but in the more commonly used regimes the treatment triggers a 90% cell loss that becomes stable in 2–3 months. A treatment of 1 mg/kg for 3 days has been used to produce partial lesions of about 60% to generate an earlier stage of the disease; these animals exhibit minor motor alterations that do not respond to L-dopa (Iravani et al., 2005). With a few intermittent injections, behavioral recovery may take place after several months. This is not the case when lower daily doses (0.2 mg/kg vs. 2 mg/kg) are administered over 2–3 weeks (Bezard et al., 1997; Meissner et al., 2003). With this regimen there is a period of time during which motor symptoms are absent despite an actual dopaminergic impairment, thus resembling the asymptomatic stage in PD; this period permits the investigation of compensatory mechanisms (Bezard et al., 2001a,b). Other regimens have been proposed to induce a more progressive neurodegeneration with low, intermittent doses administered once or twice per week (Hantraye et al., 1993; Mounayar et al., 2007). However, others failed to demonstrate an active ongoing neurodegenerative process after cessation of the MPTP injections (Garrido-Gil et al., 2009). It must be taken into account that primates exhibit inter-animal variability in MPTP susceptibility with the systemic administration. One of the advantages of the intracarotid injection is the reduction of this inter-animal variability. Age, as in mice, plays an important role in susceptibility to MPTP, with older primates generally more susceptible to MPTP (Ovadia et al., 1995).

*Motor symptoms.* MPTP produces a severe parkinsonian syndrome in monkeys, which is characterized by all of the cardinal features of PD, including tremor, rigidity, slowness, postural instability, and even freezing. Nonetheless, the typical 4-Hz resting tremor of PD has only been demonstrated convincingly in the African green monkey (Tetrud et al., 1986); other species of monkeys are more likely to exhibit a postural/action tremor.

The intensity of the motor deficits induced by MPTP depends on the regimen, the species, and the age of the animals used. Within a particular species, behavioral variability is also observed with a mild to severe range of symptoms (Jakowec and Petzinger, 2004). As in the mouse MPTP model, there is an initial stage that commences within hours of the intoxication that is due to the peripheral and central effects of MPTP. This stage may

include emesis, hypersalivation, exaggerated startle, seizure-like activity, and dystonic posturing of trunk and limbs (Jenner et al., 1987). Weight loss and hypothermia may require tube feeding and placing the animal in an incubator. Body weight and blood biochemical parameters should be stabilized to evaluate the parkinsonian symptoms of the animal to ensure that the general health status of the animal is not an interfering factor (Jakowec and Petzinger, 2004). Several ratings scales to measure the motor impairments exhibited by MPTP-intoxicated monkeys have been proposed, but all are similar to the Unified Parkinson's Disease Rating Scale (UPDRS) (Imbert et al., 2000; Goetz et al., 2008). These scales are subjective and the scores may vary depending on the scale used. To avoid the subjectivity associated with these scales some groups have developed objective methods. A good example of this is a device that is capable of recording resistive forces as a measure of rigidity while moving the arm between two specified angles (Mera et al., 2009). Nevertheless, the complete picture of the parkinsonian symptoms only can be obtained by clinical observation. These symptoms can have their particular nuances depending on the species being investigated. For instance, changes to the marmoset's natural jumping behavior and the righting reflex have been suggested as measures of akinesia and rigidity in this species (Verhave et al., 2009).

MPTP-intoxicated monkeys respond very well to antiparkinsonian treatments such as L-dopa (Burns et al., 1983). However, as in PD patients (Kostic et al., 1991), long-term treatment with L-dopa leads to dyskinesia, which can be as disabling as the parkinsonian symptoms themselves. The MPTP monkey model has emerged as an invaluable tool to investigate the molecular basis of these drug-induced abnormal movements and to test therapeutic strategies to control them (Blanchet et al., 2004). Interspecies differences are also evident in the manifestation of these abnormal movements; these should be taken in account as the Old World species exhibit easier to detect dyskinesias than the much more active New World species (Fox and Brotchie, 2010). Along with dyskinesia, the wearing-off and the beginning- and end-of-dose worsening phenomena can also be studied in the MPTP primate model (Jenner, 2003b).

*Non-motor symptoms.* MPTP-intoxicated monkeys exhibit 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 are also observed in these animals (Decamp and Schneider, 2004; Pessiglione et al., 2004). As in PD patients, these cognitive alterations are not reversed by L-dopa (Decamp and Schneider, 2009). Instead, monkeys can 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 (Fox and Brotchie, 2010). Some authors have proposed a neuropsychiatric behavior scale to rate this psychotic-like behavior (Fox et al., 2010).

MPTP-monkeys can also experience some of the sleep disorders that PD patients endure. Although a single MPTP injection completely suppresses rapid eye movement (REM) sleep, chronic MPTP with a regimen that induces Parkinsonism produces progressive sleep deterioration with an increase of sleepiness during the day. Interestingly, the deregulation of REM sleep and increased daytime sleepiness occur before the emergence of motor symptoms as in the case of PD in humans (Barraud et al., 2009).

*Usefulness of the primate MPTP model.* The MPTP monkey model remains the classic tool for the assessment of novel strategies to treat PD motor symptoms and L-dopa-associated motor complications. All the dopaminomimetic drugs used in clinical practice to ameliorate PD motor symptoms, such as L-dopa, bromocriptine, pergolide, and ropinirole, are effective in MPTP-treated monkeys. Also, potentiation of the L-dopa effect by MAO-B and catechol-O-methyl transferase (COMT) inhibitors that is observed in PD patients is also observed in these monkeys (Burns et al., 1983; Close et al., 1990; Jenner, 2003a). Indeed, the usefulness of this model is evidenced by its implication in the development of the partial dopamine agonists pardoprunox and aplindore that are currently being evaluated in clinical trials (Jackson et al., 2010; Johnston et al., 2010; Tayarani-Binazir et al., 2010). However, inhibitors of monoamine reuptake that were effective in reverting the motor impairment in MPTP-intoxicated monkeys (Hansard et al., 2002) failed in clinical trials (Bara-Jimenez et al., 2004; Hauser et al., 2007; Rascol et al., 2008).

Regarding dyskinesia, it has been shown that combining 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists (Muñoz et al., 2008) or a mu-opioid receptor antagonist (Koprich et al., 2011) with L-dopa reduces dyskinesias without affecting the anti-parkinsonian effect of L-dopa in MPTP-monkeys. Along with pharmacological strategies, gene therapy has also been successfully tested on MPTP-monkeys to improve L-dopa's therapeutic window, as in the case involving the overexpression of L-amino acid decarboxylase (Hadaczek et al., 2010). This model is less commonly used to test neuroprotective treatments, but it has for example been demonstrated that overexpression of the glial derived neurotrophic factor (lenti-GDNF) increases fluorodopa uptake in the striatum of monkeys that received a single intracarotid infusion of MPTP (Emborg et al., 2009). Emerging data testifies that this model will also be suitable to study some of the non-motor symptoms of PD and non-motor L-dopa side effects. One recent study demonstrated that the inhibition of the fatty acid amide hydrolase reduces L-dopa-induced hyperactivity that may be related to impulse control disorders (Johnston et al., 2011).

One of the most important findings translated from preclinical research into clinical practice came from electrophysiological studies on MPTP monkeys that demonstrated that hyperactivity of the subthalamic nucleus (STN) is a key factor in the development of bradykinesia and rigidity (Bergman et al., 1990). This finding allowed STN deep brain stimulation to be developed, which consists of blocking abnormal nerve signals of the STN by using high-frequency electric stimulation in an attempt to ameliorate the motor function of PD patients with intractable symptoms (Limousin et al., 1998; Zaidel et al., 2010).

## ROTENONE RODENT MODELS: TUNING TO MODEL PD

Rotenone (Fig. 3) is an insecticide and piscicide extracted from *Leguminosa* plants (Hisata, 2002) that has been used extensively as a prototypic mitochondrial toxin in cell cultures; exposure to it has been linked to a higher risk of PD (Dhillon et al., 2008; Tanner et al., 2011). Rotenone impairs oxidative phosphorylation in the mitochondria by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase activity through its binding to the PSST subunit of the multipolypeptide enzyme complex I of the electron transport chain (Schuler and Casida, 2001). Aside from its action on mitochondrial respiration, rotenone also inhibits the formation of microtubules from tubulin (Fig. 5) (Marshall and Himes, 1978; Brinkley et al., 1974; Choi et al., 2011). Proteasome inhibition has been reported after chronic rotenone exposure *in vitro* and *in vivo* (Betarbet et al., 2006), but it has been suggested that this is secondary to oxidative stress and probably to microtubule dysfunction (Chou et al., 2010). However, complex I inhibition may be sufficient since MPP$^+$ triggers proteasome inhibition *in vitro* independently by oxidative stress or ATP depletion (Caneda-Ferrón et al., 2008).

During the last decade, the use of rotenone to model PD *in vivo* has been promoted by Greenamyre and collaborators (Betarbet et al., 2000; Sherer et al., 2003; Cannon et al., 2009). Although stereotaxic injection was the first approach to model PD (Heikkila et al., 1985), systemic administration is providing more valuable results. Rotenone is highly lipophilic and is able to cross the blood–brain barrier. After a single i.v. injection, rotenone reaches maximal concentrations in the CNS within 15 min and decays to about half this level in less than 2 h (Talpade et al., 2000). Its brain distribution is heterogeneous (Talpade
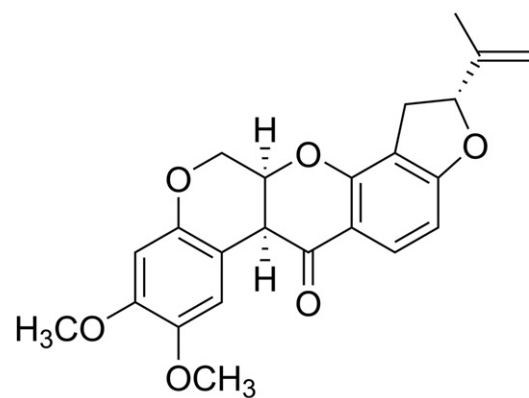


**Rotenone**

**Fig. 3.** Chemical structure of rotenone.

et al., 2000), paralleling regional differences in oxidative metabolism (Talpade et al., 2000). Rotenone also freely crosses all cellular membranes and can accumulate in subcellular organelles such as the mitochondria. However, unlike MPTP, it triggers a systemic complex I inhibition. The first attempts to model PD with the systemic administration of rotenone failed to induce an actual dopaminergic lesion and demonstrated the high lethality of this compound. Rats treated for 1 week with 10−18 mg/kg/d i.v. showed neuronal loss and gliosis in the striatum and the globus pallidus, but an unaffected dopaminergic pathway (Ferrante et al., 1997). Similarly, s.c. injection of either 15 mg/kg rotenone once only or 1.5 mg/kg on multiple occasions failed to affect the striatal dopaminergic content in mice (Thiffault et al., 2000).

**Rotenone rat model**

The turning point of PD modeling with rotenone in rats came with the use of osmotic pumps that allowed a chronic and sustained delivery of the drug to the animal (Betarbet et al., 2000; Sherer et al., 2003). Intravenous (permanent jugular cannulation) and s.c. infusion of 2–3 mg/kg/d of rotenone for 3 weeks to rats does produce nigrostriatal dopaminergic neurodegeneration. Although the initial descriptive studies did not report any striatal lesion (Betarbet et al., 2000), the numbers of dopamine-regulated phosphoprotein-32 projecting neurons, cholinergic interneurons, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase-positive neurons in the striatum were all found to be significantly reduced following the infusion of rotenone in rats (Hoglinger et al., 2003; Lapointe et al., 2004), especially in those rats exhibiting a central striatal loss of tyrosine hydroxylase immunoreactivity (Zhu et al., 2004). Even at doses that did not damage the nigrostriatal dopaminergic pathway, a significant loss of intrinsic striatal neurons could be found. Moreover, rotenone infusion is associated with a 35% reduction in serotonin transporter density in the striatum, a 26% reduction of noradrenergic neurons in the locus coeruleus, and a 29% reduction of cholinergic neurons in the PPN (Hoglinger et al., 2003). These results indicate that rotenone exerts a much more widespread neurotoxicity than was initially thought. Although PD is a multisystemic disease, intrinsic striatal neurons seem not to be affected. The fact that these regimens of intoxication induce a multisystemic lesion makes it difficult to determine if the motor impairments exhibited by the rats were exclusively due to the impairment of the dopaminergic system. The motor impairments observed included reduced mobility, flexed posture, and in some cases rigidity (Sherer et al., 2003; Hoglinger et al., 2003). As discussed by us in a previous review (Bove et al., 2005a), the administration of rotenone by means of an osmotic pump gives rise to a highly variable dopaminergic lesion, ranging from virtually absent to close to complete (Betarbet et al., 2000; Sherer et al., 2003; Hoglinger et al., 2003; Lapointe et al., 2004; Zhu et al., 2004). Around half of the animals die, and in the best-case scenario, half of them exhibit more or less extensive dopaminergic neurodegeneration. Therefore, we

concluded that the inconsistent and unpredictable effect of rotenone on the dopaminergic system prevents the chronic administration of this compound as a routine model of PD.

Taking into consideration the importance of chronicity in the treatment regimen, other groups have carried out daily i.p. or s.c. injections over 21, 48, or 60 days with a dose of 1.5–2.5 mg/kg/d in rats demonstrating dopaminergic neurodegeneration (Fleming et al., 2004; Alam et al., 2009; Alam and Schmidt, 2002, 2004). With respect to its effects on behavior, this regimen induces catalepsy, decreases the total locomotor activity, and diminishes the number of rearings. Interestingly, these motor impairments were relieved by L-dopa treatment (Alam and Schmidt, 2004). Even though motor impairments also occur in rats that do not exhibit an apparent decrease in the striatal tyrosine hydroxylase immunoreactivity (Fleming et al., 2004), compensatory sprouting may account for this discrepancy since dopaminergic cell loss in the SNpc can take place with no apparent striatal denervation (Cannon et al., 2009).

The last improvement to model PD in rats with rotenone has been the use of Mygliol as a vehicle in conjunction with a slight increase in the rotenone dose up to 3 mg/kg/d (Cannon et al., 2009; Tapias et al., 2010). With these modifications, the mortality rate is reduced dramatically and the loss of dopaminergic neurons in the SNpc (45%) is homogeneous. However, the stereological counting was done at different time points for each animal because they were euthanized when a severely debilitated phenotype was reached. Indeed, variability is apparent with the onset of this phenotype that is minimized with age. All rats exhibited postural instability and decreased rearing behavior after just 6 days of rotenone treatment, which were reversed by apomorphine, thus indicating their dopamine-related nature. A reduction of the striatal dopamine levels was accompanied by a loss of dopamine terminals, this being particularly evident in the dorsolateral quadrant of the striatum.

In light of these results, systemic rotenone intoxication in rats could be considered useful as a PD model. Nevertheless, it has to be clarified if other areas of the brain such as the striatum and the locus coeruleus are affected with repeated injections of rotenone since, unlike with the osmotic pump delivery method, this has so far not been studied systematically. In this regard, Höglinger and collaborators suggested that chronic rotenone administration may model atypical parkinsonism rather than PD (Hoglinger et al., 2006). Moreover, analysis of the time course of dopaminergic neuron death should be conducted to determine when the lesion is stable and when it is most homogeneous among the rats treated with rotenone. We consider that euthanizing the rats at different time points is far from ideal, particularly in studies of neuroprotective treatments. It is not clear if the dopaminergic lesion is already unequivocal and homogeneous at 6 and/or 9 days, when apomorphine-sensitive motor impairments are clearly evident. If so, animals should be euthanized at that time point and not when they exhibit debilitating symptoms that compromise survival. It is possible that the inherent

systemic toxicity of rotenone and not exclusive, parkinsonian-like symptoms are behind this debilitating condition. One possibility to better standardize the model could be to stop the intoxication and let the lesion progress until it is stable.

The highlight of the use of chronic rotenone treatment of rats to model PD has been the generation of proteinaceus 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 osmotic pump administration experiments as Lewy body-like inclusions, positive for ubiquitin and $\alpha$-synuclein, and composed of a dense core with fibrillar peripheral elements (Betarbet et al., 2000; Sherer et al., 2003). These findings were subsequently confirmed by a separate laboratory (Hoglinger et al., 2003, 2005) in which spherical deposits of $\alpha$-synuclein in some cells with more abundant tau immunoreactive striatal cells were reported. It remains to be determined if the daily injection regimen triggers the formation of the same kind of inclusions and/or tauopathy, although Cannon et al. reported an accumulation of $\alpha$-synuclein resistant to proteinase K and formic acid in the SNpc (Cannon et al., 2009).

**Rotenone mouse model**

Once it was demonstrated that systemically administered rotenone was able to induce dopaminergic neurodegeneration in rats, it was then also tested in mice. The first experiments conducted failed to show any lesion in the dopaminergic system with acute or chronic rotenone administration (Thiffault et al., 2000; Richter et al., 2007). However, Inden and collaborators demonstrated that chronic oral administration of rotenone does cause nigrostriatal degeneration (Inden et al., 2011, 2007). They demonstrated a dose- and time-dependent dopaminergic cell loss in the SNpc with optimum results obtained using a dose of 30 mg/kg. Along with the cell loss they described a decrease in the striatal dopamine levels (Inden et al., 2011) and a diminution of the striatal innervation (Takeuchi et al., 2009). Moreover, they did not detect any change in the levels of choline acetyltransferase, glutamic acid decarboxylase, or serotonin in the striatum, suggesting that rotenone exerts a selective toxic effect on the dopaminergic neurons (Inden et al., 2011). The dopaminergic lesion triggers a motor dysfunction that is evidenced by a reduction in endurance time and in the percentage of mice remaining on the rotarod. This impairment was reversed with different drugs that reduced the dopaminergic cell loss (Inden et al., 2007, 2009; Takeuchi et al., 2009), although it has not as yet been evaluated if the motor dysfunction can be reversed by dopaminomimetic drugs. Although the presence of protein aggregates was not reported, these authors found an increase of $\alpha$-synuclein in the remaining dopaminergic cells with the absence of tauopathy. This group already used this model successfully to test several neuroprotective strategies with a 28-day treatment regimen (Inden et al., 2007, 2009; Takeuchi et al., 2009), whereas more recently they have demonstrated that a 56-day treatment regimen elicited a more profound dopaminergic degeneration that was accompanied by poorer performance on the rotarod and an increase in the cytoplasmic accumulation of $\alpha$-synuclein in the surviving dopaminergic neurons (Inden et al., 2011).

Another group reported dopaminergic neurodegeneration with orally administered rotenone in mice; however, the low dose that they used (5 mg/kg/d) prevents rotenone from reaching the brain and inhibiting complex I activity there (Pan-Montojo et al., 2010). Their hypothesis was that $\alpha$-synucleopathy spreads from the enteric nervous system through synaptically connected structures including the SNpc. They demonstrated $\alpha$-synuclein accumulation or aggregation in several areas of the nervous system that are also affected in PD. This aggregation was not necessarily associated with cell loss as in the dorsal motor nucleus of the vagus or the intermediolateral nucleus of the spinal cord. However, the accumulation of large $\alpha$-synuclein aggregates in surviving neurons in the SNpc was accompanied by a 15% dopamine cell loss after gavaging 1-year-old mice for 3 months. Based on these results, Pan-Montojo and collaborators suggested that this regimen models the progression of PD postulated by Braak (Braak et al., 2002). Interestingly, the i.p. administration of rotenone in rats also induces $\alpha$-synuclein inclusions in the enteric nervous system (Drolet et al., 2009).

## THE MOUSE PARAQUAT MODEL: THE HERBICIDE THAT KILLS DOPAMINERGIC NEURONS

Paraquat (N,N=-dimethyl-4,4=-bipyridinium dichloride) is an herbicide that was used worldwide until relatively recently, and which was banned in the European Union in 2007. It is known that it exerts its deleterious effects through oxidative stress (Przedborski and Ischiropoulos, 2005). 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 ROS (Day et al., 1999) (Fig. 5). As its chemical name indicates, paraquat is a pyridinium that shares structural similarities with another herbicide used in the past called cyperquat, which happens to be $MPP^+$ (Fig. 4). This fact suggested that both neurotoxicants may also share some mechanisms of neurotoxicity, though the exact details of this are still a matter of debate (Miller, 2007; Cory-Slechta et al., 2008; LoPachin and Gavin, 2008).

Several cases of lethal poisoning resulting from ingestion of or dermal exposure to paraquat have been reported (Smith, 1988). Over the course of many years, experimental studies using paraquat focused on its effects on the
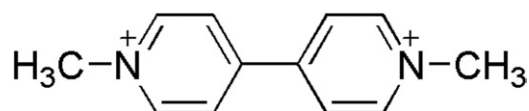


**Fig. 4.** Chemical structure of paraquat.
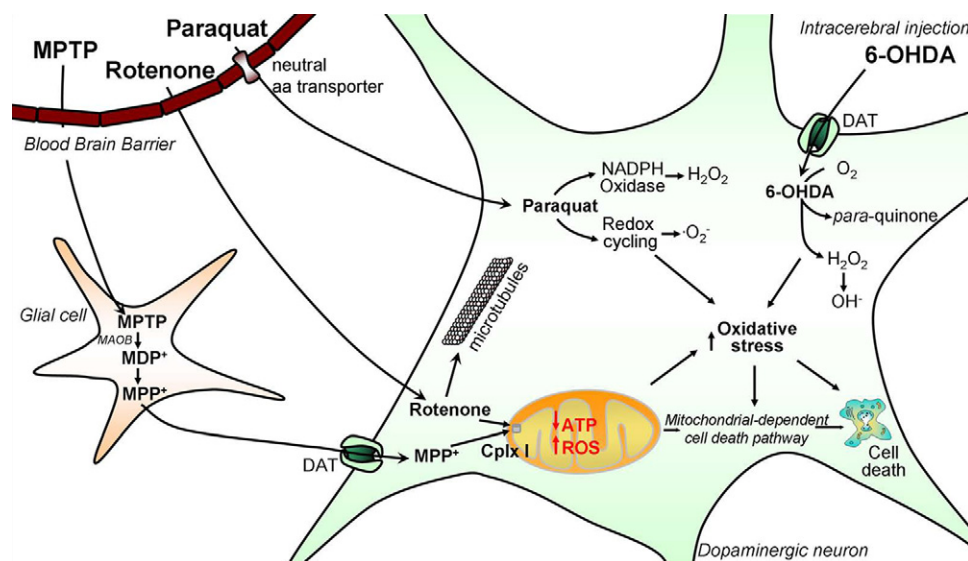
**Fig. 5.** Mechanism of action of neurotoxins used to model Parkinson's disease. 6-hydroxydopamine (6-OHDA) enters neurons via the dopamine transporter (DAT). Following several consecutive reactions it is oxidized, yielding hydrogen peroxide ($H_2O_2$) and para-quinone. It thereby induces neuronal cell death via the production of reactive oxygen species (ROS). MPTP and rotenone, as lipophilic compounds, can cross the blood–brain barrier. Once inside the brain, MPTP is metabolized into the toxic cation 1-methyl-4-phenylpyridinium ($MPP^+$) by the enzyme monoamine oxidase B (MAOB) in glial cells. $MPP^+$ enters dopaminergic cells via the DAT. $MPP^+$ and rotenone accumulate in the mitochondria where they inhibit complex I (Cplx I) of the mitochondrial respiratory chain. Complex I inhibition leads to a decrease of ATP levels, an increase of ROS production, and ultimately to the activation of a mitochondria-dependent cell death pathway. Rotenone can also induce depolymerization of cellular microtubules, which participate in the cell death process. Paraquat enters the brain with the assistance of neutral amino acid (aa) transporters and catalyzes the formation of ROS via two mechanisms: (1) redox cycling and (2) activation of ROS-generating enzymes such as NADPH oxidases.

lung, liver, and kidney, probably because the toxicity induced by this herbicide in these organs is responsible for death after acute exposure. However, significant damage to the brain is seen in individuals who died from paraquat intoxication (Grant et al., 1980; Hughes, 1988). Indeed, paraquat reaches the brain via neutral amino acid transporters (Shimizu et al., 2001; McCormack and Di Monte, 2003), but unlike rotenone is not able to freely cross the blood–brain barrier due to its hydrophilicity. Although the association of exposure to paraquat and higher risk of developing PD has been debated for several years (Liou et al., 1997; Berry et al., 2010), two recent epidemiological studies demonstrated that participants with PD were more likely than controls to have been exposed to paraquat in the past (Tanner et al., 2011; Wang et al., 2011). Those exposed to paraquat, along with exposure to the fungicides ziram and maneb, experienced the greatest increase in PD risk (Wang et al., 2011).

Mice treated with paraquat have been commonly used to model PD. Although some groups have been able to demonstrate dopaminergic cell loss, others failed to reproduce this neurotoxicity after systemic administration. Although the underlying mechanism for this lack of reproducibility remains unclear, the age and the strain of mice used are probably implicated since it has been demonstrated that older mice are more sensitive to paraquat (McCormack et al., 2002) and that the half-life of this neurotoxin varies among different strains (Prasad et al., 2009). In this regard, using the same mouse strain that we normally use to conduct experiments with the MPTP mouse model, we failed to detect cell loss when we followed a regimen of

intoxication that elicited a 25% cell loss in another strain (McCormack et al., 2002; data not published). One example that illustrates the difficulty associated with detecting dopaminergic degeneration following exposure of mice to paraquat is that some authors who eventually demonstrated cell loss (McCormack et al., 2002; Manning-Bog et al., 2002) initially failed to see any behavioral abnormality or nigrostriatal dopaminergic pathway damage in those animals (Thiruchelvam et al., 2000a,b). The authors suggested that the use of a less-sensitive technique than stereological counting may explain the initial failure (McCormack et al., 2002). Paraquat (Fernagut et al., 2007) and MPTP intoxication (German et al., 1992) both trigger a non-homogeneous pattern of cell loss. In both cases the ventral area is the most damaged area of the SNpc, as in PD. Different investigators have also reported reduced motor activity and dose-dependent losses of striatal dopaminergic nerve fibers and substantia nigra neuronal cell bodies in paraquat-treated mice (Brooks et al., 1999). However, in the majority of cases there is not a patent decrease of the dopaminergic innervation of the striatum and there is only a transient diminution of the striatal tyrosine hydroxylase content (Manning-Bog et al., 2002; Prasad et al., 2009). Actually, a significant reduction of dopamine levels in the striatum is only seen after 24 doses of paraquat (administered two or three times per week) (Prasad et al., 2009). The most commonly used regimen of intoxication to induce dopaminergic degeneration is the one that requires one injection per week for 3 weeks, although McCormack and collaborators have demonstrated that the third injection does not trigger further cell loss (McCormack et al., 2005).

It has been demonstrated that microglial activation is pivotal in paraquat-induced degeneration (Purisai et al., 2007; Peng et al., 2009). A single injection of paraquat is enough to trigger microglial activation and predispose dopaminergic neurons to degenerate with the subsequent injections. Neurodegeneration can be prevented when this initial inflammatory reaction is inhibited with minocycline, which is in agreement with the fact that paraquat is only able to kill dopaminergic mesencephalic neurons in the presence of microglia (Peng et al., 2009). In addition, it has been demonstrated that paraquat triggers oxidative stress *in vivo* (Peng et al., 2005; Mc-Cormack et al., 2005) and that microglia may be the source of ROS since paraquat triggers superoxide production in microglia *in vitro* in a concentration-dependent manner (Wu et al., 2005). It seems that induction of microglial NADPH-oxidase may account for ROS production and neurodegeneration since pharmacological (Peng et al., 2009) or genetic (Purisai et al., 2007) inhibition of NADPH-oxidase protects dopaminergic neurons against paraquat.

Regarding the molecular pathways of cell death, it has been demonstrated that paraquat-induced cell death *in vivo* is Bak-dependent (Fei et al., 2008) and is mediated by the activation of the JNK/c-Jun pathway and caspase-3 (Peng et al., 2004). Moreover, *in vitro* studies indicate that paraquat triggers cytochrome c-induced cell death (Fei et al., 2008).

The role of $\alpha$-synuclein has also been explored in this model. An up-regulation of $\alpha$-synuclein in both the frontal cortex and ventral midbrain 2 days after each administration of paraquat has been described (Manning-Bog et al., 2002). Thiofalvine-S-positive $\alpha$-synuclein fibrils have been detected in the dopaminergic neurons 2 days after the third administration (Manning-Bog et al., 2002). These deposits are still apparent 7 days after the third administration, and their pattern is not influenced when human wild-type or mutated (A53T) $\alpha$-synuclein are overexpressed (Manning-Bog et al., 2003). Contradictory results have been published by the two main laboratories working with this model regarding the effect of these forms of $\alpha$-synuclein on the neurodegeneration induced by paraquat. The first work assessing this issue reported that the overexpression of one form or the other protects against paraquat (Manning-Bog et al., 2003). However, a more recent study reported that at 2 and 12 months of age, A53T $\alpha$-synuclein adult mice have the same susceptibility to paraquat as wt mice, and that 23-month-old mice are more susceptible (Peng et al., 2010). Another study also found the same susceptibility to paraquat in young mice overexpressing human wild-type $\alpha$-synuclein compared with control mice (Fernagut et al., 2007).

Paraquat has been combined with neonatal exposure to iron (Peng et al., 2007) and more commonly with the fungicide maneb (Thiruchelvam et al., 2000a,b; Kachroo et al., 2010) to potentiate paraquat neurotoxicity, with this effect occurring in an age-dependent manner.

## CONCLUDING REMARKS

An animal model of a particular disease is validated when the data it generates can be translated to the actual disease to better understand its pathology and to develop new therapeutic strategies. Any one particular model rarely displays all aspects of the disease in question. This is especially true when the pathophysiology of the disease is not fully understood and the etiology is unknown, as is the case in PD. Therefore, trying to model all the aspects of PD is futile until it is known what is causing the neurodegeneration. In contrast, animal experimentation may help to discriminate which elements are relevant to the etiopathogenesis of the disease. We consider it important to promote the use of drugs/toxins with the same or different mechanisms of action and/or transgenic animals to help elucidate which cell death pathways are relevant to PD. From our point of view, these different models will serve as the basis by which it will be possible to identify the unknown variables that are the causative agents of neurodegeneration in PD (Table 1). For instance, we propose to develop a model with Lewy body-like inclusions to study their role in the neurodegenerative process, or to induce neurodegeneration with drugs that interfere with the autophagic degradation system. Pharmacological inhibition of the proteasome degradation system has been previously used to model PD but the initial enthusiasm wore off with the lack of reproducibility of the initial results published (Bove et al., 2006b). A constant validation process must be undertaken that involves contrasting the data generated with models to that seen with human samples/imaging. In this process, it is important to take into account the interspecies differences and the age of the animals under investigation. Age has turned out to be an important factor in PD models since aged animals are more susceptible to neurotoxins and more prone to show histological or behavioral differences compared with young animals. Animal welfare is also an important issue to be considered in animal modeling. The discomfort of the animal must always form part of the process to evaluate animal models.

The 6-OHDA and MPTP models have already enabled us to better comprehend the consequences of dopaminergic cell loss in PD and to develop symptomatic treatments. In other words they have been validated for these purposes. Regarding the molecular mechanisms of cell death, the toxin-based models have highlighted apoptosis, oxidative stress, mitochondrial dysfunction, and microglial activation as key players in PD-related neurodegeneration. All these elements have been identified in PD (Hartmann et al., 2000; Gerhard et al., 2006; Alam et al., 1997a,b; Schapira et al., 1990). Moreover, in all the models reviewed here, and consistent with PD, an accumulation of $\alpha$-synuclein in the dopaminergic neurons is observed. In the MPTP mouse model in particular, elevated $\alpha$-synuclein mRNA levels in dopaminergic neurons have been demonstrated (Vila et al., 2000). This was also seen subsequently in PD brains (Gründemann et al., 2008), suggesting that $\alpha$-synuclein up-regulation may play a role in the neurodegenerative process. These are only some examples of how

**Table 1.** Characteristics of toxic-based animal models of Parkinson's disease

| Toxic model | Behavioral alterations | Nigro-striatal damage | Lewy bodies/$\alpha$-synuclein inclusions | Uses of the model |
|---|---|---|---|---|
| Unilateral 6-OHDA injection into rodent MFB | Quantifiable turning behavior after systemic administration of a dopaminergic agonist. Bradykinesia and impaired paw use on the contralateral side. | Massive loss of dopaminergic neurons (>90%). Dose-dependent loss of striatal dopamine innervation. | No intracellular inclusions. | Test symptomatic therapies. Used in l-dopa-induced dyskinesia and motor fluctuation models. Study of the consequences of dopaminergic denervation on the basal ganglia circuitry. |
| Unilateral 6-OHDA injection into rodent striatum | Quantifiable turning behavior after systemic administration of a dopaminergic agonist. Bradykinesia and impaired paw use on the contralateral side after multiple 6-OHDA injections. | Partial and less acute loss of dopaminergic neurons (30–75%). Circumscribed loss of striatal dopamine innervation at injection site. | No intracellular inclusions. | Test of symptomatic therapies. Study of mechanisms of cell death. |
| Acute MPTP mouse model | Coordination and motor impairments patent in challenging situations. | Massive loss of dopaminergic neurons (about 70%). Reduced dopamine levels in the striatum. | No intracellular inclusions. | Test neuroprotective strategies. Study of mechanisms of neuroinflammation. |
| Chronic MPTP mouse model | Less obvious motor and coordination impairments that are in some cases only detectable in aged mice. | Partial loss of dopaminergic neurons (30–50%). Reduced dopamine innervation in the striatum. | Increased $\alpha$-synuclein immunoreactivity in the substantia nigra. No intracellular inclusions. | Test neuroprotective strategies. Study of cell death mechanisms. |
| Nonhuman primate MPTP model | Motor impairments resembling PD symptoms. Cognitive impairments. | Loss of dopaminergic neurons depending on the route of administration and regimen (60–90%). Reduced dopamine innervation in the striatum. | Inclusions resembling Lewy bodies in the locus coeruleus of older squirrel monkeys. | Test of symptomatic therapies. Used in l-dopa-induced dyskinesia and motor fluctuation models. Study of the consequences of dopaminergic denervation on the basal ganglia circuitry. |
| Rotenone in rodents | Reduced motor activity (rats) and reduction in the endurance time in rotarod (mice). | Loss of dopaminergic neurons (20–75%). Reduced dopamine innervation in a circumscribed area of the striatum (rat). Reduced TH levels in the striatum (mouse). | $\alpha$-synuclein aggregation in dopaminergic neurons and other neuronal populations. | Test neuroprotective strategies. Study of mechanisms of cell death. |
| Paraquat in mice | No clear motor impairments detectable. | Little or no cell loss of dopaminergic neurons (<25%). Little or no measurable changes in striatal dopaminergic denervation. | Increased $\alpha$-synuclein immunoreactivity in the substantia nigra. No intracellular inclusions. | Test neuroprotective strategies. Study of cell death mechanisms. |

data generated by the toxin-induced mammalian models related to putative mechanisms of cell death have been validated against human samples or imaging. However, this validation has its limitations since it does not provide information concerning the chronology or hierarchy of events in the neurodegeneration process.

Thus far, none of the neuroprotective strategies that have worked in the preclinical stage have been translated to clinical practice. There are two main possible explanations for this failure. The first one, as suggested by several authors, is that toxin models are not really useful tools for the screening of neuroprotectants, probably because of the acute nature of cell death in comparison with the progression of the diseased state. The second reason is that the design of clinical trials is not always the most optimum. Indeed, given the lack of validated biomarkers for PD it is very difficult to measure disease progression, meaning that any possible beneficial effects of therapeutic intervention can only be measured at the symptomatic level. Other parameters that could compromise the success of clinical trials are the use of sub-therapeutic doses and the use of groups of patients that are too heterogeneous. Standardizing and/or stratifying clinical populations before their inclusion in a given clinical trial based on symptomatology or on specific characteristics such as complex I activity or the presence of specific mutations linked to higher PD risk, may decrease the variability of the effect of a candidate neuroprotective drug in a subgroup of patients.

The fact that there is no drug or other therapeutic strategy that has a neuroprotective profile in PD patients makes it impossible to validate a particular model. We propose to use epidemiological data linking lower risk of developing PD with nutritional/consumption habits or a specific chronic drug therapy to validate these models. Among the factors that have been associated with a lower

risk for PD is that of smoking, with a 60% reduction of the risk. Epidemiological studies indicate that the duration of the habit is more important than the average number of cigarettes smoked daily (Ritz et al., 2007; Thacker et al., 2007; Chen et al., 2010). It has been demonstrated that cigarette smoke protects dopaminergic neurons in the MPTP mouse model (Parain et al., 2003), indicating that smoking does indeed have a neuroprotective effect. Nicotine has been suggested to be behind this effect since it is also neuroprotective in the MPTP mouse model (Parain et al., 2003; Janson et al., 1988; Maggio et al., 1998), the primate MPTP model (Huang et al., 2009; Quik et al., 2006), the 6-OHDA rat and mouse models (Ryan et al., 2001; Visanji et al., 2006), the rotenone mouse model (Takeuchi et al., 2009), and the paraquat mouse model (Khwaja et al., 2007). Transdermal nicotine treatment has been tested in PD patients, with some studies reporting no beneficial effect on symptom alleviation (Lemay et al., 2004; Ebersbach et al., 1999; Vieregge et al., 2001). In contrast, when higher doses of nicotine and longer treatments were employed, there was an improvement of motor scores that enabled dopaminergic treatment doses to be reduced, and there was also a higher than expected dopamine transporter density in the striatum compared with non-treated PD patients (Itti et al., 2009; Villafane et al., 2007). However, this observational study involved only a few patients and no controls. A randomized, placebo-controlled, double-blind, multi-center trial to assess the disease-modifying potential of transdermal nicotine treatment in early PD is being carried out in Germany and the USA. This trial is assessing for the first time if nicotine can retard PD progression. If nicotine turns out to be neuroprotective in PD patients, all of the models discussed in this issue will have correctly predicted its neuroprotective effect.

Coffee consumption has also been linked with a lower risk of PD (Ascherio et al., 2001) even though some studies failed to show a significant negative association (Facheris et al., 2008). A recent systematic review and meta-analysis of observational studies showed a dose–response-related 25% reduction in the risk of PD among caffeine consumers (Costa et al., 2010). Caffeine has been reported to be neuroprotective in the MPTP mouse model (Chen et al., 2001; Xu et al., 2010), the 6-OHDA rat model (Joghataie et al., 2004; Aguiar et al., 2006), and the paraquat/maneb mouse model (Kachroo et al., 2010). Caffeine probably exerts its protective effect by antagonizing adenosine A2A receptors since A2A antagonists mimic this protection in the MPTP and 6-OHDA models (Chen et al., 2001; Ikeda et al., 2002; Pierri et al., 2005; Bove et al., 2005b). Regardless of the preclinical data, the neuroprotective effect of caffeine has not been proved thus far in clinical trials with early PD patients (Simon et al., 2008). However, consistent with preclinical data (Bove et al., 2006a; Rose et al., 2006, 2007; Pinna et al., 2007; Tronci et al., 2007), new A2A antagonists appear to be good candidates as antiparkinsonians when used as a monotherapy or as adjunct treatment with L-dopa (Pinna, 2009). Therefore, it would be of great interest to assess if A2A antagonists are able to relieve not only parkinsonian symptoms but also to halt the progression of the disease.

A better understanding of PD at the molecular level may lead to the development of better animal models. Notwithstanding, the MPTP and the 6-OHDA models have been extensively replicated by different investigators and are generalized models with a high predictive value for symptomatic treatments. In addition, these models along with the paraquat and rotenone models are contributing to the elucidation of mechanisms of cell death in PD.

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