Nonhuman primate (NHP) models of Parkinson’s disease (PD) play an essential role in the understanding of PD pathophysiology and the assessment of PD therapies. NHP research enabled the identification of environmental risk factors for the development of PD. Electrophysiological studies in NHP models of PD identified the neural circuit responsible for PD motor symptoms, and this knowledge led to the development of subthalamic surgical ablation and deep brain stimulation. Similar to human PD patients, parkinsonian monkeys are responsive to dopamine replacement therapies and present complications associated with their long-term use, a similarity that facilitated the assessment of new symptomatic treatments, such as dopaminergic agonists. New generations of compounds and novel therapies that use directed intracerebral delivery of drugs, cells, and viral vectors benefit from preclinical evaluation in NHP models of PD. There are several NHP models of PD, each with characteristics that make it suitable for the study of different aspects of the disease or potential new therapies. Investigators who use the models and peer scientists who evaluate their use need information about the strengths and limitations of the different PD models and their methods of evaluation. This article provides a critical review of available PD monkey models, their utilization, and how they compare to emerging views of PD as a multietiologic, multisystemic disease. The various models are particularly useful for representing different aspects of PD at selected time points. This conceptualization provides clues for the development of new NHP models and facilitates the clinical translation of findings. As ever, successful application of any model depends on matching the model to the scientific question to be answered. Adequate experimental designs, with multiple outcome measures of clinical relevance and an appropriate number of animals, are essential to minimize the limitations of models and increase their predictive clinical validity.

Key Words: dopamine; MPTP; neuroprotection; neurorestoration; neurotoxicity; Parkinson’s disease; striatum; substantia nigra

Introduction

Nonhuman primate (NHP\(^1\)) models of Parkinson’s disease (PD\(^1\)) are a key tool to unravel PD pathophysiology and evaluate therapeutic strategies for the disease. The motor and cognitive skills of NHPs as well as their neuroanatomical complexity closely resemble those of humans and thus can provide insight on issues that have clinical impact (Capitanio and Emborg 2007). An example relevant to PD is the comparative neuroanatomy of the striatum. In monkeys and humans cortical fibers are grouped in the internal capsule that separates the striatal mass into the caudate nucleus dorsomedially and the putamen ventromedially; in rats, by contrast, the striatum appears as a single mass pierced by cortical fibers (Parent 1986). As white matter tracts affect the distribution of intracerebrally injected compounds, cells, or vectors, research on NHPs (but not rodents) can shed light on the consequences of specific intrastratal targeting of a therapy that may affect its translation to humans.

This article provides a critical review of current PD monkey models, their applications, and methods of evaluation, with emphasis on the importance of matching the model to the scientific question to be answered, the role of experimental design, and the use of multiple outcome measures of clinical relevance to maximize the value of the resulting data set.

Defining Parkinson’s Disease

Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder (after Alzheimer’s disease). In the United States approximately 1 million persons...
manage PD at any given time and 3 to 4 million people are undiagnosed (Korell and Tanner 2005).

The cause of PD remains unclear. Mutations occur in rare, familial cases of PD (Hardy et al. 2006), but most cases of PD are sporadic. Researchers have identified several risk factors, including exposure to pesticides, head trauma, and aging (Allam et al. 2005)—PD most commonly affects people during or after their sixth decade (only 15% of cases are diagnosed before age 50).

The four primary symptoms of PD are resting tremor, rigidity, bradykinesia, and postural instability. As these symptoms become more pronounced, patients may have difficulty walking, talking, or completing other simple tasks. Nonmotor symptoms include urinary problems, constipation, skin problems, sleep disruptions, orthostatic hypotension, and depression and other emotional changes (Pfeiffer 2000). Individual variability in the occurrence of symptoms and in the disease progression and intensity is typical of PD.

The pathologic hallmark of the disease is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of intracytoplasmic inclusions called Lewy bodies, which are accumulations of alpha-synuclein and ubiquitin. Astrocytic gliosis, extracellular neuromelanin, neuromelanin-laden macrophages, and pale bodies are also present in the substantia nigra (SN) of PD patients (Robinson and Rajput 2005). A decrease of 80% or more of dopamine (DA) in the striatum, the main brain area of projection of DA neurons, is responsible for the occurrence of the motor syndrome. This reduction is associated with a loss of 50% or more of dopaminergic cells, mainly in the SNpc. The nigrostriatal system compensates for smaller losses, probably through DA receptor supersensitivity, increased DA production by remaining cells, and/or other plastic adaptations (Przedborski et al. 2003). Other brain areas that may show varying degrees of involvement include the following (Robinson and Rajput 2005):

- the locus coerules, which is the main source of noradrenergic innervation in the central nervous system;
- the nucleus basalis of Meynert, which is the main source of cholinergic innervations of most forebrain areas;
- the dorsal motor nucleus of the vagus;
- the Edinger-Westphal nucleus;
- the paranigral nucleus, which is a component of the dopaminergic mesocortical limbic pathway;
- the intermediolateral cell column of the spinal cord;
- the serotonergic raphe nuclei;
- the pedunculopontine nucleus;
- the reticular formation of thepons and midbrain;
- the hypothalamus; and
- peripheral sympathetic ganglia.

Lewy bodies occasionally appear in the neocortex unassociated with dementia in idiopathic PD patients (Jellinger 1991). Based on these findings, Braak and Braak (2000) proposed that PD is a widespread multisystem degenerative illness that affects the human central, peripheral, and enteric nervous system. This group has also suggested that nonmotor signs may precede motor parkinsonism and that the individual variable expression of PD may be related to the extent and progression of the disease at defined sites (Wolters and Braak 2006).

A presymptomatic method of PD diagnosis does not yet exist. Many of the signs proposed as indicative of pre-PD are not specific to the disease. Current diagnostic examinations generally include assessments of genetic background, autonomic function, and olfaction (Montgomery et al. 2000a,b; Wolters and Braak 2006), either with or without in vivo imaging of the dopaminergic system (the sole use of the latter as a diagnostic or prognostic tool has become controversial; Ravina et al. 2005). Analysis of changes in specific gene expression signals in the blood may also reveal possible biomarkers of PD (Scherzer et al. 2007).

Current PD treatments are symptomatic in nature (Rajput and Rajput 2006). The mainstay of therapy is DA replacement. Because DA cannot cross the blood-brain barrier, DA precursor levodopa is administered. The enzyme dopa decarboxylase converts levodopa to DA. Bloodstream metabolism of levodopa to DA activates the area postrema, potentially causing nausea and vomiting. Inhibitors of dopa decarboxylase, such as carbidopa or benserazide, do not cross the blood-brain barrier and when given with levodopa block its conversion to DA, limiting peripheral side effects (Jurkowski and Stacy 2005). Dopaminergic agonists (e.g., apomorphine, bromocriptine, pramipexole, ropinirole) can be used alone or in combination with levodopa. As symptoms grow worse, drug-related side effects such as dyskinesias increase and the efficacy of therapies declines, leaving many patients severely disabled (Bezard et al. 2001). Surgical options include disruption of the neural circuitry by targeted ablations or, more recently, deep brain stimulation (DBS; e.g., Liang et al. 2006).

**Inducing PD in Nonhuman Primates**

An ideal animal model of PD would include the actual cause of the disease as well as behavioral signs, pathology, and a time course similar to those of human PD. The more similarities between a model and PD, the higher the predictive validity for clinical efficacy of the model’s results. But how can researchers replicate PD in animals without knowing its cause? Is it even possible to mimic this complex progressive, multisystem, multisymptom syndrome that presents with individual differences in age of onset, speed of development, preferential signs, and complications related to treatment? The development of a parkinsonian model that resembles early PD and worsens over time is particularly relevant for the assessment of therapies that are supposed to slow or stop disease progression. However, the capacity of the nigrostriatal system to adapt to relatively small DA losses—adaptations that appear as spontaneous recovery of the system—is a modeling challenge. Is it possible to create an effective model of early PD?
The motor syndrome first described as “paralysis agitans” in 1817 by James Parkinson, and the associated dramatic nigrostriatal DA loss identified by Hornykiewicz and colleagues (Hornykiewicz 1963; Ehringer and Hornykiewicz 1960), are the most striking features of PD and have been the core of PD models. As scientists’ understanding of PD etiology and pathophysiology continues to deepen, there is a better appreciation of both the possibilities and the limitations of available PD modeling systems and of the features that need improvement, which affect the development and testing of new therapies. Most current NHP models focus on aging, gene transfer, and neurotoxins to mimic different characteristics of PD.

Aging

Studies have identified old age as a critical risk factor for the development of PD in humans (Tanner et al. 1999). Although there are no reports of spontaneous development of PD in monkeys, like humans they present age-related dysfunction of the nigrostriatal system that is associated with motor impairments, such as slight tremor, stooped posture, and hand, gait, and balance disturbances (Bachevalier et al. 1991; Emborg et al. 1998; Irwin et al. 1994; Zhang et al. 2000). Studies have described the following dopaminergic nigrostriatal changes with age in monkeys:

- loss of striatal dopamine (Collier et al. 2007; Goldman-Rakic and Brown 1981; Irwin et al. 1994; Wenk et al. 1989),
- decreased DA D2 receptor function (Arnsten et al. 1995; Morris et al. 1999),
- DA transmission deterioration (Gerhardt et al. 1995),
- loss of tyrosine hydroxylase (TH); limiting enzyme of DA synthesis)-positive striatal fibers (Collier et al. 2007),
- decreased number of TH and dopamine transporter (DAT1) immunoreactive nigral neurons counted by stereological methods (Emborg et al. 1998; Siddiqi et al. 1999),
- smaller size of TH- and DAT-positive nigral neurons (Collier et al. 2007; Emborg et al. 1998),
- decreased TH optical density in nigral neurons (Collier et al. 2007),
- lipofuscin accumulation (Kanaan et al. 2007; Siddiqi and Peters 1999), and
- abnormal dendritic morphology (Siddiqi and Peters 1999) of TH-positive nigral neurons.

In vivo imaging has shown decreased striatal fluorodopa uptake measured with positron emission tomography (PET; De Jesus et al. 2001), and researchers have used magnetic resonance imaging (MRI) to demonstrate that increased iron accumulation with age predicts declines in motor function (Cass et al. 2007). The changes listed above were obtained in rhesus monkeys (Macaca mulatta), with the exception of those reported in Irwin et al. (1994), which utilized squirrel monkeys (Saimiri sciureus).

The listed changes resemble an early PD state that worsens over time, which is difficult to achieve in other modeling systems. But aging is a natural process, not a disease, and symptoms present subtle differences between individuals. Positive response to levodopa treatment can be measured only with sensitive behavioral tests (Grondin et al. 2000). In addition, criteria for the definition of “aged” animals vary among different species (e.g., postmenopausal age for rhesus monkeys is over 25 years while for squirrel monkeys it is approximately 15 years) and can influence data comparisons between age groups and species. It is therefore important to carefully consider such factors in the study design, species selection, and methods of evaluation. For example, stereological counts of TH-positive neurons in the SN of 26- to 28-year-old rhesus monkeys showed significant loss (Emborg et al. 1998), whereas Irwin and colleagues (1994) did not find changes in 15.8- to 21-year-old squirrel monkeys’ TH nigral numbers by serial section cell counts, although they noted significant loss of DA in the SN.

As NHPs have a lifespan of several decades, and aged monkeys require specialized care, funding of aging colonies is expensive, a practical factor to consider when choosing to work with this model. Aged NHPs may develop diseases typical of old age such as cancer, arthritis, or diabetes, as well as cognitive impairment that is evident in delayed-response tasks (Roberts 2002). These changes may affect the experimental results in a PD study, similar to the complications that many PD patients encounter as they age. Aged NHP models nonetheless provide a framework to study the effect of therapies in an aged system (e.g., Kordower et al. 2000; Maswood et al. 2002) and the role of aging in the development of PD (e.g., Kanaan et al. 2007).

Targeted Gene Transfection

Five percent of all PD cases are familial, and researchers have identified several genetic mutations related to the disease (Hardy et al. 2006; Mariés et al. 2003). Alpha-synuclein is one of the major components of Lewy bodies and Lewy neurites in sporadic PD (Spillantini et al. 1997, 1998), and two missense mutations in the alpha-synuclein gene (A53T and A30P) have been identified in several families with autosomal dominant parkinsonism (Kruger et al. 1998; Polymeropoulos et al. 1997). But there are no reports of monkeys with similar spontaneous mutations, and it is difficult to breed NHPs with targeted genetic mutations. In addition to the challenges that usually accompany genetic engineering, monkeys have small litters (one or two babies per pregnancy), and their prolonged lifespan (e.g., over 30 years for rhesus monkeys in captivity) makes it difficult to evaluate the induction of a disease that is associated with the aging process.
As an alternative to assess the role of alpha-synuclein in nigral cell death and develop a new PD monkey model, Kirik et al. (2003) and Esllamboli et al. (2007) used gene transfer methods to introduce the alpha-synuclein gene in the brain of adult common marmoset monkeys (Callithrix jacchus) (National Institutes of Health safety guidelines for viral vector manipulation are available at www.nih.gov). In the earlier of these two studies (Kirik et al. 2003), under sterile surgical conditions monkeys received two stereotaxic injections in the right substantia nigra of 3 μl of recombinant adeno-associated viral (rAAV) vector 2 encoding for (a) alpha-synuclein wild type (n = 2), (b) A53T mutation (n = 2), or (c) green fluorescent protein (GFP; n = 4) (titer: 8.2 × 10^{11}, 1.4 × 10^{12}, and 1.5 × 10^{11} infectious units/ml respectively) at a rate of 0.25 μl/min by using a 29-gauge injection needle that remained in place for 4 minutes after each injection. The researchers monitored the animals for 16 weeks. Although the NHPs had an inconsistent response to amphetamine administration to assess DA receptor supersensitivity (typically observed in unilaterally DA-lesioned animals as rotations ipsilateral to the side of the lesion), the alpha-synuclein-treated monkeys showed head position bias, which worsened over time, consistent with an increase in rAAV2 gene expression. Postmortem histological analysis of the brains of GFP-treated monkeys euthanized at 3 (n = 2) and 16 (n = 2) weeks showed increased GFP expression over time. The brains of the alpha-synuclein treated monkeys euthanized 16 weeks after injection revealed the presence of alpha-synuclein-positive cytoplasmic inclusions and granular deposits in the substantia nigra neurons as well as swollen, dystrophic, and fragmented neurites, associated with decreased striatal immunostaining of the dopaminergic markers tyrosine hydroxylase (TH) and vesicular monoamine transporter 2 (VMAT2) in the striatum and a significant loss (by stereological counts) of TH and VMAT2-positive nigral neurons. Nissl immunostaining confirmed nigral cell loss.

In a subsequent study (Esllamboli et al. 2007), 24 adult common marmoset monkeys received 2 stereotaxic injections in the substantia nigra of 2 μl of rAAV2/5 encoding for (a) alpha-synuclein wild type (n = 10), (b) A53T mutation (n = 10), or (c) GFP (n = 10). The animals, which were followed for up to 52 weeks, remained asymptomatic for the first 9 weeks, and then head position bias and spontaneous rotations appeared in the wild-type alpha-synuclein-expressing animals between 15 and 27 weeks. In the later phase, the animals overexpressing the A53T mutation, in particular, showed a gradual worsening of hand motor performance in six- and two-tube choice tasks and on the Hill and Valley staircase tasks as well as increased gait motor coordination errors. Histological analysis from animals overexpressing either the wild-type or A53T alpha-synuclein showed degeneration of dopaminergic fibers in the striatum. In the ventral midbrain, however, the dopaminergic neurodegeneration was more prominent in the A53T group than in the wild-type group, suggesting differential toxicity of these two proteins in the NHP brain. Antibodies to the pathological form of phosphorylated alpha-synuclein stained the surviving cell bodies and their processes in the substantia nigra. Alpha-synuclein aggregates were also positive for ubiquitin. Alpha-synuclein expression occurred not only in neurons but also in astro- and oligodendroglia. In that regard, animals of the wild-type (four of eight) and the A53T (two of seven) alpha-synuclein groups followed for 1 year showed loss of oligodendroglial cells and myelin in the cerebral peduncle.

Replication of this model by other laboratories is still needed. It is important to note that this model requires a surgical setting and trained personnel to perform stereotaxic brain surgery and to provide care for the primates afterward. Additional heating and soft food (e.g., cut-up fruit, moistened monkey chow) are minimal postsurgical requirements. Magnetic resonance imaging (MRI) for surgical nigral targeting is recommended for application of this model to other NHP species such as rhesus monkeys. It should be kept in mind that, if the study requires a comprehensive model of PD, unilateral stereotaxic delivery of the vectors into the substantia nigra does not fully reproduce the multisystem, multisymptom syndrome that characterizes either sporadic or familial PD.

A unilateral model is advantageous as the animals present an internal control in the other brain hemisphere and can feed and groom themselves in case of severe DA loss. At the same time, a unilateral model lacks features of bilateral PD, which theoretically could be solved by injecting vectors in the right and left substantia nigra. But careful evaluation of bilateral transfection is essential in case the procedure results in severe impairment that may require additional care.

An attractive feature of this gene transfer model is that the use of AAV vectors induces a progressive increase in protein expression that takes several weeks (symptoms became evident at 9 weeks) to reach its peak, with the lesion developing over a similar period of time. It is important to recognize that this characteristic is vector dependent, as for example lentiviral vectors reach peak levels of expression in a few days (e.g., Lo Bianco et al. 2002) and different rAAV vectors display different transfection efficiency and tropism (Burger et al. 2004).

Researchers should carefully consider the selection of the rAAV vector type, as alpha-synuclein cytotoxicity and rAAV2/5 higher dispersion (compared to rAAV2) and tropism may cause oligodendrocyte death and demyelination. As Esllamboli and colleagues (2007) reported, six of fifteen marmosets treated with alpha-synuclein (either wild-type or mutated) presented demyelination in the cerebral peduncle. This white matter structure, located immediately ventral to the SN, is formed by the motor corticospinal pathway. Demyelination of this tract affects motor performance and adds a confounding factor in the evaluation of the motor symptoms.

With these caveats in mind, this model presents an opportunity to analyze the role of overexpression of wild-type...
or mutated alpha-synuclein in PD pathology and to assess specific neuroprotective or restorative therapies.

**Neurotoxins**

The neurotoxins 6-hydroxydopamine (6-OHDA) and 1 methyl-4 phenyl-1,2,3,6 tetrahydropyridine (MPTP) are the most used toxins for modeling PD in animals.

**6-OHDA**

6-OHDA, the first identified catecholaminergic neurotoxin (Jonsson and Sachs 1975; Thoenen and Tranzer 1968; Ungerstedt 1968), induces cell death via oxidative stress after uptake by the catecholamine transport system of DA and norepinephrine. 6-OHDA is unable to cross the blood-brain barrier and requires intracerebral administration to exert its toxic effects. The compound is light sensitive and should be shielded from light. As 6-OHDA in solution oxidizes easily, it is important to freshly prepare for its administration (approximately one new batch per hour for prolonged or multiple procedures), by dissolving the powder in saline and ascorbic acid (0.2 mg/ml in 0.9% saline), usually at a concentration of 4 to 5 μg/μl. Although 6-OHDA cannot easily cross cellular membranes, laboratory personnel should exercise caution to avoid unnecessary exposure, including the use of gloves when handling the compound and sanitization of the working area with appropriate cleaning material.

The time course and severity of the PD model depend on the number, amount, and location (striatum, substantia nigra, or medial forebrain bundle) of the 6-OHDA injections (Emborg 2004; Zigmond and Stricker 1989). Eslamboli and colleagues (2003) have used common marmoset monkeys to model PD, with nine striatal injections of 6-OHDA. Because of the spontaneous recovery of the symptoms 10 weeks after surgery, they developed a new model using eighteen unilateral intrastriatal injections (Eslamboli et al. 2005), with each injection delivering 2 μl of 6-OHDA solution infused at 0.5 μl/min. After each injection the 28-gauge needle remained in place for 2 minutes before withdrawal. The treated animals presented a significant unilateral TH-positive nigral cell loss (by stereological cell counts) that was associated with sensorimotor neglect (evident in two- and six-tube tasks), head position bias, amphetamine-induced rotation, and fine motor skill deficits (evident in the Hill staircase task). The deficits persisted for 17 weeks, although some recovery of function occurred by the end of the observation period.

A complication of this model is the numerous intracerebral needle passages necessary to achieve appropriate 6-OHDA distribution, in order to decrease the extent of spontaneous recovery. As with other stereotoxic procedures, this model requires a surgical setting and trained personnel to perform brain surgery and to provide care for the primates afterward. Additional heating and softened food should be available during the postsurgical recovery period. Replication by other laboratories has not yet been done. MRI targeting is recommended in applications of the method to other NHP species, such as macaque monkeys. As with other stereotoxic methods, unilateral striatal delivery of 6-OHDA does not fully reproduce the multisystem, multisymptom syndrome that characterizes PD.

The unilateral syndrome has the advantage of a built-in control in each animal in the contralateral brain hemisphere. Treated monkeys can feed and groom themselves, even in the case of severe DA loss. While this model lacks features of bilateral PD, bilateral 6-OHDA injections might achieve this effect, although dose titration may be necessary to avoid severe debilitation. With all its limitations, this model induces a retrograde, relatively progressive (over approximately 2 weeks) nigrostriatal lesion, which may provide a window of time to study oxidative stress and neurodegeneration and to test neuroprotective strategies (e.g., Eslamboli et al. 2005).

**MPTP**

MPTP-induced parkinsonian models are the most widely used NHP models of PD. MPTP is a mitochondrial complex I inhibitor that was discovered in accidentally exposed humans (Davis et al. 1979; Langston et al. 1983). It is highly lipophilic and easily crosses the blood-brain barrier. It becomes toxic through its transformation to MPP+ by the enzyme MAO-B. The DA transporter system then transports the MPP+ into DA neurons.

Investigators and animal caretakers should avoid chronic exposure to even small amounts of MPTP because of the long-term increased risk of developing PD (Langston 1996). Careful adherence to handling and usage guidelines reduces the risk of exposure; NIH safety guidelines for MPTP use are available at the National Institutes of Health website (www.nih.gov, Procedures for Working with MPTP or MPTP-Treated Animals). Before using MPTP, investigators should ensure that there is an institutional plan to deal with MPTP-related issues, including quarantine quarters for monkeys after MPTP injections, disposal of contaminated material, and training for the safe handling and use of the neurotoxin (Przedborski et al. 2001).

MPTP-treated monkeys develop a parkinsonian motor syndrome that replicates key PD features such as rigidity, bradykinesia, and postural instability and that is responsive to conventional dopamine replacement treatments (e.g., Stephenson et al. 2005). Similar to PD patients, MPTP-treated monkeys (in particular, those with bilateral parkinsonism) may also present nonmotor signs, including frontostriatal cognitive deficits (Schneider and Kovelowski 1990; Schneider and Pope-Coleman 1995; Taylor et al. 1999) and changes in sleep pattern (Almirall et al. 1999; Barcia et al. 2003). Researchers have also documented temporary autonomic disturbances affecting noradrenergic cardiac innervation (Goldstein et al. 2003).

Biochemical analysis of striatal DA in PD monkeys re-
veals differences depending on the method of administration and, to a lesser degree, the individual. A common finding is the generalized DA depletion in the caudate and putamen nucleus; this pattern differs from human PD, in which DA loss is more prevalent in the caudal putamen nucleus and rostral caudate (Kish et al. 1988; Pifl et al. 1988). MPTP also affects levels of other brain monoamines. Pifl and colleagues (1991) compared the levels of dopamine, noradrenaline, and serotonin in 45 brain regions in nine rhesus monkeys treated with chronic systemic MPTP. As expected, the most significant alterations occurred in the nigrostriatal dopamine system. However, many extrastriatal regions of the subcortex and brainstem also suffered significant loss of dopamine, with the noradrenaline loss in the regionally subdivided brainstem being less widespread, and the serotonin levels least affected. In the cortex, noradrenaline loss was greater than serotonin, which was greater than dopamine. In the cerebellar cortex, dopamine and noradrenaline concentrations were significantly reduced, whereas the serotonin level remained unchanged. An interesting finding was that many of the subcortical and cortical changes in symptomatic monkeys also appeared in asymptomatic animals, suggesting individual differences in functional compensatory mechanisms.

Morphologically, the most striking feature after MPTP administration is the loss of dopaminergic nigral neurons associated with loss of dopaminergic striatal terminals that can be observed with antibodies against TH or VMAT2. Although typical Lewy body structures have not been found, researchers have reported eosin-positive inclusion bodies in the substantia nigra, locus coeruleus, nucleus basalis of Meynert, dorsal motor nucleus of the vagus, and raphe nucleus of an aged, chronic systemic MPTP-treated squirrel monkey (Forno et al. 1986, 1993). There are also reports of alpha-synuclein overexpression in the substantia nigra of baboons treated with chronic MPTP (Kowall et al. 2000) and rhesus treated with intracarotid MPTP (Emborg et al. 2003a). We also note a study using mice, in which chronic delivery of low MPTP doses via osmotic pumps induced Lewy body–like structures (Fornai et al. 2005), suggesting that alpha-synuclein aggregation may be a result of slow, persistent nigral deterioration. Researchers have observed nigral inflammation in systemic (Barcia et al. 2004; McGeer et al. 2003) and intracarotid (McGeer et al. 2003) MPTP-treated monkeys years after the intoxication. This finding suggests an ongoing pathological process triggered by the neurotoxin that resembles PD progression in human patients (Miklossy et al. 2006).

Depending on the method of administration, treated monkeys present a unilateral or bilateral syndrome. The predictability, stability, and speed of onset of the symptoms depend on the dosing regime, although individual variability affects the response to MPTP. It is important to adjust the amount of MPTP and the frequency of administration based on the age, weight, and monkey species in order to induce a successful PD syndrome without severe debilitation.

**Systemic administration of MPTP.** Intramuscular (IM), subcutaneous (SC), or intravenous (IV) injections are appropriate methods of systemic administration in monkeys. Intraperitoneal injections to NHPs are not advisable as they increase the risk of infection or gastrointestinal complications. Administration of 0.3 to 0.6 mg/kg IM for 4 to 5 consecutive days to St Kitts African green monkeys (Cercopithecus aethiops sabaeus) can induce severe bilateral dopaminergic nigrostriatal loss, which is associated with a severe bilateral PD syndrome. Resting tremor (a difficult feature to replicate in NHPs) may also be present (Tetrud et al. 1986). Yet this regimen has high individual variability and animals under the same treatment may be asymptomatic or only mildly affected and may later present spontaneous recovery (Elsworth et al. 2000). It is important to evaluate animals after each dosing for PD signs and general effects of MPTP intoxication in order to stop administration if necessary. Common marmosets have also been treated with acute MPTP dosing (2.0 mg/kg SC for 4 to 5 consecutive days; Kupsch et al. 2001). Their evaluation during the first week after intoxication showed decreased motor activity associated with dopaminergic nigrostriatal loss. Bezard and colleagues (1997, 2001) tested an alternative systemic method in cynomolgus monkeys (Macaca fascicularis), with daily dosing of 0.2 mg/kg IV of MPTP until a predefined PD syndrome became evident, after 2 to 3 weeks of daily injections.

Chronic administration of MPTP (0.2 to 2.0 mg/kg IM, IV, or SC) 1 to 2 times per week every 1 or 2 weeks for several weeks or months is another method to mimic the progression of PD and decrease the animals' general debilitation as they have time to recover between doses. Administration of the neurotoxin continues as needed to develop the desired level of disability. The amount and frequency of MPTP administration also define the speed of onset of the signs, with lower dosing requiring many months of treatment. Chronic MPTP administration has been effective for inducing different degrees of parkinsonism in baboons (Papio papio), rhesus, and squirrel monkeys (Brownell et al. 1998; Langston et al. 2000; Perez-Otaño et al. 1994; Schneider and Kovelowski 1990; Stephenson et al. 2005). A clear advantage of a very slow, chronic regimen is that it induces a protracted degeneration. Chronic MPTP administration in baboons induces an uneven loss of dopaminergic striatal fibers and mesencephalic dopaminergic neurons that is similar to that observed in PD and is not found in acute models (e.g., Hantraye et al. 1993; Vararet et al. 1994). This slow method of inducing a PD state has less impact on overall health and less individual variability, as the dose is titrated to effect. Presymptomatic stages may present cognitive impairments, and motor disabilities become evident after subsequent doses and persist for several years (Brownell et al. 1998). A validated scale to rate parkinsonian symptoms (see Imbert et al. 2000) is useful for evaluating monkeys before dosing to determine their tolerance to MPTP as well as their motor disability. If acute toxicity to MPTP requires a monkey to have special care, it is appro-
ropriate to delay subsequent doses and to reevaluate the dosing frequency. Individual dose amounts and the total number of doses can be titrated to produce similar degrees of parkinsonian disability (measured with the rating scale) as defined by the experimental design.

The care and feeding of monkeys is critical during MPTP treatment. There is great variability in the nature of systemic MPTP acute toxicity and NHPs may or may not present PD signs after the first 2 to 3 MPTP dosings. Particularly with low dosing, signs may take months to become evident. The first signs to appear are slowness (brady-kinesia) and reduced amount of movement (hypokinesia). Stooped posture, gait disturbances, and impairments in motor skills may follow subsequent dosings. These early symptoms may be difficult to spot without appropriate training and knowledge of both normal monkey behavior and the characteristic signs of PD. It is critical to closely monitor the animals (e.g., body weight, feces characteristics) and to provide supportive care as needed. Animals that are in good condition after a dosing may be extremely sick after the next one. High dosing of MPTP may cause total akinesia and gastrointestinal ulcers (Ueki et al. 1989).

The general effects of MPTP or the resulting difficulty moving may cause animals to decrease their food and fluid intake and therefore lose weight. Researchers should anticipate this effect and take care of the animals before they lose significant body weight (>10%). There are several methods to address this problem. Offering a variety of novel food items will entice the animals to eat. Monkey biscuits softened in orange juice or yogurt are easier to eat than dry biscuits. Training the animals before MPTP dosing to drink liquid diet supplements (which supply additional fluids, calories, and nutrients) from a syringe offered by a trusted animal caretaker can facilitate post-MPTP care. Nasogastric delivery of liquid diet supplements and fluids may be appropriate in certain cases (Stephenson et al. 2005). In addition, supplementary heating should be available, as the treated animals may become hypothermic (Satoh et al. 1987) due to a combination of decreased caloric intake, general debilitation, and probably the effect of MPTP in the hypothalamus, similar to the thermo-dysregulation that occurs in some human PD patients (Davis 2005). It is usually not necessary to continue the supplemental care after the MPTP phase of treatment, although severe parkinsonian monkeys, like humans with advanced PD, require sustained additional care. DA replacement therapy can facilitate animals’ care, although it may affect experimental results, in particular in studies of dyskinesias. Investigators should also be aware of the increased risk to themselves and personnel of exposure through long-term handling of MPTP, MPTP-treated monkeys, and their excreta.

Systemic MPTP models present the advantage of inducing a bilateral syndrome, which makes it possible to use each brain hemisphere for different analysis and to affect other systems in addition to the nigrostriatal system. Surgical procedures are not necessary. Slow, chronic systemic models are especially fitting for studies on the progression of PD, as they closely resemble the behavioral, biochemical, and pathological features of PD, including the development of typical L-dopa-induced dyskinesias. Once the syndrome is stabilized (usually 2 to 3 months after the last dose of MPTP), interventions such as restorative strategies by drugs, gene transfer, or cell therapy can be successfully studied in these models. Investigators looking for fast results and/or to apply this model in efficacy studies should keep in mind that the chronic slow model requires many months to obtain monkeys with equivalent parkinsonian signs, and that this increases total housing cost for the monkeys. Although acute systemic administration can induce a PD syndrome in 4 to 5 days, its main limitations are severe animal debilitation and lack of predictability. As mentioned above, titrated dosing of MPTP overcomes inter-individual reproducibility, but the inability to predict the resulting onset of PD syndrome for each animal complicates the application of this model for neuroprotective studies. The neurotoxin may adversely affect therapies, in particular cell-based treatments that begin before the end of MPTP administration. Therapies may also affect MPTP metabolism and toxicity, and this effect should be evaluated as a potential confounding factor in neuroprotective studies.

**Intracarotid administration of MPTP.** Intracarotid administration of MPTP is an appealing model because it decreases the risks to animals and investigators as well as the variability associated with the systemic model. In expert hands this procedure has a high success rate (over 70%) of inducing a unilateral parkinsonian syndrome with almost no mortality. The original MPTP-HCl dose of 0.4 mg/kg used in rhesus monkeys (Bankiewicz et al. 1986) has to be adjusted for the monkey's species and age (Ovadia et al. 1995). Based on the experience of my laboratory and other colleagues (e.g., Emborg et al. 2001, 2006; Kordower 2000, 2006), the administration of a total amount of 3 mg to rhesus or cynomolgus males or females over 5 years old and weighing 5.5 to 7 kg is safe and ensures a stable lesion. Animals present signs such as contralateral arm rigidity, brady-, and hypokinesia 24 to 72 hours after surgery and remain parkinsonian for many years (Embrog-Knott and Domino 1998).

The procedure takes place in sterile surgical conditions with the monkey supine and its neck hyperextended and slightly turned left or right. An incision is done along the medial edge of the sternocleidomastoid muscle. The common carotid artery, internal jugular vein, and vagus nerve are identified. A vascular clip is temporarily used to clamp the external carotid artery and its primary branch, the superior thyroid artery. A 27-G butterfly needle inserted in the common carotid artery in a direction retrograde to the blood flow infuses 20 ml of saline containing MPTP dose at a rate of 1.33 ml/min for 15 minutes (Emborg et al. 2006). Upon completion of the infusion, a 3-ml postflush of saline is delivered through the system followed by retrieval of the clamp. After withdrawal of the needle, a small piece of Gelfoam is used to apply focal pressure to the penetrated vessel. The musculature, subcutaneous tissues, and skin are...
then closed in routine fashion. As in all surgical procedures, vital signs, including temperature, O₂, and CO₂ respiration, and heart rate are monitored and recorded. Animals receive cefazolin (25 mg/kg IM) and buprenex (0.01 mg/kg IM) both before and then 6 to 12 hours after surgery and remain in quarantine for the following 72 hours.

Age increases animals’ susceptibility to MPTP (Ovadia et al. 1995), and for successful dosing we recommend the use of animals over 5 years old. Dosing of aged rhesus monkeys (over 25 years old) should be no more than two-thirds of the original dose (Collier et al. 2005; Moirano et al. 2006). Capuchin (Cebus apella; Emborg and Colombo 1994) and squirrel (Pinkston et al. 1995) monkeys usually require higher MPTP doses (approximately 1.2 mg/kg) to obtain a similar hemiparkinsonian syndrome.

In all cases and in particular for aged monkeys, supplemental care should be available as needed during the first week after neurotoxin administration. The two main items that should be ready for all NHPs as they emerge from surgery are additional heating (mainly during the first few hours of recovery) and moistened monkey chow and pieces of fruit to encourage eating.

Intracarotid artery administration of MPTP presents the advantage of inducing a unilateral model in which the animals require minimal or no critical care, the syndrome develops in days, and the model is predictable and reproducible. All of these features make this model suitable for quick studies on PD pathophysiology, neuroprotection, or restoration with enough animals to give significant statistical power. Due to the specificity of MPTP’s toxic metabolite MPP⁺ for DAT and affinity for neuromelanin (D’Amato et al. 1986), most of the neurotoxic uptake by DA nigral neurons occurs in the first passage and negligible amounts reach the contralateral brain hemisphere, inducing a unilateral syndrome. Limitations of the intracarotid model are that it requires a surgical setting and expertise to perform the intracarotid delivery. Personnel require training to care for the animals after surgery in quarantine quarters. The model is unilateral, acute, and does not develop typical dyskinesias associated with chronic L-dopa treatment.

Researchers have performed bilateral intracarotid artery administration in two stages (Smith et al. 1993) to induce a bilateral syndrome. This model has limited application mostly because of the combination of two surgeries that debilitate the animals and the extensive acute dopaminergic loss, which generates a severe PD syndrome that requires supplemental care.

Combined systemic and intracarotid MPTP administration. Combinations of systemic and intracarotid artery dosing (Eberling et al. 1998; Oiwa et al. 2003) present an interesting alternative to obtain a bilateral but asymmetrical syndrome in a shorter period of time than by systemic administration alone. The severity of the syndrome depends on the amount of systemic MPTP and the individual sensitivity of the animal. Resting tremor occurs in animals that develop an advanced PD syndrome (Emborg et al. 2003b). A combination of intracarotid and IV administration of MPTP accelerates the onset of the bilateral syndrome, and the asymmetry of the two methods results in one brain hemisphere with a severe lesion (useful for assessing restorative strategies) and the other with a moderate lesion (useful for testing neuroprotective or trophic strategies). Titrated systemic MPTP administration reduces inter-individual differences associated with systemic dosing but compromises the predictability of the effects.

Alternative Parkinsonian Agents

In addition to 6-OHDA and MPTP, other potential parkinsonian agents, such as pesticides, have been tested in animals (Emborg 2004). They include the following:

- Saphenous vein injection of the microorganism Noocardia asteroides to cynomolgus monkey induced nigral intracellular inclusions (reactive to alpha-synuclein and ubiquitin) and inflammation (Chapman et al. 2003).
- Isoquinoline derivatives are endogenous metabolites, weak inhibitors of the mitochondrial complex I. Systemic chronic administration of this compound to common marmosets (Nagatsu and Yoshida 1988) and squirrel monkeys (Yoshida et al. 1990) induced a PD syndrome responsive to L-dopa treatment associated with a decline in nigral DA and TH activity, although loss of nigral neurons with Cresyl violet staining was not observed.
- Chronic inhibition of complex I by the lipophilic pesticide rotenone causes highly selective nigrostriatal dopaminergic degeneration in rodents, associated with hypokinesia and rigidity (Betarbet et al. 2000). Nigral neurons in rotenone-treated rats accumulate fibrillar cytoplasmic inclusions that contain ubiquitin and alpha-synuclein. These findings indicated that chronic exposure to a common pesticide can induce a PD state. There have been no NHP studies of rotenone because of its high individual variability and potential general toxicity, but other pesticides (e.g., paraquat, maneb; Thiiruchelvam et al. 2002), alone or in combination, may be effective PD agents and warrant application in NHP models.
- Impaired proteasome function is a potential mechanism for dopaminergic neuron degeneration. One study reported that the systemic administration to rodents of the proteasome inhibitor epoxomicin induced a progressive PD syndrome, nigral cell loss, and Lewy body–like inclusions (McNaught et al. 2002). But several laboratories have failed to replicate the model in rats (Kordower et al. 2006; Manning-Bog et al. 2006) or induce a PD syndrome in cynomolgus monkeys (Kordower et al. 2006), indicating poor reproducibility of the model (Beal and Lang 2006; Bove et al. 2006).

Evaluating Parkinsonism in Monkeys

Monkey models of PD present behavioral and neurochemical deficits associated with the pathologic degeneration that
is characteristic of humans with PD. The use of multiple outcome measures can provide a multidimensional insight of the model, therapies, and their shortcomings.

Behavioral Tests

Behavioral tests provide an external measure of the brain pathology and can be used to select animals for a specific study or correlate function to deficits. The ultimate clinical goal of developing a strategy for PD is to cure the parkinsonian symptoms, and this should be a primary outcome measure when assessing treatments in animals. For translational research purposes, the relevance of the data will depend on the parkinsonian sign under study and the similarity between the animal tests and clinical evaluation. Again, the model and species determine the characteristics of the syndrome and the ability to assess particular features, such as changes in fine motor skills. Moreover, the sensitivity of the tests depends on their adaptation to the model and defines, for example, their capability to detect a positive response to DA replacement treatment. A battery of behavioral tests that assess different aspects of the syndrome becomes a powerful tool for clinical prediction. Evaluation of parkinsonian features using a rating scale, fine motor skill tasks, and activity monitoring are relevant tests to assess the PD motor syndrome. Depending on the goal of the study, additional tests, including cognitive and motor planning evaluation as well as studies of sleep pattern or autonomic function, may be relevant (see Table 1; Emborg 2004). General measures of health—such as regular weight records, frequency and characteristics of feces, standard blood and chemistry panels, gross and microscopic examination of body tissues—can provide valuable measures of the general effects of parkinsonian agents or antiparkinsonian treatments that may assist in the preparation of a formal submission of a novel therapy.

In Vivo Imaging

Investigating DA function in vivo using neuroimaging allows for the evaluation of an endpoint in monkeys that is exactly the same as in the clinic. Neuroimaging functional

<table>
<thead>
<tr>
<th>Behavioral measure</th>
<th>Pros</th>
<th>Cons</th>
<th>References</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotations</td>
<td>Simple evaluation</td>
<td>No clinical value, low sensitivity to small changes in striatal DA</td>
<td>Bankiewicz et al. 1986, Eslamboli et al. 2003</td>
<td>Rhesus C. marmoset</td>
</tr>
<tr>
<td>Clinical rating</td>
<td>Clinical value</td>
<td>Sensitivity depends on scale and rater</td>
<td>Imbert et al. 2000, Langston et al. 2000</td>
<td>Macaque</td>
</tr>
<tr>
<td>Activity monitoring</td>
<td>Standardized equipment, circadian rhythm can be evaluated, quantifies a clinical observation</td>
<td>Unilateral models can have increased activity due to spontaneous rotations</td>
<td>Irwin et al. 1994, Emborg et al. 1998, Palfi et al. 2000</td>
<td>Squirrel, Macaque, Capuchin</td>
</tr>
<tr>
<td>Fine motor skills tasks</td>
<td>Clinical value, very sensitive to striatal DA loss</td>
<td>Training and testing are involved and time-consuming</td>
<td>Kordower et al. 1995, Zhang et al. 2000, Eslamboli et al. 2005</td>
<td>Macaque, Macaque, C. marmoset</td>
</tr>
<tr>
<td>Resting tremor recording</td>
<td>Standardized equipment (electromyography or tremor monitor), quantifies a clinical observation</td>
<td>Electromyography is invasive, training is time-consuming, requires animal to be restrained</td>
<td>Bergman et al. 1994, Emborg et al. 2003b</td>
<td>Macaque</td>
</tr>
<tr>
<td>Spontaneous blink rate</td>
<td>Quantifies a clinical observation, simple evaluation</td>
<td>Relative clinical value</td>
<td>Elsworth et al. 1991</td>
<td>Vervet</td>
</tr>
<tr>
<td>Cognitive testing</td>
<td>Clinical value</td>
<td>Training and testing are involved and time-consuming</td>
<td>Taylor et al. 1990a,b, Schneider et al. 1995, Lipina and Colombo 2007</td>
<td>Macaque, Macaque, Capuchin</td>
</tr>
</tbody>
</table>
methods can be effective tools for estimating dopamine cell degeneration, sympathetic denervation, adaptive responses to injury, and, importantly, the effect of therapeutic interventions (Goldstein et al. 2003; Sanchez-Pernaute et al. 2002). Different tracers (F-dopa, F-meta-tyrosine, (-)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane [CFT], tetrabenazine, raclopride) labeled with emission positrons, such as 18F or 11C, provide insight at different levels of the integrity of the DA system (see Table 2; Doudet et al. 2006).

An alternative to the use of radio-labeled markers of the dopaminergic pathway is the study of the pattern of resting glucose metabolism as measured by [18F]2-fluoro-2-deoxy-D-glucose (FDG; Eidelberg et al. 1995; Emborg et al. 2007; Moeller et al. 1999). FDG patterns can be associated with electrophysiologic recordings to assess neuronal activity of distinctive brain areas (Kazumata et al. 1997). Correlations with the results of awake electrophysiologic recordings, although difficult to perform in monkeys (e.g., Wichmann et al. 1999), could further help understand the physiologic changes induced by disease-modifying strategies.

Magnetic resonance imaging (MRI) is a useful method to determine precise coordinates for intracerebral procedures, as well as to provide information on the opening of the blood-brain barrier, presence of edema, inflammation, previous lesions or alterations caused by the parkinsonian agent (Miletich et al. 1994), or the accumulation of iron in the brain (Hardy et al. 2005). The same magnet can be used for proton magnetic resonance spectroscopy (MRS), functional MRI (fMRI), and diffusion magnetic resonance imaging (dMRI) to assess in vivo dynamic changes associated with neurodegeneration and antiparkinsonian treatments (e.g., Andersen et al. 2002; Brownell et al. 1998). Pharmacological MRI (phMRI) is proposed as a method to map DA function in primates by analyzing relative changes in cerebral blood flow after drug administration, such as amphetamine, and could become a diagnostic tool for PD (Chen et al. 2005; Jenkins et al. 2004).

Table 2 Radiopharmaceutical tracers used to evaluate in vivo the dopaminergic nigrostriatal system of parkinsonian monkeys

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Target</th>
<th>Monkey model (macaque monkeys)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-dopa</td>
<td>AADC/vesicles</td>
<td>Intracarotid MPTP</td>
<td>Pate et al. 1993</td>
</tr>
<tr>
<td>F-meta-tyrosine</td>
<td>AADC</td>
<td>Intracarotid MPTP</td>
<td>Eberling et al. 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracarotid + IV MPTP</td>
<td>Eberling et al. 1997</td>
</tr>
<tr>
<td>CFT</td>
<td>DA transporter</td>
<td>IV MPTP</td>
<td>Brownell et al. 1998b</td>
</tr>
<tr>
<td>Tetrabenazine</td>
<td>VMAT2 (vesicles)</td>
<td>Intracarotid MPTP</td>
<td>DaSilva et al. 1993</td>
</tr>
<tr>
<td>Raclopride</td>
<td>D2 receptor</td>
<td>Intracarotid MPTP</td>
<td>Leenders et al. 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC MPTP</td>
<td>Doudet et al. 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intracarotid MPTP</td>
<td>Doudet et al. 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV MPTP</td>
<td>Brownell et al. 1998b</td>
</tr>
</tbody>
</table>
discussion of counting methods in Geuna 2000). Morphological quantifications also include measurements of dopaminergic fibers in the striatum, to assess terminal damage (e.g., Emborg et al. 2001).

Depending on the PD model and the experimental design, it can be useful to include in the morphological assessment areas of the brain different from the dopaminergic nigrostriatal system (e.g., the cortex or hypothalamus), with different neurotransmitters (e.g., noradrenaline, acetylcholine) or markers of neurodegeneration (e.g., alpha-synuclein, ubiquitin), as well as areas of the body (such as the gut or heart) that are affected in PD. Assessments of these areas can assist in the understanding of the models and the effects of new therapies.

Do NHP Models of PD Have Clinical Validity?

Despite some limitations, NHP models of PD have changed the field of PD research. The identification of MPTP as a dopaminergic neurotoxin was possible because of tests of the drug in monkeys, as the compound does not affect rats (Burns et al. 1983; Langston et al. 1984), and this finding brought awareness of the impact of environmental toxins as risk factors for PD. Electrophysiological studies in MPTP-treated monkeys (DeLong 1990) found that nigral DA deficiency induces increased output from the globus pallidus pars interna and SN pars reticulata as well as excessive inhibition of the brainstem and thalamocortical neurons, resulting in PD motor symptoms. This knowledge led to subthalamic surgical ablation and deep brain stimulation (Bergman et al. 1990; Baron et al. 2002). MPTP-lesioned primates, like human PD patients, respond to antiparkinsonian medications and display the same motor complications associated with long-term drug use (Bezard 2001; Jenner 2003b). Researchers have developed symptomatic treatments like DA agonists (Jenner 2003a) and are evaluating new generations of compounds (e.g., D3 DA agonists, A2a antagonists, 5-HT1a antagonists) using MPTP monkey models as testing platforms.

NHP PD models have also been effective in studies assessing dopaminergic cell replacement (Redmond 2002), trophic therapy (Lapchak et al. 1997), and gene transfer for restoration (Bankiewicz et al. 2000, 2006; Emborg et al. 2007) or neuroprotection (Eslamboli et al. 2005; Kishima et al. 2004; Kordower et al. 2000, 2006). NHP studies using these novel technologies have paved the way for several clinical trials, some of them currently under way (Capitanio et al. 2004; Kordower et al. 2000, 2006). NHP models have also been described in systemic MPTP models.

In this context, it is important to recognize the need for exchange between the clinical and laboratory setting in order to further understand what causes PD and develop targeted treatments. The story of MPTP identification as a dopaminergic toxin is an example of successful translation from the patient bedside, where the effects of the toxin were first described (Davis et al. 1979; Langston et al. 1983), to the laboratory bench, where MPTP was recognized and used to develop animal models (Burns et al. 1983; Langston et al. 1984), which now are a tool to test new therapies that go back to the clinic.

The predictability of primate studies that lead to trials depends on the similarities between preclinical and clinical conditions (e.g., Roitberg et al. 2004; Salvatore et al. 2006; Sherer et al. 2006), appropriate statistical analysis, and the search for and identification of potential complications or limitations of the proposed technology and, most importantly, of the models.

Applying NHP Models of PD

Given the variety of NHP models of PD described in this article, it is clear that one model cannot replicate the complex and individual variability of PD. Accumulated evidence suggests that PD is not a single entity but rather is clinically, pathologically, and etiologically diverse. Its cause is probably a combination of genetic and environmental factors. There is no single definition of the different PD entities (e.g., tremulous, akinetic-rigid; Wolters and Braak 2006), and so any clinical study should indicate the characteristics of the patient population being studied (Litvan et al. 2007).

Parkinson’s researchers should consider the diversity of human PD when looking for a PD model, because there is also variability in NHP models of the disease. As discussed above, the various methods of inducing parkinsonism in monkeys affect the characteristics of the syndrome. The different NHP models can be considered as samples of sets of PD features (or syndrome subtypes) being studied at a specific time point in the progression of the disease. In that context, NHP models provide a useful framework to understand PD and to test therapies.

The selection of the model according to the scientific question to be analyzed is the first step to minimize a model’s limitations (Table 3). Aged monkeys with or without toxic treatments are an obvious model of choice to evaluate the role of aging in PD. Gene transfer models can address questions related to familial PD. Studies of disease progression will benefit from models with protracted cell loss, as seen with the chronic systemic delivery of MPTP. Motor complications such as L-dopa-induced dyskinesias are well mimicked in systemic MPTP models. Nonmotor symptoms, including cognitive and autonomic dysfunction, have also been described in systemic MPTP models.

Neuroprotective therapies for PD mainly attempt to prevent the loss of dopaminergic nigral cells and the functional consequences of this loss, and are most effective when they are administered early in the course of the disease. Their evaluation requires models that induce a replicable nigral lesion without spontaneous recovery. The model should also provide a window of opportunity in which the neuroprotective strategy can work. Intracarotid MPTP, 6-OHDA, or rAAV alpha-synuclein wild-type or mutated models may fit these requirements.
Restorative strategies try to reestablish lost function by replacing DA or restoring balance among affected nuclei and they require replicable, stable models. Any MPTP, 6-OHDA, or rAAV models are applicable if the animals are selected according to PD signs after a period of 2 to 3 months to ensure the stability of the symptoms, and then are randomly distributed between treatment groups. Trophic treatments can be considered neuroprotective or restorative depending on the timing of their administration, and the selection of testing model platform depends on that approach.

The choice of monkey species in which to model the disease affects the research, and there are various factors to consider when selecting a species. All the NHP species named in this review are nonendangered and can effectively reproduce in captivity. Small species, such as common marmosets or squirrel monkeys, compared to macaques or baboons, present the benefits of reduced housing costs, a high reproduction rate in captivity, and easy handling (Eslamboli et al. 2007). However, as the phylogenetic differences between humans and the selected species increase, so do the behavioral, physiological, and anatomical differences (Soderstrom et al. 2004). It should also be mentioned that Asian macaques are natural hosts of *Cercopithecine herpesvirus 1* (B virus), an alphaherpesvirus closely related to herpes simplex virus that can induce neurological damage and/or death in humans. Human infection with B virus is low, a uncommon result of macaque-related injuries, whether by direct exposure to secretions (e.g., through a bite, scratch, or mucous splash) or by indirect exposure (e.g., through injury from an object that was in contact with an infected monkey); it can be prevented by proper use of personal protection and, in the event of exposure, treatment with antiviral medications (for guidelines see Cohen et al. 2002; Holmes et al. 1995; Huff and Barry 2003).

Some of the limitations in NHP models depend on how the models are used. Investigators can minimize the limitations of a model by selecting a model and experimental design that fit the scientific question being asked, incorporate multiple outcome measures, use an appropriate number of animals per group, and include acquisition and analysis of the data by investigators blind to the treatment group. It is important to adjust the timeline of the experiments, including the period between the end of the intoxication and the proposed intervention and necropsy, to the model and to the testing paradigm.

As monkeys are a relatively restricted and expensive resource that requires expert care, many studies have used a limited number of animals. The use of few monkeys is suitable for pilot studies assessing proof-of-principle or the feasibility of an event or treatment (e.g., Kirik et al. 2003; Kordower et al. 1999). But experiments that seek definitive answers to questions of treatment efficacy require statistically powered analysis in order to be valid.

**Future Directions: Are New NHP Models of PD Needed?**

A significant challenge to PD research is the fact that Parkinson’s disease is not a single entity resulting from dopaminergic deficit but rather a multifactorial, multisystemic, multisymptom disease. In addition, researchers are identifying new genetic mutations that increase the risk of PD. Growing awareness of the complexity of repairing neuronal circuits has increased interest in the development of strategies to prevent neurodegeneration and sustain function, with a focus on developing PD treatments for use as early as diagnostic methods allow. These challenges highlight the limitations of current NHP models and the need for new ones.

Gene transfer methods with appropriate vectors to locally introduce mutated genes associated with PD will assist in the understanding of the effect of these proteins in the NHP brain and in PD development and will provide a model to test therapies that target specific forms of familial PD.

If PD is indeed induced by several causes, models that

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Table 3 Proposed suitability of PD monkey models for different studies, depending on the model characteristics. The experimental design plays an essential role to further define the suitability of the model to assess a particular scientific question (see text for details).a

<table>
<thead>
<tr>
<th>Model</th>
<th>PD etiology</th>
<th>Neuroprotective</th>
<th>Restorative</th>
<th>LID</th>
<th>Nonmotor PD signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>1–2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Alpha-synuclein gene transfer</td>
<td>1–2</td>
<td>1–2</td>
<td>1–2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6-OHDA intrastrital</td>
<td>1–2</td>
<td>1–2</td>
<td>1–2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>MPTP systemic acute</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MPTP systemic intermediate</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MPTP systemic chronic</td>
<td>1–2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MPTP intracarotid</td>
<td>2</td>
<td>1–2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MPTP intracarotid + IV</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*a1 = best, 5 = worst; LID = L-dopa-induced dyskinesias*
combine PD agents or risk factors (Carvey et al. 2006; Manning-Bog and Langston 2007) are an attractive option. Administration of MPTP to older adults (e.g., rhesus over 15 years) and aged monkeys has already shown the increased sensitivity with age to toxic insults and is helping understand the role of aging in PD development and therapy effects (e.g., Ovadia et al. 1995). Administration of pesticides (e.g., maneb or paraquat) alone or in combination with other age, gene transfer, or neurotoxic models may provide insight on the effect of accumulated risk.

The multisystem issue is problematic. Systemic MPTP delivery induces nonmotor symptoms (Almirall et al. 1999; Barcia et al. 2003; Schneider and Pope-Coleman 1995; Taylor et al. 1999), some of which seem to present spontaneous recovery when lesions are limited (Goldstein et al. 2003). Again, new pesticide models or a combination of toxins (e.g., MPTP for PD signs plus intravenous 6-OHDA for sympathetic cardiac denervation) may be an alternative to obtain global models of PD rather than models of dopaminergic nigrostriatal lesions.

RepliCity and progression of small lesions are necessary in order for investigators to attempt neuroprotection over time to rescue dying cells. The intracarotid MPTP model presents a chance to assess neuroprotection, but the period of time after application of the neurotoxin is short (approximately 1 week) and the neurotoxin induces a severe unilateral lesion, which restricts the kinds of agents that can be successfully used. In that regard, stable NHP PD models (i.e., those that do not include spontaneous recovery) are usually reported to have striatal DA losses of over 80% (Soderstrom et al. 2006). Striatal 6-OHDA requires a high number of injections to induce a relatively stable model, and the time frame for effective intervention is approximately 2 weeks. Transfection of gene mutations that induce nigral degeneration with vectors that reach a peak of gene production over several weeks may be an alternative.

If PD starts by affecting nonmotor areas (as suggested by Braak and Braak 2000), investigators should modify their models of early PD accordingly. The nature of the disease’s onset also affects the therapies proposed. Nigrostriatal targeted treatments, such as localized delivery of trophic factors, will not be sufficient to stop disease progression; instead, systemically administered or combination treatments will be more appropriate for global protection. Once again, assessing new PD agents and combining models may be a solution.

Basic researchers’ capacity to develop experimental designs that closely resemble the clinical conditions in which an event or treatment will be evaluated depends on early interaction between preclinical and clinical investigators. Again, the essential tools to minimize the limitations of current and future models of PD and to ensure their predictive clinical validity are selection of the NHP model of PD based on the scientific question to be answered, development of experimental designs with multiple outcome measures of clinical relevance, acquisition and analysis of data by investigators blind to the treatment conditions, and use of an appropriate number of animals for the phase of the study.

Should current NHP models of PD be abandoned in favor of new models? Definitely not. They can provide answers to questions about PD etiology and treatments, and some have already proven their utility. Furthermore, new NHP models of Parkinson’s disease will require funding and years of characterization, development, and validation, and even then will represent only certain aspects of the disease. Patients are still waiting for better treatments, if not for a cure.

Acknowledgments

The author acknowledges funding support from the Kinetics Foundation and Grant P51 RR000167 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), to the Wisconsin National Primate Research Center, University of Wisconsin-Madison. The author also gratefully acknowledges Judith Peterson for her assistance in the writing of this manuscript. This research was conducted in part at a facility constructed with support from Research Facilities Improvement Program grants RR15459-01 and RR020141-01. This article’s contents are solely the responsibility of the author and do not necessarily represent the official views of NCRR or NIH.

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